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Publication data: Fiziologia (Physiology) is issued quarterly
Subscription rates: Subscriptions run a full calendar year. Prices are give per volume, surface postage included.
Personal subscription: Romania - 100 RON, Outside Romania - 35$ (must be in the name of, billed to, and paid by an individual. Order must be marked "personal subscription")
Institutional subscription: 50$ (regular rate)
Single issues and back volumes: Information on availability and prices can be obtained through the Publisher.
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Bibliographic indices: We hope this journal will be regularly listed in bibliographic services, including "Current Contents".
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Editura **EUROSTAMPA**
Tel./fax: 0256-204816
ISSN 1223 – 2076

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CUPRINS

REVIEW

STEM CELLS AND THE PLACENTAL STEM CELLS

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ABSTRACT

Stem cells are cells that divide to produce one daughter cell that is a stem cell and another daughter cell that produces differentiated descendants. The definition fits a newly fertilized egg but begins to unravel as we move along the developmental pathway. Totipotent embryonic stem (ES) cells can readily be shown to differentiate into essentially all cell phenotypes, whereas most isolates of adult stem cells (multipotent stem cells) from sources as bone marrow stroma, fat, muscle, and nervous tissue have a more limited potential for differentiation. Most of the adult stem cells, but not all isolates, have a more limited life span in culture than the ES cells have. Moreover, the progression from a fertilized egg to an ES cell to an adult stem cell to a differentiated cell may be a continuum in which few if any steps are irreversible. Placental stem cells are at the crossroads of adult stem cells and ES cells, so that their increased potential should be explored in more detailed for further therapeutic strategies.

Key words: embryonic stem cells, adult stem cells, totipotent, multipotent

OVERVIEW OF STEM CELLS

Stem cells differ in their differentiation plasticity or developmental potential but they all have the ability to self-renew. Depending on the developmental potential, there are more types of stem cells, for instance pluripotent or multipotent stem cells, the unipotent and induced pluripotent stem cells. *Pluripotent stem cells* can be cultured and isolated from the inner cell mass of the blastocyst or morula stage, and have the ability to differentiate into cell types from all three embryonic germ layers. These cells are also referred to as embryonic stem cells (ESCs). Furthermore, *multipotent stem cells* can derive cell types from lineages other than their germ layer of origin such an example are/represent the mesenchymal stem cells. They can differentiate into multiple cell types that can span the ectodermal and mesodermal lineages. Other stem cells isolated from the child or adult have a limited potential in the differentiation process. The *unipotent stem cells* are proliferative stem cells that are committed to a single lineage and can only differentiate into cells of a related lineage, except for neural progenitor cells which can develop into both neuronal and glial cell types. Likewise, primordial germ cells are unipotent and develop into germ cells in the adult. In contrast to stem cells, *nullipotent cells* are committed somatic cells. These types of cells that are reprogramed *in vitro* to a less differentiated state are called *induced pluripotent stem cells (iPSCs*).

EMBRYONIC-LIKE STEM CELLS

Embryonic stem cells (ESCs) are very important cells that can be used in the treatment of different diseases such as: spinal cord injury, heart disease, diabetes etc. because of their potential to develop into many cell types, including chondrocytes, cells of the neural lineage, retinal cells and cardiomyocytes. Human ESCs are obtained from poor quality embryos donated from in vitro fertilization programs that would have otherwise been discarded, but, nonetheless, there are ethical and political challenges to be taken into account. The cells are isolated from the inner cell mass of five-to-seven day old blastocysts and are cultured with a fibroblast feeder layer. The tumorigenicity of the ESCs has yet to be resolved (1).

Embryonic germ cells (EGCs) have been used to repair bladder defects, cartilage degeneration and motor neuron injuries in rat models. However, research is trying to define a culture condition that facilitates the best derivation of EGSs. These cells are derived from PGSs, primordial germ cells who are difficult to obtain, considering that they are present in the fetal genital ridge by the fifth week of human development. In the female, the mitotic expansion begins at the 5th week and in the male at the 8th week so PGSs in both sexes arrest their expansion at week 10.

Germ-line stem cells (GSCs) and their potential in clinical applications is still ongoing. These cells are associated with fewer ethical concerns because they are derived from testicular biopsies in men, and have a potential adult source of pluripotent stem cells. The spermatogonia stem cells have the ability to self-renew and differentiate into sperm. However, the use of GSCs is limited by the possible restriction for use only in male patients, the uniparental epigenetic imprint (1).

Induced pluripotent stem cells (iPSCs) are derived from somatic, differentiated adult cells such as fibroblasts, keratinocytes, hepatocytes, neural stem cells and gastric epithelial cells. These iPSC cells are useful not only for *in vitro* human models in order to study the molecular mechanisms involved in human

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disease, but also for patient specific cell based treatments, as well as studying a multitude of other afflictions. Current studies have developed strategies for transdifferentiation, in which a cell of specific lineage is converted to another lineage and then into another cell type without reverting to a common progenitor state. Complications, however, do occur because of the use of genetic integrated viruses, as vectors in the reprogramming strategies. These complications include: inflammatory response, replication, virulence, tumorigenicity, immunogenicity, random integration, and reactivation. Future research focuses on establishing safe and effective methods for reprogramming cells (1).

PLACENTAL COMPARTMENTS

The human placenta is a feto-maternal organ. At term, it has a discoid shape with a diameter of 15-20 cm and a thickness of 2-3 cm (3). The placenta has a maternal and a fetal side. The maternal side has 16-20 cotyledons that are divided by the placental septa. The fetal adnexa are composed of the placenta, fetal membranes and umbilical cord. The fetal membranes amnion and chorion, extend from the margins of the chorionic disc which enclose the fetus in the amniotic cavity, and the endometrial decidua. The chorionic plate faces the amniotic cavity and consists of two different structures: the amniotic membrane and the chorion. The villi originate from the chorionic plate and anchor the placenta through the trophoblast of the basal plate and maternal endometrium. Some villi terminate freely in the intervillous space, while others anchor the placenta to the basal plate. In the term placenta, the stem villi show an inner core of fetal vessels with a distinct muscular wall and connective tissue. Mature intermediate villi and term villi consist of capillary vessels and thin mesenchyme. The stromal core is separated from the syncytiotrophoblast by the basement membrane. Between the syncytiotrophoblast, which is an uninterrupted multinucleated layer, and its basement membrane are single aggregated Langhans cytotrophoblastic cells also called cytotrophoblast cells. The amnion is a thin avascular membrane and is contiguous over the umbilical cord with the fetal skin. Microscopically, the amnion is composed of an epithelial layer of columnar and cuboidal cells, which lie on top of the mesodermal layer consisting of an upper acellular compact layer and a deeper layer containing dispersed fibroblasts. The chorionic membrane consists of a mesodermal layer and a layer of extravillous trophoblast cells (2,4,7).

Embryological development of the placenta

In humans, the placenta starts to develop during the implantation window, by days 6-7 after fertilization, when the blastocyst implanted. The blastocyst consists of the outer wall, blastocystic cavity and inner cell mass. The outer wall surrounds the blastocystic cavity while the inner cell mass, is represented by a small group of larger cell apposed to the inner surface of the trophoblastic vesicle. By days 8-9 the bilayered trophoblast is build. The inner layer becomes the cytotrophoblast, the inner cells remain temporarily unfused. The outer layer is the syncytiotrofoblast, formed by the fusion of neighboring trophoblast cells. Small vacuoles appear in the syncytiotrophoblastic mass, these vacuoles grow, become confluent and form a system of lacunae. The syncytiotrophoblast and cytotrophoblast form the trabeculae, which intermingle with the hematic lacunae. The primordial villi are generated by these trabeculae. Those which are in contact with the decidua basalis give rise to the chorion frondosum while villi in contact with the decidua capsularis build/form the chorion leave. The epiblast and hypoblast are differentiated from the inner cell mass at day 8-9 after fertilization. Small cells from the epiblast appear between the trophoblast, and the embryonic disc becomes the amniotic cavity. The exocoelomic membrane and its cavity modify to form the yolk sac, between the hypoblast and cytotrophoblast. The amniotic and chorionic mesoderm is built by the extra embryonic mesoderm that surrounds the yolk sac and amniotic cavity. The gastrulation process takes place (the bilaminar disc differentiates into ectoderm, mesoderm and endoderm) and, during the 3rd week after fertilization, a defined form develops with a right-left, cranio-caudal and dorsal-ventral body axes (2,5,6).

PLACENTAL TISSUE CELL POPULATION

Mesenchymal stem cells are defined as stem cells that can differentiate into multiple cell lineages and have the potential to self-renew (8,9). Moreover, they secret antiapoptotic and proangiogenic (10) cytokines and possess immunosuppressive properties (11). Caplan coined the term mesenchymal stem cells (MSCs) in 1991 (12). Bone marrow MSCs represent the primary source of MCSs and are most commonly used (13). In the 1860s Cohnheim was the first to hypothesize the existence of MCSs in the bone marrow (14). Maximov (1920) assumed the important role of the marrow stromal tissue in supporting the development and maintenance of blood and hematopoietic organs (15). Friedenstein demonstrated for the first time in 1960s that stromal cells could be isolated from whole bone marrow aspirate, based on differentiation adhesion to tissue culture plastic dishes (16). Mesenchymal stem cells can also be grouped into: adult MSCs and fetal MSCs. The adult MSCs are one of the most studied populations of multipotent ASCs (adult stem cells), they are isolated from bone marrow, peripheral blood. However MSCs from the bone marrow have to be obtained through invasive procedure and stem cell numbers decrease significantly with the age of the individual (3,4,17-19). This brought research to look for easily accessible sources of multipotent stem cells, in other tissues for example: peripheral blood (3,20), umbilical cord blood (3, 21-24) and more recently, deciduous tooth (25) and umbilical cord mesenchyme (26). There is still a debate if in some sources stem cells do exist (27, 28) and cell volume from these tissues can be limited (3,22, 25).

The fetal MSCs are isolated from placenta, amniotic fluid, umbilical cord and umbilical cord blood (2,29). The human placenta is a fetomaternal organ which has both maternal and fetal tissue. After birth, the term placenta is discarded which means that no invasive procedure is necessary. Furthermore, there are

no ethical conflicts and the tissues can be effectively used for clinical application, as well as research. Although they do not have the same proliferative and differentiation potentials of ESCs, the placenta-derived multipotent cells (PDMCs) may be superior to Adult stem cells and are of fetal origin (2,3).

MCS of maternal origin

Vikram Sabapathy and colleagues isolated placental mesenchymal stem cells from cotyledons of the maternal side while the placental membrane from the maternal side was cut open. About 80 g of cotyledon was exercised and washed with PBS, cut off into small pieces and blood clots were removed. The minced placenta was washed with physiological saline and subjected to digestion with trypsin and collagenase I, and then the tissues were incubated with 0.25% trypsin for 1hour at 37 °C. After the trypsinization, the sample was filtered through 250µm metal sieve. The supernatant was put up for a second digestion with 12.5 U/mL collagenase I for 1 hour at 37 °C. Collagenase I digested tissue was passed first through 100µm metal sieve and filtrate collected was then passed through 100µm cell strainer. The filtrate with the cell suspension was subjected to centrifugation at 300g for 10min after dual filtration stages. The cell pellet was resuspended in RBC lysis buffer and centrifuged at 300g for 10 minutes. At the end the cell pellet was resuspended in Mesenchymal expansion medium (alphaMEM + 10%FBS+ 50µg/mL streptomycin + 1Mm L-glutamine) and plated into two 75 cm^2 flasks (2). The isolated MSCs formed a homogenous monolayer of adherent spindle-shaped fibroblast-like cells, which are generally term placenta-derived multipotent mesenchymal stem cells. The colonies became visible after 7 days. By day 14 the MSC colonies started to proliferate, the flask was almost 60-70% confluent and ready for splitting. Flow analysis established MCSs phenotypic characteristics staining positively for mesenchymal lineage surface markers CD29+, CD73+, CD90+, CD105+, and negative for hematopoietic marker CD34-, CD45-, CD14-, HLA-DR-. The expression profile confirms the criteria generally defined for multipotent mesenchymal stem cells. By visualizing large lipid vacuoles, mineralized bone with calcium deposits and Saffranin O positive collagen matrix results the ability of MSCs to differentiate into adipocyte, osteocytes, chondrocytes, consequently, mesodermal cell lineage but also translineage differentiated towards pancreatic progenitor cells, neural cells and retinal cells displaying plasticity. Furthermore these cells maintained a normal karyotype while cell cycle or apoptosis pattern did not alter significantly, are Naïve for stimulatory factors CD80 and CD86 and have limited expression of MHC-II antigens. Moreover the soft agar assays showed that placental MSC do not have the ability to form invasive colonies (2).

Linju B and collaborators concluded that after the plating, the initial growth consisted of cells with two different morphologies: a non-fibroblastoid, an epitheliod, polygonal morphology and a fibroblastoid, spindle-shaped morphology. The fibroblastoid population continued to proliferate after numerous passages, while the non-fibroblastoid population disappeared after passing with trypsin. The tissue that was harvested from the placentas, were washed several times in PBS, mechanically minced and enzymatically digested with 0.25% trypsin- EDTA for approximately 10 minutes at 37 °C. The homogenate was then centrifuged and suspended in Dulbecco's modified Eagle's medium, supplemented by 10% fetal bovine serum. Cell cultures were maintained at 37 °C with water-saturated atmosphere and 5% CO_2. One to two times every week the medium was replaced. The cells were recovered with 0.25% trypsin / EDTA and replated at dilution of 1:3, when they were confluence more than 80%. Immunologically, PDMCs were negative for HLA-DR and positive for HLA-ABC, ESC-associated cell surface markers of SSEA-4, TRA-1-60, and TRA-1-81 (30-32). Furthermore the immunophenotyping of the PDMCs cells were positive for: SH-2/CD105, SH-3, SH-4, CD29, CD44, CD90/Thy-1, and CD166. These markers are common to MSCs (6, 21). In addition they are negative for hematopoietic surface markers of CD14, CD34, CD45, CD117/c-kit, AC133/2, endothelial cell markers of von Willebrand factor and Flk-1 and glycophorin A. Also the expression of the costimulatory molecules CD40L, CD80, and CD86 are negative and not only, there were no markers found in trophoblastic cells like HLA-G and cytokeratin 7 but the cells were positive for the nontrophoblastic markers of CD9 and vimentin.

The β-human chorionic gonadotropin (β-hCG) was measured by immunometric assay and after the second passage; β-hCG levels ranged from 2.16 IU/L to undetectable and in the culture media of JEG-3 cells, levels of up to 260 IU/L were detected (3).

MCSs of fetal origin

From the fetal placenta, four regions can be distinguished: amniotic epithelial, amniotic mesenchymal, chorionic mesenchymal and chorionic trophoblastic from which cell populations are isolated: human amniotic epithelial cells (hAEC), human amniotic mesenchymal stromal cells (hAMSC), human chorionic mesenchymal stromal cells (hCMSC), and human chorionic trophoblastic cells (hCTC). Human Amniotic Epithelial Cells have the ability to differentiate toward all three germ layers, demonstrated in vitro and express stem cell markers (33-36). They have unique characteristics and, currently, there is strong in vitro and in vivo evidence of neural, pancreatic and hepatic differentiation of hAEC. The mesenchymal stromal cells from the amnion and chorion are thought to be derived from the extraembrionic mesoderm (37). The distinct morphology of cultured hAMSC, hCMSC and hAEC is shown by phase micrographs, hAMSC is distinguished from hAEC by the expression of CD49d (α4 integrin). Also, hAMSC has a hybrid phenotype; the cells have mesenchymal and epithelial characteristics (shown by electron microscopy). This feature is interpreted as a sign of multipotentiality and is not found in hCMSC which are metabolically quiescent and more primitive (38). Human amniotic and chorionic cells successfully persistently engraft in multiple organs and tissues in vivo after either intravenous or intraperitoneal transplantation into neonatal swine and rats (39).

Table I. Isolation protocols, phenotype and *in vitro* differentiation potential of each cell type

	Human amniotic epithelial cells	Human amniotic mesenchymal stromal cells	Human chorionic mesenchymal stromal cells
Isolation protocols	Mechanical peeling of amnion from the underlying chorion followed by digestion with the following: dispase II (2,4 U/ml) for 1 hour or trypsin EDTA (different concentrations and incubation times). Centrifugation (200g for 10 minutes) of solution containing released cells, discarding the remaining membrane	Mechanical peeling of amnion membrane from the underlying chorion followed by digestion with various concentrations of collagenase (0.75-2mg/Ml) AND dnASE (20-75µg/ml) for 30-120 minutes at 37C	Mechanical removal of surrounding layers after treatment with 2.4 U/ml dispase II at 37C, followed by 1-3 hours treatment with Worthington collagenase II (270 U/ml) or collagenaseA (0.83 mg/ml)
Phenotype at passages 2-4	Mesenchymal and hematopoietic markers: CD105+, CD90+, CD73+,CD44+, CD29+, HLA-A,B,C+, CD13+, CD10+, CD166+, CD49d-, CD49e+, CD117 (+/- very weak signal), CD14-, CD34-, CD45-, HLA-DR-	Mesenchymal and hematopoietic markers: CD105+, CD90+, CD73+,CD44+, CD29+, HLA-A,B,C+, CD13+, CD10+, CD166+, CD49c+, CD49d+, CD49e+, CD54+,CD166+, CD271 low, CD14-, CD34-, CD45-, CD31- HLA-DR-, CD133-,CD3-	Mesenchymal and hematopoietic markers: CD105+, CD90+, CD73+,CD44+, CD29+, HLA-A,B,C+, CD13+, CD166+, CD49e+, CD-271low, CD10+,CD117-, CD14-, CD34-, CD45-, CD133-, HLA-DR-
Differentiation potential	Adipogenic: DMEM high glucose (or DMEM/Ham's F-12 medium), 10% FBS, 0.5mM isobutylmethylxanthine, 1µM dexamethasone, 10 µM insulin, 200µM indomethacin Chondrogenic: DMEM high glucose, 1% FBS, 6.25µg/ml insulin, 10ng/ml TGF-β1, 50ng/ml fresh ascorbic acid Osteogenic: DMEM high glucose (or DMEM/Ham's F-12 medium), 10% FBS, 10µM dexamethasone, 10 Nm 1-α,25-dehydroxyvitamin D3, 50 µg/ml ascorbic acid, 10 Mm β-glycerophosphate MesenCult Human Osteogenic Stimulatory Kit (StemCell Technologies, Vancouver, BC, Canada, http://www.stemcell.com	Adipogenic: DMEM, 10% FCS, 0.5mM isobutylmethylxanthine,200µM indomethacin, 10-6M(10 la -6) dexamethasone, 10µg/ml insulin Bullet Kit Adipogenic Differentiation Media (Cambrex, Walkersville, MD, http://www.cambrex.com) Chondrogenic: DMEM high glucose, 1%FBS, 6.25µg/ml insulin,10ng/ml TGF-β1, 50ng/ml fresh ascorbic acid DMEM, 6.25 µg/ml insulin, 6.25µg/ml transferrin, 6.25µg/ml selenous acid, 5.33µg/ml linolenic acid, 1.25mg/ml BSA,0.35Mm proline, 1mM sodium pyruvate, 10-7M dexamethasone, 0.1mM L-ascorbic acid-2-phosphate supplemented with 10ng/ml transforming growth-β-3 DMEM, 100nM dexamethasone, 50µg/ml L-ascorbic acid 2-phosphate, 1mM sodium pyruvate, 40 µg/ml proline, ITS (5µg/ml insulin, 5µg/ml transferrin, 5ng/ml selenous acid), 5ng/ml TGF-β1 Osteogenic: DMEM high glucose, 10% FBS, 10mM β-glycerophosphate, 50-200µg/ml ascorbic acid, 10-8 M dexamethasone, 1,α25-dihydroxyvitamin-D3 Commercial media: MesenCult Human Osteogenic Stimulatory Kit (StemCell Technologies); Bullet Kit Osteogenic Differentiation Media (Cambrex)	Adipogenic: DMEM high glucose, 10% FBS, 0.5mM isobutylmethylxanthine, 1µM dexamethasone, 10 µM insulin, 200µM indomethacin Bullet Kit Adipogenic Differetiation Media (Cambrex) Chondrogenic: DMEM high glucose, 1% FBS, 6.25µg/ml insulin, 10ng/ml TGF-β1, 50ng/ml fresh ascorbic acid DMEM, 100 nM, dexamethasone, 50µg/ml L-ascorbic acid-2phosphate, 1mM sodium pyruvate, 40µg/ml proline, ITS (5 µg/ml insulin, 5µg/ml transferrin, 5 ng/ml selenous acid), 5ng/ml TGF-β1 Osteogenic: 10µM dexamethasone, 10Nm 1α, 25-dihydroxyvitamin D3, 50 µg/ml ascorbic acid, 10Mm β-glycerophosphate

Pieternella S. IN 'T Anker and collaborators indicate that both fetal and maternal MSCs can be isolated from the human placenta, and that the amnion is also a good source for MSCs. Placenta and amniotic fluid were derived from human second-trimester and third-trimester pregnancies. Term third-trimester placenta tissue and amniotic fluid was harvested from women who underwent elective cesarean section for breech presentation and the amniotic fluid was collected by puncture through the membranes after opening the uterine wall during the operation. Decidua parietalis was collected by scraping it fom the chorion and term amnion was obtained by removing it from the membranes. From the central region of the maternal-facing surface of the placenta, decidua basalis tissue was dissected macroscopically. Second trimester amniotic fluid and placenta were obtained from women undergoing socially indicated termination of pregnancy, while decidua tissue was collected by ultrasound-guided transcervical biopsy with a uterine curette; amniotic fluid was collected by ultrasound transabdominal puncture using a 22-gauge spinal needle and amnion was collected after the abortion. Tissue specimen of decidua basalis and decidua parietalis from approximately 1 cm 3 an amnion from approximately 1cm2 were washed in PBS. Single –cell suspension of decidua basalis, decidua parietalis and amnion were made by mincing and flushing the tissue parts through a 100µm nylon filter (Falcon, Becton, Dickinson, San Jose, CA) with washing medium. The amniotic fluid was centrifuged for 10 minutes at 1,283 rpm and pellets were resuspended in Iscove's modified Dulbecco's medium containing 20 U/ml penicillin and 20µg streptomycin (P/S) and 2% heat-inactivated fetal calf serum that is washing medium. All single-cell suspensions were cultured in M199 supplemented with 10% heat-inactivated FCS, P/S, endothelial cell growth factor and heparin (8 U/ml). The maternal and fetal origin of the cells was determined by molecular human leukocyte antigen typing. There has been no difference detected among the expression of MSCs markers from different sources. The second trimester AF and amnion is a rich source of MCSs, and the growth curves of the both were very similar. Although the second trimester AF is an abundant source of MSCs, compared to the third trimester where proportion of viable cells decreased with GA, the risk of amniocentesis prohibits the use for routine clinical use (4).

CELL AND TISSUE BANKING

The European Community directive 2003/94/EC lays down the principles and guidelines of good manufacturing practice (GMP) for medical products for human use, but also other institutions such as The European Association of Tissue Banks and the Joint Accreditation Committee-ISCT& EBMT also publish standards for cell and tissue banking and interpreting laws (40-42). Amniotic membranes are widely used in skin transplantation (43), treatment of burned and ulcerated skin (44,45), connective tissue defects (46), ophthalmology, surgery and wound healing (47,48). Its use has a history of almost 100 years. They can be freeze-dried, gamma-sterilized, decellularized, glycerol-preserved and cryopreserved. A comparison of the cryopreservation of the membranes with the storage in glycerol at 4 °C showed a

retained cell viability and release of angiogenic growth factors and cytokines in the first method while in the glycerol method resulted immediate cell death (49). It has been confirmed that other methods like irradiation influences the growth factor content of amniotic membrane (50). The most experience in preservation of placental tissue derived cells has been gained with cord blood. Because of the efficiency of cord blood transplantation many banks were established offering collection and banking for public and private use. Cord blood contains both mesenchymal stem cells and hematopoietic stem cells and it can be procured from natural births or caesarean section (51). It has been proven that placentae collected from natural birth are more likely to be contaminated with aerobic or anaerobic bacteria then the placentae collected from caesarean sections. Therefore it may be preferential to collect placentae from caesarian sections (2). Cord blood products containing cryoprotectans are frozen at a controlled-rate and can be stored in liquid nitrogen for at least 15 years without the loss of their engraftment potential in vivo (51,52) while fetal membrane stem cells are presently preserved mainly for research.

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CELULELE STEM SI CELULELE STEM PLACENTARE

REZUMAT

Celulele stem sunt celule care se divid pentru a genera o celula progenitoare, care este o celula stem, si o alta celula progenitoare, care produce descendenti diferentiati. Definitia se poate aplica embrionului fecundat, dar nu mai are aplicabilitate pe masura ce ne indreptam spre caile de dezvoltare celulara. Celulele embrionare (ES) totipotente se pot diferentia cu usurinta spre toate fenotipurile celulare esentiale, in timp ce celulele stem adulte (multipotente) izolate din diverse surse tisulare – stroma maduvei osoase hematogene, tesut adipos, muschi, tesut nervos – prezinta un potential de diferentiere limitat. Majoritatea celulelor stem adulte, prezinta un ciclu de viata redus comparativ cu celulele ES. Mai mult, progresia de la embrionul fecundat, la ES si apoi la celulele stem adulte si celule diferentiate ar putea fi un proces continuu, in care doar unele etape sa fie ireversibile. Celulele stem placentare se afla la intersectia dintre celulele stem adulte si celulele ES, astfel incat potentialul crescut al acestora ar trebui explorat in detaliu, pentru utilizarea lor in viitoare terapii celulare.
Cuvinte cheie: celule stem embrionare, celule stem adulte, totipotent, multipotent

THE TAURINE EFFECT ON ELECTRICAL CEREBRAL SIGNAL IN OLD RATS

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ABSTRACT
In the aging process, there are a series of structural and functional brain changes that cause modifications in electrical activity. Taurine is an amino acid which has a osmoregulator, neuroprotector and antioxidant actions in the central nervous system. The aim of the experiment was to test the effects of taurine on brain electrical activity in old rats. Two groups of Wistar rats, 26 months old were formed.They were chronically cortical implanted. Taurine was administrated on a period of two months in drinking water (1g/kgc). The electroencephalographic recordings were made at the beginning of the experiment, at one month and at two months. The results indicated the increase in spectral power density in the delta and theta bands and the improvement of the correlation coefficient between the two cerebral hemispheres.
Keywords: taurine, oxidative stress, cerebral electrical signal, old rats

INTRODUCTION

Aging is accompanied by multiple structural and functional cortical changes. Among these, may be mentioned the decrease in the number of neurons (1, 2), the loss and modification of synapses (3). It was also noticed a reduction in neurons receptor surface due to decreased number of dendrites and shortening process (4). Changes in the brain electrical activity can be computed through quantitative electroencephalography analysis.

Taurine is a widespread amino acid in the body, including the central nervous system. At this level has osmoregulator, neuroprotective and antioxidant actions. In the brain, taurine is found both in neurons and astrocytes.The main pathway for taurine biosynthesis is that of cisteinsulfuric acid decarboxylase (5). Neurons lacks this enzyme and base on taurine acquisition (6). Cisteinsulfuric acid decarboxylase is primarily found in glial cells (7). Taurine content decreases in the aging brain (8).This aminoacid is taken from plasma through a transport system based on Na^+ and Cl^- gradient, which is located in luminal and basal membranes of endothelials cells from hematoencephalic barrier (9).The purpose of this article is to present the results obtained by quantitative electroencephalographic analysis of changes in brain electrical signals, induced by taurine in old rats.

Electroencephalographic analysis

Electroencephalography is the recording method of the electrical activity of the brain. In 1875, Caton R. published the first paper about registering spontaneous brain electrical activity in experimental animals (10). In 1924, Berger H. made the first electroencephalographic recording in humans, which was published in 1929 (11).

Brain signals are complex electrical signals. Frequencies spectrum of electroencephalographic waves was divided into five bands: delta (0.5 - 4 Hz), theta (4 - 8 Hz), alpha (8 - 13 Hz), beta (13 - 30 Hz), gamma (30 - 70 Hz). Delta band waves are generated in the cortex and thalamus (12). They occur in deep sleep, stages 3 and 4 (13) and in brain lesions. Theta rhythm is seen in superficial sleep, stages 1 and 2 and REM sleep. In adult humans, in wakefulness, there are two types of theta rhythms: the first is associated with decreased sensory perception (drowsiness and impaired information processing) and the second occurs in the frontal midline area and is associated with attention and mental effort (14). In rats, hippocampal theta rhythm prevails. It occurs when the animal is involved in a motor activity and during REM sleep (15). Alpha rhythm is recorded in wakefulness, relaxing state, with closed eyes. It has the largest amplitude in the occipital region and it is blocked by the eyes opening and mental concentration. Beta waves present mainly a fronto - central distribution and are associated with activity, with thinking and concentration. Gamma rhythm is associated with intense cortical activation and object recognition.

Quantitative electroencephalography analysis deals with mathematical processing of brain electrical signals, the most frequently used being the spectral analysis and coherence analysis.

Fourier Transform is used for data analysis, because it allows signal decomposition into sinusoid constituents of different frequencies. For a given sample of data, Fourier analysis is performed using Fast Fourier Transform.

A parameter that can be calculated using Fast Fourier

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Transform is spectral power density. Spectral power density indicates the distribution of the power at different frequencies. It represents the Fourier Transform of the autocorrelation function and has the following formula:

$$Sx(f) = \int_{-\infty}^{\infty} Rx(\tau) e^{-2\pi i f \tau} d\tau$$

The autocorrelation function describes how a signal changes over time and what is the signal correlation in two different moments in time. It has the following equation:

$$Rx(\tau) = E[x(t)x(t+\tau]$$

To avoid convergence problems, it is considered a signal recorded on a finite interval of time, T, where $x_T = x(t)w_T(t)$

, where $$w_T = \begin{cases} 1 \, for \, 0 \le |t| \le \frac{T}{2} \\ 0 \, for \, |t| > \frac{T}{2} \end{cases}$$

Fourier Transform is applied to x_T

The result obtained is

$$X_T(f) = \int_{-T/2}^{T/2} x(t) e^{-2\pi i f t} dt$$

The variables are replaced in the equation of the autocorrelation function and the spectral power density formula is obtained.

Studies on analysis methods of quantitative electroencephalography showed that this is highly reliable. Salinsky et al., 1991, demonstrated that the reliability for 20 seconds epochs is 82% and for 60 seconds epochs is 92% (16). Gasser et al., 1985, indicated that in electroencephalographic analysis 20 seconds epochs are enough to reduce signal variability (17).

MATERIALS AND METHODS

The experiment consisted of recording cortical electrical activity in studied animals before and after administration of taurine. All procedures respected the directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

The experiment was conducted on two groups, one test and one control, of ten Wistar male rats each. Rats, 26 months old, were bred in standard conditions in the Biobase of the University of Medicine and Pharmacy „Carol Davila". The animals were anesthetized with chloral hydrate, 400 mg / kgc (Novachim Trading S.R.L.) and were chronically implanted under stereotaxic control. The electrodes were placed after the model introduced by Zagrean L., as follows: two electrodes at 5 mm anterior to bregma, at 3 mm lateral right and left of the midline and two electrodes at 6 mm posterior to bregma, at 4 mm lateral right and left of the median line (18). The internal part of the electrodes was placed on the cerebral cortex and the external part was connected to a pin array. The system was secured to the skull with dental acrylic cement. The scalp was sutured and the animals received antibiotic. They were placed individually in transparent cages and were allowed a period of a week to recover from operator stress. The rats were exposed to a cycle of 12h light, 12 hours dark, with free access to food and water at a temperature of 20 Celsius degrees. Taurine (Novachim Trading S.R.L.) was administered over a period of two months in drinking water in a volume of 20 ml, which contained the daily dose of 1g/kgc (19). For the rest of the day there was access to taurine - free water. Taurine administration was started after the first electroencephalographic recording, which was performed at one week after implant. The other two recording sessions were performed at one and two months from taurine first administration. To exclude the effects of circadian tests were done at the same time. The recording system was composed from MP150 units and Acqknowledge 4.2.0 software (BIOPAC System, Inc.).

RESULTS

Electrocorticographic recordings were made in anterior - posterior axis, using bipolar derivations. The quantitative processing of the electrical signal was made with aid of the Acqknowledge 4.2.0 software. Statistical analysis of the data was made using Kingsoft Spreadsheets software (Kingsoft Office Software Corporation Limited).

The average magnitudes of the waves for the five frequency bands were computed using Fast Fourier Transformation.The results obtained indicated statistically significant increases in waves magnitudes in delta and theta bands for the test group. For the control goup there were no changes in time. The p was computed using the values obtained from recordings at control and at two months moments. The p value for the right hemisphere was 0.0017 for the delta band and 0.002 for the theta band. For the left hemisphere the p value was 0.002 for the delta band and 0.0023 for the theta band. The changes obtained were represented as percentage in Figures 1 and 2 for each hemisphere. Each percentage was calculated compared to the control. It should be noted that the most significant increases from baseline were recorded at one month.

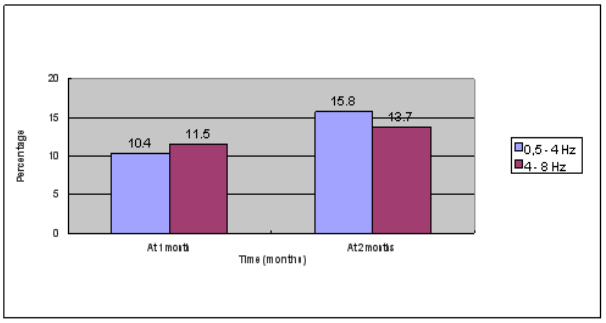

Fig.1. Percentage changes of waves magnitudes in right cerebral hemisphere. It was noticed the increased magnitudes of cerebral waves of the delta and theta bands at 1 month and at to 2 months.

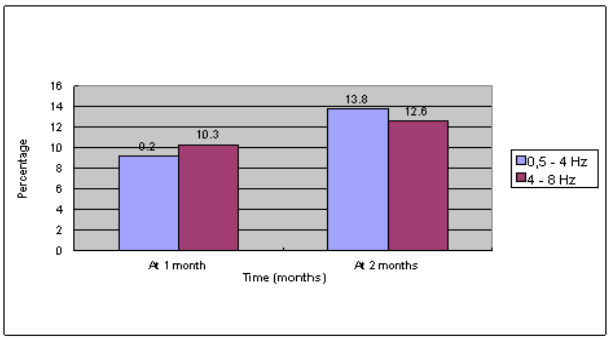

Fig.2. Percentage changes of waves magnitudes in left cerebral hemisphere. It was noticed the increased magnitudes of the two bands at the two registration moments.

Another analyzed parameter was the spectral power density (PSD). This was computed with aid of the Acqknowledge 4.2.0. software witch used a Welsh approximation algorithm to average epochs of the signal and reduce noise effect and a Hamming window tapering. It was calculated the average power spectral density for each frequency band. Statistically significant increases were observed for the density of delta and theta bands in the test group. For the control goup there were no changes in time. The results are shown in Figures 3 and 4.

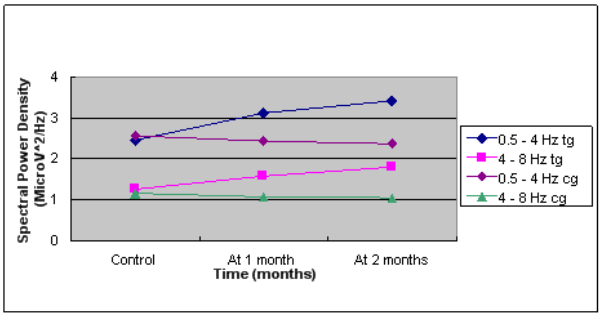

Fig.3. Spectral power density in right cerebral hemisphere (MicroV^2/Hz). It can be observed the progressive increase of PSD values in the test group compared to the control group.The most important change was observed for the delta band. The p value for delta band was 0.0004 and for the theta band was 0.0013.

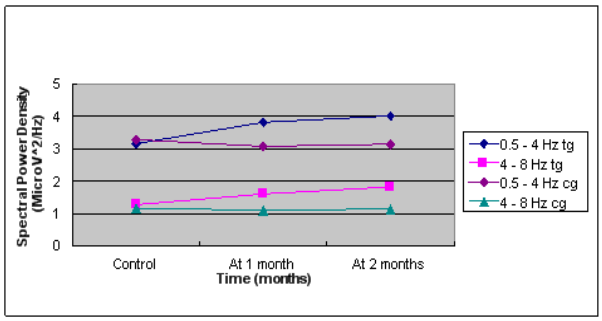

Fig.4. Spectral power density in left cerebral hemisphere (MicroV^2/Hz). It can be observed the increased values of PSD for delta and theta bands, indicating that at 1 month the increase was the most important. The p value for the delta band was 0.0006 and for the theta band was 0.0014.

Correlation coefficient (r) is an important statistical index and shows the degree of correlation between the two phenomena and it can be applied to signal analysis. The absolute value of r ranges between 0 and 1. The degree of correlation is proportional with the r absolute value. The correlation coefficient between the two hemispheres was computed with the aid of Acqknowledge 4.2.0 software. Its value increased at one month and two months compared to control. The results are shown in Figure 5.

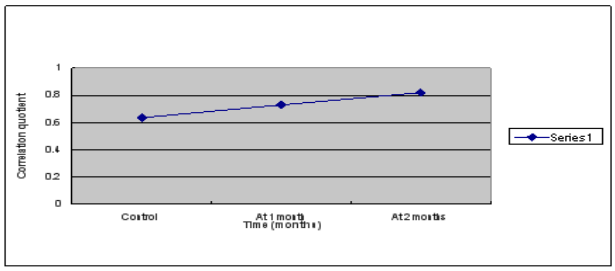

Fig.5. Correlation coefficient between the two hemispheres. It can be noticed that the value of correlation coefficient increased gradually.

DISCUSSION

The aging process is accompanied by a series of brain changes such as loss of neurons in various structures and loss of synapses. From functional point of view, it was noticed a decreases in the discharge rate of cortical neurons (20). Electroencephalographic recordings shows a decrease in the magnitude of electric signal from the delta and theta bands (21).

Brain aging is associated with increased levels of reactive oxygen species (22), that react with nucleic acids, proteins and membrane lipids generating gene mutations, impairments or loss of enzyme activity, abnormal protein clearance and altered cell membrane permeability (23, 24, 25).

Protein modifications include carbonyl formation and covalent modification of cysteine, lysine, and histidine residues by the lipid peroxidation product 4-hydroxynonenal. In aged brain have been detected high levels of lipid peroxidation products (26).

Taurine is one of the most abundant amino acids in the organism, being found in the brain and spinal cord. Taurine is an osmoregulator, a neuroprotector and antioxidant agent.

Endoplasmic reticulum stress due to the accumulation of misfolded proteins interferes with neuronal signaling. In PC12 cell cultures and primary neuronal cultures, in oxidative stress and excitotoxicity conditions, taurine reduces the endoplasmic reticulum stress by decreasing the levels of certain proteins such as Grp 78, CHOP/GADD153, p-IRE and p-eI0046–2 alpha protein (27, 28). Taurine acts as antioxidant also by increasing the levels of protective enzymes against reactive oxygen species, as glutathione peroxidase and superoxide dismutase (29).

Administration of taurine improved alterations in electrical activity. Recordings made at one month and at two months from the start of taurine administration indicated an increase in waves magnitude from delta and theta bands. Another result was an increase in the power spectral density of the two bands for the group test. Improving the synchronization of electrical activity between the two hemispheres has been

demonstrated by the progressively higher values of the correlation coefficient.

CONCLUSION

Taurine has benefic effects over cortical electrical activity. The changes induces by taurine administration over a period of two months in analyzed parameters, may due to its antioxidant activity in central nervous system.

ACKNOWLEDGEMENT

The experiment presented, including procedures, were part of TD grant, number 449/2007, which was approved by the Board of University of Medicine and Pharmacy „Carol Davila" and by the National Council of Scientific Research in Higher Education from Romania.

DISCLOSURE STATEMENT

There are no conflicts of interest between authors. The article is not submitted to other journal for publication.

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EFECTELE TAURINEI ASUPRA ACTIVITATII ELECTRICE CEREBRALE LA SOBOLANII BATRANI

REZUMAT

In procesul de imbatranire apar o serie de modificari cerebrale structurale si functionale care determina modificarea activitatii electrice. Taurina este un aminoacid, care la nivelul sistemului nervos central are rol osmoreglator, neuroprotector si antioxidant. Scopul experimentului a fost testarea efectelor taurinei asupra activitatii electrice cerebrale la sobolanii batrani. Au fost constituite doua loturi, unul control si unul test, de sobolani Wistar in varsta 26 luni. Acestia au fost implantati cronic cortical. Administrarea taurinei s - a facut pe o perioada de doua luni in apa de baut (1g/kgc). Inregistrarile electroencefalografice s-au realizat la inceputul experimentului, la o luna si la doua luni. Rezultatele au indicat cresterea densitatii puterii spectrale in benzile delta si theta si imbunatatirea gradului de corelatie intre cele doua emisfere cerebrale la lotul test.

Cuvinte cheie: taurina, stres oxidativ, semnale electrice cerebrale, sobolani batrani

HYDATID CYST OF THE KIDNEY: PRECLINICAL AND CLINICAL PARTICULARITIES

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ABSTRACT

Renal echinococcosis is rarely occurring in comparison with liver and lung localization. Kidney involvement reveals a percentage of 6% in confirmed cases of hydatid disease. In this paper we point the elements of physiology, preclinical and clinical aspects of hydatid cyst of the kidney. In the same manner are presented the diagnostic steps with the differential connotations. Since the availability of nuclear medicine and clinical laboratory brings new insights in the diagnostic accuracy, medical and surgical treatment remains the most important benefit for the patient.

Key words: kidney, echinococcosis, immunology, diagnostic, surgery, considerations

INTRODUCTION

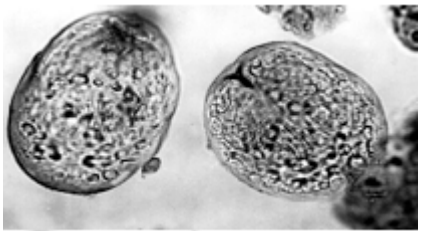
A renal hydatid cyst is a parasitic infection (benign) caused by larval growth of the cestode *Echinococcus granulosus*.

Synonym: *Taenia echinococcus*

Disease: *Hydatiosis, Hydatid disease, Echinococcosis.*

Elements of morphology

Adult parasite, specially occurred in dogs, is the lowest cestode of medical importance. Its measure is from 2.5 to 9 mm, consisting from a scolex, a neck and only three attached segments (proglottids). The first proglottid is short and contains immature genital organs. In the second proglotte, a bit elongated, the genital organs are completely developed. The scolex is granular, measuring 0.3 mm in diameter equipped with 4 suckers and 28-40 hooks arranged in two rows (1). The uterus reaches the maximum development in the last segment being composed from short diverticles which as filled with eggs become wider, taking the aspect of a bag full of eggs. After the proglotte's rupture, the eggs are eliminated in feces. The definitive host can accommodate thousands of adult parasites, their lifespan is between 6 months and 2 years. The eggs are typical for most *taeniid* species, small and round 34-41μm in diameter, thick-shelled, containing a hexacanth (6-hooked) embryo (oncosphere) (Figure 1).



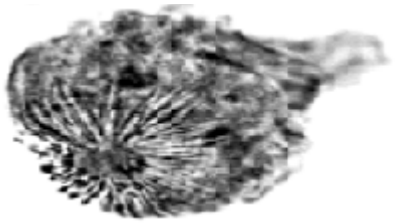


Fig.1. Invaginated and exvaginated ptotosleces (after Steriu D)

The evolutive cycle

Definitive hosts are carnivores such as dogs, wolfs, foxes, hyena. Gravid proglottids or released eggs are shed in faeces and after their ingestion by the host; a oncosphere larva is released and penetrates the intestinal epithelium into lamina propria. The passively pass through blood or lymph into the target organs, where it develop into hydatid cyst. The hydatide is compose of a thick cuticle coated inside by a proligere membrane and inside by a adventia membrane. Inside are found the hydatic liquid and scoleces. At the contact zone between parasite and infected organ there is a dense coating which can reach a thickness of 1-2 mm, produced by the condensation and infiltration of the organ that develop hydatid cyst. The growth of hydatid cyst is concentric with a rate of 1-5cm/year (Figure 2).

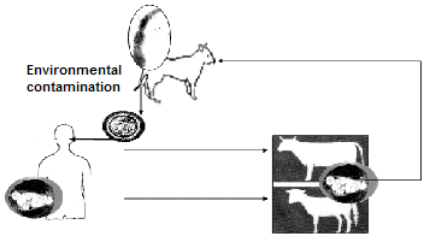


Fig.2. The evolutive cycle (after D.Steriu)

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In humans the cysts can reach a volume of several liters and they can contain hundreds of thousands protoscoleces. The infectious potential of hydtid cyst is enormous, one single proligere vesicle contains between 10-120 scoleces, 1cm^3 of sand contains 400.000 scoleces, and a hydatid cyst cam contain 3-6 cm^3 of hydatid sand.

Patogenesis

Hydatid cyst can develop in humans in two situations: by ingestion of eliminated embriosphere from dog, it forms a primary hydatida, located in different organs, depending on blood stream passive migration of the parasite (1). Most embryos are retained at the level of hepatic capillary network (60-70%) especially in the right lobe; 20-30 % retired in the pulmonary filter and only 10% passes in the circulation, being disseminated in muscles (4%), spleen (2%), brain (1%), kidney (1%), orbit (0.5%), bones (1-2%). Usually they are located in one single organ but there are clinical cases described with plurivisceral localization. It depends presumably by the intensity of the contamination. Other modality in production of hydatiosis is the rupture of the primary hydatid cyst followed by the protoscoleces release.

The protoscoleces are converted in new hydatids, generating local secondary echinococcosis or if disseminate at a distance of the primitive focus, it produces systemic secondary echinococcosis (2). The parasited organ will influence its further development determining the shape and latent period, when becomes symptomatic. The rhythm of hydatid cyst development is very different and may vary depending on the host, the parasitic tissue being as well conditioned by the cyst fertility and organ tolerance. The hydatid cyst can damage profoundly the infested organ through the action of parenchyma destruction accompanied by the hypertrophy of free territory. The hydatid cyst once constituted can describe three evolutive stages: spontaneous involution, rupture, infection. Initially the cyst is univesicular and after 6-8 months the vesicles becomes fertile (proligere vesicles and protoscoleces). The thickness of the cuticle grows gradually by adding new lamellar structures increasing the liquid quantity till 5 to 800 cm^3. After a variable number of years the hydatide may die, replaced by a formation well tolerated by the body. The hydatid cavity will be surrounded by a fibrous capsule charged with limestone salts. The cyst rupture can be caused by a trauma or an increased renal pressure.

Clinical manifestations

Most of the infections progresses asymptomatic. In other cases, the clinical manifestations are determined by the localization of the cyst, by dimension and it state: intact, cracked, infected. Renal hydatid cyst can remain asymptomatic for many years and can be discovered accidentally (3).

Cardinal symptoms: flank pain (lumbar and abdominal), perirenal reactions suggests the appearance of "fixed tumor" indicate a solid tumor actually. Digestive symptoms: subcostal pain, abdominal distension and vomiting.

Hydatiuria (the presence of daughter vesicles in urine) can be recognized macroscopically, being as well the only pathognomonic evidence of a hydatid cyst that has ruptured in the urinary tract.

Other: renal colic, fever, allergic reactions.

DIAGNOSTIC

Immunoserologic studies

Immunoserological diagnosis is helpful in determination of parasitic etiology, if the factor that influences the result is known. Firstly, a negative result does not exclude the diagnosis of hydatiosis because some cyst carriers have no detectable antibodies. The presence of a detectable immune response is conditioned by the localization, integrity and vitality of the cyst. The immunological diagnosis consists in detection of anti-E. Granulosus antibodies can be detected from the patient's sera, via indirect hemagglutination, immunoelectrophoresis, ELISA, Casoni's test including immunofluorescence. The highest specificity is done by the test based on the detection of antibodies against 5 specific antigens.

Radiologic studies

These studies are the most helpful tool. During the 1960's and 1980's retrograde pyelography and less often aretiography were used. Plain abdominal radiography can be sometimes useful. The classification of the hydatid cysts was done, according with Gharbi studies (Table I) (3):

Table I. Garby ultrasonographic classification

Type	Criterion
I	Well limited liquid cyst, with parietal echo backing
II	Cyst with a detached membrane or a floating membrane
III	Partitioned cyst with a honeycomb picture
IV	Heterogeneous echo structure formation, sometimes with liquid predominance and sometimes with solid predominance
V	Dense reflecting pictures with a posterior shade cone, corresponding to the calcified cyst

Computed tomography usually after ultrasonography is mandatory in most of the cases. Renal echography should be performed as well.

Surgical terminology and procedures

Treatment of cyst → cystectomy

Resection of the preeminent dome → partial peri-cystectomy

Total cystectomy → Total pericystectomy

Epidemiology

Humans are occasionally infected: infection is caused by occupational factors, the more exposed people are hunters

and the persons which process the foxes furs (1). The dogs are infected by capturing and eating rodents which can carry the larval form of the parasite, becoming sources of infection for humans trough the eggs eliminated via feces.

Methods of prevention

Infection can be avoided by removing the eggs from the fruits, vegetables or other foods. It is proved that the eggs are destroyed after exposure for three hours at 45°C or -80°C (1). In endemic areal the measures of hygiene recommends', avoiding the contact with foxes and their excrements, in order to protect the people having a high risk of contamination. It is indicated for hunters to wear rubber gloves when processing the fox's fur and for other people is indicated to wash the fruits and boil the vegetables before consuming/prepare them.

CONCLUSIONS

Renal hydatiosis due to Echinococcus is an endemic parasitic zoonosis characterized by worldwide distribution. The most commonly involved anatomical locations are the liver and the lung. The renal cases are rare.

The immunoserological diagnosis may contribute in order to monitor the patient after the surgical intervention.

CT is recommended in the pre-operatory phase, suggesting to surgeon a indispensable vascular cartography, if it is decided a nephrectomy, especially for the retroperitoneal procedures, giving information on the benign nature of the process.

The exploratory puncture is formally contraindicated due to the risk of secondary seeding.

Imagistic techniques are important in the diagnosis of renal hydatiosis and should be corroborated with laboratory data.

ACKNOWLEDGEMENTS

We would like to acknowledge to Prof. Univ. Dr. Lotus Elena Mester for the important contribution in scientifical support with regards to biology of the parasites. We also would like to acknowledge to Mihaela Radu.

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CHISTUL HIDATIC RENAL: PARTICULARITATI PRECLINICE SI CLINICE

REZUMAT

Echinococoza renala este rar intalnita in comparatie cu cea hepatica si cea pulmonara. Implicarea renala arata un procentaj de 6% din totalul cazurilor confirmate. In acest articol subliniem elemtele de fiziologie, aspectele preclinice si clinice ale chistului hidatic renal. In aceeasi maniera sunt prezentate etapele diagnosticului cu conotatii diferentiale. Intrucat disponibilitatile moderne ale medicinei nucleare si laboratorului clinic aduc noi perspective in acuratetea diagnosticului, tratamentul medical si chirurgical raman beneficiul primordial pentru pacient.

Cuvinte cheie: rinichi, echinococoza, imunologie, diagnostic, chirurgie, consideratii

SUBCLINICAL ATHEROSCLEROIS IN RHEUMATOID POLYARTHRITIS

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ABSTRACT

RP is considered an autoimmune disease due to the presence of autoantibodies, such as anti-cyclic citrullinated peptide antibodies (tested as anti-cyclic citrullinated peptide), that may be present many years before clinical manifestation of RP. Atherosclerosis has common features with autoimmune diseases, as showed in the study in which β_2-glycoprotein I-reactive transfer lymphocytes increase atherosclerosis in diseased mice. Cardiovascular diseases (CVD) are a major cause of comorbidity and mortality, reportedly related to approximately 60% of the mortality risk in RP.

Aim: The aim of this paper is to assess atherosclerotic lesions by carotid ultrasound and to characterise the factors responsible for their onset in PR patients. Material and methods: This study has been conducted on a group of based on the criteria set by American College of Rheumatology-ACR/European League Against Rheumatism-ELA revised in 2010. Medication used by patients consisted of methotrexate and sulfasalazine. The control group was composed of 17 healthy women of age similar to RP patients. Both groups underwent carotid ultrasound by ALOKA ProSound 4000 echograph, using 10 MHz linear probe. Biochemical and immunological investigation consisted in determination of: total cholesterol (Abbott photometry), C reactive protein (CRP - Turbidimetry) triglycerides (Abbott reagent), anti-cyclic citrullinated peptide antibodies (Microparticle enzyme immunoassay), rheumatoid factor (photometry) and platelet sedimentation rate (PSR - Electro Optical System Technologies).RP activity has been appreciated by the means of DAS28 (Disease Activity Score) index.

Results: Carotid IMT in RA patients has been strongly correlated with: DAS28 index ($r = 0.869$, $p < 0.01$), disease evolution time ($r = 0.944$, $p < 0.01$), systolic blood pressure value ($r = 0.946$, $p < 0.01$). Medium correlations were recorded between this parameter and diastolic blood pressure value ($r = 0.568$, $p < 0.05$), number of relapses ($r = 0.669$, $p < 0.01$), and value of total cholesterol ($r = 0.678$, $p < 0.01$), respectively. Carotid IMT has been weakly correlated with the values of triglycerides, anti-cyclic citrullinated peptide antibodies, PSR and CRP.

Conclusion: Subclinical atherosclerosis has high incidence among patients with RP. Besides the traditional cardiovascular risk factors, other factors related to the main disease, as well as to its treatment occur in these patients. Vascular ultrasound of common carotid artery is a non-invasive mean to identify and quantify subclinical atherosclerosis in order to apply therapies to prevent the worsening of disease and the onset of clinically manifest atherosclerosis.

Keywords: subclinical atherosclerosis, rheumatoid arthritis

INTRODUCTION

Rheumatoid polyarthritis (RP) is a chronic systemic inflammatory disease marked by joint inflammation and sensitivity, as well as by synovial joint destruction, leading to severe disability and premature mortality (1). RP is considered an autoimmune disease due to the presence of autoantibodies, such as anti-cyclic citrullinated peptide antibodies (tested as anti-cyclic citrullinated peptide), that may be present many years before clinical manifestation of RP (2).

Austrian pathologist Carol von Rokitansky (1840) and Rudolf Virchow were the first to describe the nature of inflammation in atherosclerosis. Although Rokitansky believed the inflammation is secondary to other processes, Virchow promoted atherosclerosis as a primary inflammatory disease (2).

Atherosclerosis is an inflammatory process in the large and medium arteries, characterized by infiltration of monocytes/macrophages and activated T cells into intima. Atherosclerosis has common features with autoimmune diseases, as showed in the study in which β_2-glycoprotein I-reactive transfer lymphocytes increase atherosclerosis in diseased mice (2). Cardiovascular diseases (CVD) are a major cause of comorbidity and mortality, reportedly related to approximately 60% of the mortality risk in RP. Traditional risk factors for CVD and inflammation-related factors appear to play a critical role in the increase of CVD risk in RP patients.

Higher CVD risk has been reported in RP (3). Dyslipidemia is often positive in RP, but seems to be somewhat different. An increased prevalence of small dense atherogenic LDL particles has been determined in RP, this being considered an early atherogenesis stage. Role of corticosteroids and other treatments needs to be considered. Methotrexate treatment appears to be associated with an increase in CVD risk. Substitution with folate on the other hand seems beneficial (4). The mechanisms inducing increased mortality by CVD in RP are present early in the natural evolution of this disease (I). Several types of cardiac involvement may occur in RP (e.g., ischemic disease secondary to atherosclerosis), which are the

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main CVD death causes in RP patients (1,2). Other risk factors for subclinical atherosclerosis onset are: smoking, functional endothelial abnormalities chronic inflammation (1,2).

Ultrasound evaluation of common carotid artery (intima-media thickness, presence of atheromatous plaques) is a non-invasive method to early detect atherosclerosis. The aim of this paper is to assess atherosclerotic lesions by carotid ultrasound and to characterize the factors responsible for their onset in PR patients.

MATERIAL AND METHODS

This study has been conducted on a group of based on the criteria set by American College of Rheumatology-ACR/ European League Against Rheumatism-ELA revised in 2010 (5). Medication used by patients consisted of methotrexate and sulfasalazine. The control group was composed of 17 healthy women of age similar to RP patients.

Both groups underwent carotid ultrasound by ALOKA ProSound 4000 echograph, using 10 MHz linear probe. Evaluation of carotid intima-media thickness (IMT) was done at 20 mm before common carotid bifurcation (normal value < 0.5 mm). Atheromatous plaque has been defined as focal thickening of vascular wall, with or without calcifications.

Biochemical and immunological investigation consisted in determination of: total cholesterol (Abbott photometry), C reactive protein (CRP - Turbidimetry) triglycerides (Abbott reagent), anti-cyclic citrullinated peptide antibodies (Microparticle enzyme immunoassay), rheumatoid factor (photometry) and platelet sedimentation rate (PSR - Electro Optical System Technologies).

RP activity has been appreciated by the means of DAS28 (Disease Activity Score) index.

Data were presented as mean ± standard deviation. Statistic analysis was performed by the means of Pearson (for correlation) and t-Student (for comparison) tests, $p < 0.01$ being considered statistically significant.

RESULTS

Both groups (RP and control) were similar as regards the age, while some of the traditional cardiovascular risk factors were absent, such as: diabetes mellitus, chronic kidney disease. Average disease evolution time was 2.58±1.46 years (Table I)

Table I. Compared characteristics of study groups

Parameter	Groups	
	RP	Control
Age (years)	42.6 ±4.14 years	42.6 ±4.14 years
Average disease evolution (years)	2.58±1.46 years	0
Total Cholesterol (mg/dl)	201.24±23.965	196.2±29.02
Triglycerides (mg/dl)	161±26.7	153±46.26
Smoke (%)	23.52	17.64
Blood pressure (%)	41.17	29.41
Blood pressure (mmHg)	159.12±25.44/ 79.71±7.389	137.083±19.59/ 82.916±9.87

Results of immunological explorations and DAS28 values are presented in Table II.

Table II. Results of immunological exploration, DAS28 index

Parameter	Value
Anti-cyclic citrullinated peptide antibodies	123U/ml
Rheumatoid Factor	232U/ml
DAS28 index	4.635±0.4808
Number of relapses	2.71±1.359

Higher carotid IMT and higher incidence of atheromatous plaques were recorded in RP patients, as compared to the control group (Table III, Figures 1, 2, and 3).

Table III. Carotid parameters of two groups

Parameter	Group		p
	RA	Control	
IMT (mm)	0.706±0.2015	0.583±0.126	P<0.01
Atheromatous plaques (%)	47.05%	29.41%	P<0.01

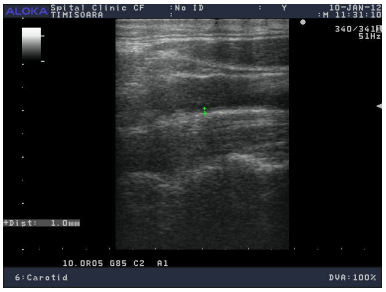
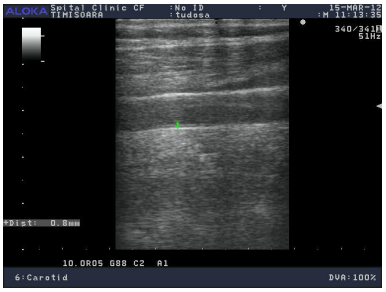


Fig.1.



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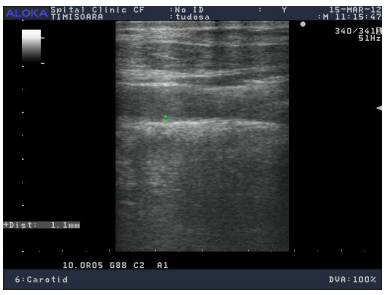

Fig.3.

Carotid IMT in RA patients has been strongly correlated with: DAS28 index ($r = 0.869$, $p < 0.01$) (Figure 4), disease evolution

time (r = 0.944, p < 0.01) (Figure 5), systolic blood pressure value (r = 0.946, p < 0.01) (Figure 6). Medium correlations were recorded between this parameter and diastolic blood pressure value (r = 0.568, p < 0.05), number of relapses (r = 0.669, p < 0.01), and value of total cholesterol (r = 0.678, p < 0.01), respectively. Carotid IMT has been weakly correlated with the values of triglycerides, anti-cyclic citrullinated peptide antibodies, PSR and CRP.

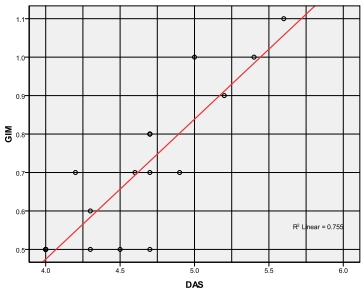

Fig.4. Correlation between IMT and DAS

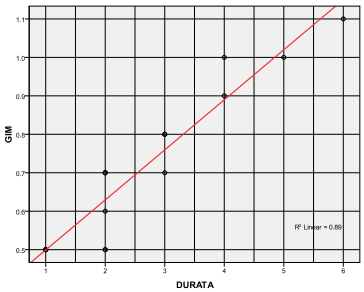

Fig.5. Correlation between IMT and disease evolution time

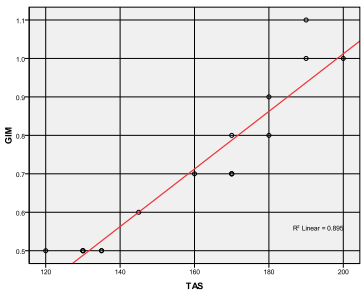

Fig.6. Correlation between IMT and systolic blood pressure

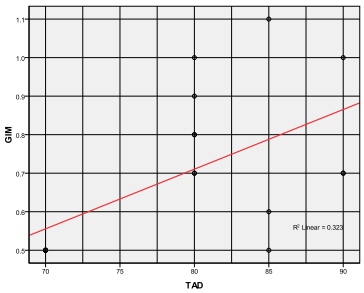

Fig.7. Correlation between IMT and diastolic blood pressure

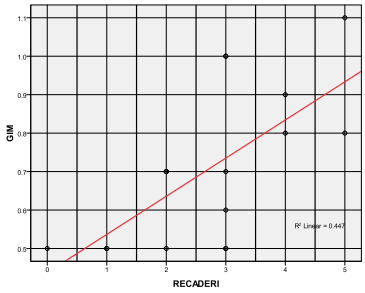

Fig.8. Correlation between IMT and number of relapses

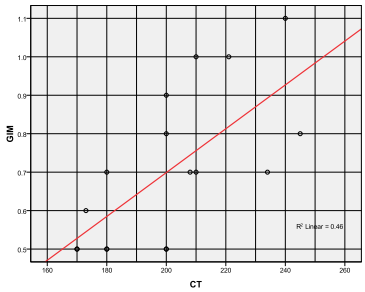

Fig.9. Correlation between IMT and total cholesterol

Carotid IMT, as well as atheromatous plaques incidence had higher values in smokers than in non-smokers (Table IV).

Table IV. Carotid parameters at smokers vs. non-smokers

	Smokers	Non-smokers	p
Number of patients	7	27	<0.01
IMT (mm)	0.8429±0.345	0.9077±0.281	<0.01
Atheromatous plaque (%)	85.71%	29.62%	<0.01

Patients with carotid atheromatous plaques had a longer disease evolution time, a higher DAS28 index compared to those without atheromatous plaques (Table V).

Table V. Clinical and biological parameters at patients with atheromatous plaques

Parameter	Atheromatous plaques		p
	With	Without	
Total Cholesterol (mg/dl)	209.66±20.25	208.71±27.08	<0.01
SBP (mmHg)	170.43±20.50	137.50±13.56	<0.01
DBP (mmHg)	80.65±6.79	79.16±7.33	<0.01
DAS28	4.65±0.50	4.26±0.23	<0.01
Disease evolution time (years)	3.46±1.68	2.16±0.93	<0.01
Number of relapses	3.17±1.33	2.00±1.20	<0.01

Patients on corticotherapy had greater intima-media thickness and higher atheromatous plaques incidence (Table VI).

Table VI. Carotid parameters according to treatment used

Parameter	Type of treatment		p
	Corticotherapy	Another therapy	
Number of patients	23	11	<0.01
IMT (mm)	0.81±0.16	0.60±0.15	<0.01
Atheromatous plaques (%)	73.26%	67.8%	<0.01

DISCUSSION

RP is a chronic inflammatory disease mainly affecting women up to 45 years of age and declining up to 75 years. Although therapeutic schemes have been significantly improved, thus increasing the survival time, both morbidity and mortality among these patients remained high, chiefly because of CVD. RP has been suggested to be comparable with type 2 diabetes mellitus as an independent risk factor for CVD (2,4). Traditional risk factors for cardiovascular diseases and inflammation-related factors seem to play a major role in explaining the increase of CVD risk in RP patients (2,4). Increased prevalence of CVD is the consequence of an early extensive atherogenesis process.

Non-invasive evaluation of subclinical atherosclerosis patients by vascular ultrasound of common carotid artery quantifies two parameters: IMT and atheromatous plaques presence. In a meta-analysis, Tyrell *et al* showed that atherosclerosis, ascertained by measurement of IMT, is indeed higher in rheumatic diseases (2). RP appears to be an important risk factor for early atherosclerosis and CVD development. Under these conditions, a number of epidemiological, clinical and laboratory investigations suggest that chronic inflammation and immune irregularities specific to RP play an essential role in atherosclerosis acceleration [1]. Permanent endothelial dysfunction predisposes to organic damage of the vascular wall, which can be detected before the disease onset by ultrasound measurements of IMT. Many investigations showed evidences of carotid IMT increase in PR (I). This cannot be explained by corticosteroid treatments, but seems instead to be associated with the markers of systemic inflammation and disease duration, thus emphasising the importance of RP as atherosclerosis risk factor (1). The role of rheumatic factors in this context is unknown, although the rheumatoid factor is often positive in smokers (4). Dyslipidemia is frequently positive in RP, with low density lipoproteins and triglycerides increased similarly to inflammatory and infectious diseases (4,7). It is possible that patients with autoimmune diseases, such as RP, to have a lower tolerance to atherosclerosis as compared to healthy individuals (7).

Carotid IMT recorded higher values in RP patients compared to the control group, the difference being statistically significant.

Strong and medium correlations, respectively, have been recorded between studied carotid parameters and traditional risk factors for atherosclerosis: hypertension, smoking, total cholesterol. Medium correlation ($r = 0.678$, $p < 0.01$) between IMT and total cholesterol value has also been reported by other researchers. Lipid profile in RP is characterised by lower LDL and HDL and higher triglycerides. LDL of RP patients had also an increased capacity to bind proteoglycans, which is deemed an early step in atherosclerosis development (7). Lipid peroxidation might play a role in RP and LDL oxidised LDL holds foamy cells that have been described in synovial RP. Moreover, the oxidative stress is higher in RP, being associated with atherosclerosis (4,8).

Hypertension is one of the most studied traditional risk factors of atherosclerosis, carotid abnormalities being correlated with the increase in blood pressure values. A strong correlation between IMT and blood pressure value (Figure 6) was found in this study. Hypertension action in atherogenesis process is complex, increased blood pressure values leading to endothelial dysfunction, changes in the biology of certain active substances in the vascular wall, LDL-cholesterol transport to the vascular wall. RA treatment and life style of RP patients may favour physical inactivity, hypertension being involved in accelerated atherosclerosis in RP (1).

IMT and atheromatous plaques prevalence were higher in smokers as compared to non-smokers. Smoking contributes to the onset of atherosclerosis by lipid profile changes (2,4).

Study of RP dependent parameters revealed strong, medium and weak with IMT and presence of atheromatous plaques, respectively.

The strongest correlation has been recorded between IMT and DAS28 index (Figure 4). The mean value of this index has been significantly higher in patients with carotid atheromatosis. DAS28 index evaluates the number of swollen, sensitive joints, while the general assessment of disease activity is performed using an analogue visual scale of 100mm and PSR or CRP (9). Atherogenesis process is gradual, the risk factors persistence inducing and worsening the existing lesions.

Strong correlation has been established between IMT and RP evolution time (Figure 5), which can be explained by the fact that a longer evolution means: older age, persistence of inflammation and risk of more evolutive attacks. Atheromatous plaques have been found in patients with prolonged evolution of RP.

Emergence of an evolutive attack of RP means severe inflammation, important immunity disturbances and higher doses of corticoids for its control. As atherosclerosis is strongly associated with inflammation, the number of attacks is higher, setting the stage for the onset of atherosclerosis (6).

As the immunological and inflammation markers vary over time, which is a consequence of post-therapy relapses and remissions, their value at a certain evolutive time does not correlate with IMT and the presence of carotid atheromatous plaques, respectively (4,6).

The role of corticosteroids and other treatments should be considered. Methotrexate treatment is associated with an increase of CVD risk. Folate substitutes were used in the past years, which are beneficial (4,7). Higher doses corticotherapy may interfere with atherogenesis by its effect on the traditional cardiovascular risk factors (dyslipidemia, hypertension, diabetes mellitus), which can be induced or worsened because this therapeutic approach involves also the existence of an active severe form of RP (4).

CONCLUSION

Subclinical atherosclerosis has high incidence among patients with RP. Besides the traditional cardiovascular risk factors, other factors related to the main disease, as well as to its treatment occur in these patients. Vascular ultrasound of common carotid artery is a non-invasive mean to identify and quantify subclinical atherosclerosis in order to apply therapies to prevent the worsening of disease and the onset of clinically manifest atherosclerosis.

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ATEROSCLEROZA SUBCLINICĂ ÎN POLIARTRITA REUMATOIDĂ

REZUMAT

Dată fiind prezența autoanticorpilor, cum ar fi anticorpii anti-peptid ciclic citrulinați proteici (testați ca anti-peptid ciclic citrulinat), care pot precede manifestări clinice a PR cu mulți ani, PR este considerat o boală autoimună. Ateroscleroza are caracteristici comune cu bolile autoimune, după cum s-a indicat în studiul în care limfocitele de transfer β_2-glicoproteice I-reactive cresc ateroscleroza la șoarecii cu această boală. BCV sunt o cauză majoră a co-morbidității și mortalității, fiind raportate a fi relaționate cu aproximativ 60% din riscul mortalității în PR.

Scop: Scopul lucrării este reprezentat de evaluarea leziunilor aterosclerotice, prin intermediul ultrasonografiei carotidiene și caracteristicile factorilor responsabili de apariția acestora la pacienții cu PR. Material si metode: Studiul a fost efectuat pe un grup de 17 paciente cu PR. Diagnosticul PR a fost stabilit pe baza criteriilor Colegiului American de Reumatologie (American College of Rheumatology-ACR)/ Liga Europeană Împotriva Reumatismului (European League Against Rheumatism-ELA), revizuite în 2010. Grupul martor a fost constituit din 17 persoane de sex feminin, sănătoase, cu vârstă asemănătoare cu cea a pacientelor cu PR. Ambelor grupuri li s-a efectuat ultrasonografia carotidiană cu ajutorul ecografului ALOKA ProSound 4000, utilizând sonda liniară de 10MHz. Aprecierea grosimii intimă-medie (GIM) carotidiană s-a efectuat la 10mm înaintea bifurcației arterei carotide comune (normal < 0,5mm). Placa de aterom a fost definită drept o îngroșare focală a peretelui vascular, cu sau fără calcificări. Investigațiile biochimice și imunologice au constat în determinarea: colesterolului total (fotometrie Abbot), proteina C reactivă (PCR) (Turbidimetry), trigliceride (reactiv Abbott), anticorpi anti-peptid ciclic citrulinat (Microparticle enzyme immunoassay), factor reumatoid (fotometrie), VSH (Electro Optical System Technologies). Activitatea PR a fost apreciată cu ajutorul indicelui DAS28 (Disease Activity Score-Scorul activității bolii). Rezultate: GIM carotidian la pacientele cu PR s-a corelat puternic cu: indicele DAS28 ($r = 0,869$, $p < 0,01$), durata de evoluție a bolii ($r = 0,944$, $p < 0,01$), valoarea tensiunii arteriale sistolice ($r = 0,946$, $p < 0,01$). Corelații medii au fost înregistrate între acest parametru și valoarea tensiunii arteriale diastolice ($r = 0,568$, $p < 0,01$), numărul recăderilor ($r = 0,669$, $p < 0,01$), respectiv valoarea colesterolului total ($r = 0,678$, $p < 0,01$). GIM carotidiană s-a corelat slab cu valoarea trigliceridelor, anticorpilor anti-peptid ciclic citrulinat, VSH, PCR. Concluzie: Ateroscleroza subclinică are o incidență crescută în rândul pacietelor cu PR. Pe lângă factorii de risc cardiovasculari tradiționali, la acești pacienți intervin și alți factori legați de boala de bază și tratamentul acesteia. Ultrasonografia vasculară la nivelul arterei carotide comune reprezintă un mijloc neinvaziv pentru identificarea și cuantificarea aterosclerozei subclinice în vederea aplicării mijloacelor terapeutice de prevenire a agravării și apariției bolii aterosclerotice și cardiovasculare clinic manifeste.

Cuvinte-cheie: ateroscleroza subclinică, artrita reumatoidă

IDENTIFICATION OF THE ANTIBIOTIC RESISTANCE PHENOTYPES OF ESCHERICHIA COLI STRAINS ISOLATED IN THE URINE SPECIMENS OF DIABETIC PATIENTS

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ABSTRACT

The survey aimed to determine the antimicrobial resistance of the *Escherichia coli* strains involved in producing urinary tract infections in diabetic patients, hospitalised in the Diabetes Clinic, from January to December 2012. The study included 109 *Escherichia coli* isolates from 570 urine specimens collected from 1470 patients. Germs identification relied on cultural and biochemical characters. Final identification and antimicrobial testing were performed using the Vitek 2 (Bio Merieux France) automatic analyzer. Out of the 570 urine specimens, 158 samples (27.71%) were positive. From these, 109 were *E. coli*. The framing of the resistance phenotypes showed a low percentage of Extended-Spectrum Beta-lactamase (ESBL) producing strains. Conclusions: The most frequently isolated germ from the total number of collected urine specimens was *E. coli.* Fortunately, we isolated only a small percentage of ESBL multidrug resistant type. 30.27% from our *E. coli* strains were included in the wild phenotype, while 47.71% were included in the high level penicillinase producing phenotype. The highest percentage of antimicrobial resistance was recorded in β-lactam antibiotics, fluoroquinolones and trimethoprim/sulfamethoxazole.

Key words: urinary tract infection, *E. coli*, antibiotic resistance, diabetes mellitus

INTRODUCTION

The urinary tract is one of the most common sites of infection. Urinary tract infections (UTIs) are usually acquired by the ascending route from the urethra to the bladder and may proceed to the kidney. Ascending infections of the urinary tract are most commonly caused by *Escherichia coli*. (1, 2).

Diabetes mellitus appears to be a risk factor for developing UTI (3). Diabetic patients may suffer more severe clinical forms of infection and when diabetic neuropathy interferes with normal bladder emptying, persistent UTI commonly occur (2). Immunologic factors are also implicated in developing an UTI: functional disorders of polymorphonuclear (PMN) granulocytes, reduced chemotaxy, impaired phagocytosis, decreased killing activity of PMN granulocytes, low levels of serum immunoglobulins, etc. (4).

In some studies, diabetic women have been found to have a higher prevalence of bacteriuria, while, in others, no difference was found in its prevalence between patients with and without diabetes. The higher prevalence of UTIs in diabetic patients in some series may be attributed to more frequent catheterization (1).

The aim of this study was to determine the resistance to antiinfectious chemotherapeutic agents of the *Escherichia coli* strains involved in the etiology of UTIs diagnosed in diabetic patients.

MATERIAL AND METHOD

The study was conducted in the Diabetes Clinic of the Emergency Clinical County Hospital Timişoara and included 109 *Escherichia coli* strains isolated from 570 urine specimens. The urine specimens were collected from 1470 diabetic patients (847 women and 623 men), both with type 1 and type 2 diabetes mellitus, admitted to the Clinic in 2012 (from January to December).

Urine specimens were collected according to sample specific collection protocols.

Quantitative bacterial culture of the urine specimens were performed by inoculating culture media (Columbia agar supplemented with 5% sheep blood and Mac Conkey agar) with a measured amount of urine with calibrated loop designed to deliver a known volume.

Germs identification was generally done according to morphological, cultural, biochemical characters and pathogenicity tests.

All the strains involved in the etiology of urinary tract infections were identified using the Vitek 2 Compact automated system (bioMerieux).

The antimicrobial resistance of the germs isolated form

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the collected samples was assessed by performing the MIC (Minimum Inhibitory Concentration) test, with automated reading and classification into resistance phenotypes by use of the Vitek 2 Compact analyzer, according to the CLSI (Clinical Laboratory and Standards Institute Inc.).

Quality control strains used in testing were *E. coli* ATCC 35218 and *E. coli* ATCC 25922.

RESULTS

Out of the 570 urine specimens, 158 samples (27.71%) were positive. Gram negative bacilli from the *Enterobacteriaceae* family were predominant. In 109 samples it was *E. coli*.

The antimicrobial resistance patterns for the *Escherichia coli* isolates are showed in Tables I, II and the resistance phenotypes in Table III.

Table I. Resistance to ß-lactam antibiotics in *Escherichia coli* isolates.

ß-lactam antibiotic	Susceptible		Intermediate		Resistant	
	No.	%	**No.**	%	**No.**	%
Ampicillin	33	30.27	1	0.92	75	68.81
Amoxicillin/ clavulanic acid	41	37.61	11	10.09	57	52.30
Piperacillin	39	35.78	30	27.52	40	36.70
Piperacillin/ tazobactam	87	79.82	16	14.68	6	5.50
Cefazolin	38	34.87	28	25.69	43	39.44
Cefoxitin	105	96.33	1	0.92	3	2.75
Cefotaxime	99	90.82	1	0.92	9	8.26
Ceftazidime	99	90.82	1	0.92	9	8.26
Cefepime	101	92.67	-	-	8	7.33
Imipenem	109	100	-	-	-	-

Table II. Resistance to other antibiotics in *Escherichia coli* isolates.

Antibiotic	Susceptible		Intermediate		Resistant	
	No.	%	**No.**	%	**No.**	%
Amikacin	108	99.08	-	-	1	0.92
Gentamicin	100	91.75	2	1.83	7	6.42
Ciprofloxacin	78	71.56	5	4.59	26	23.85
Norfloxacin	78	71.56	-	-	31	28.44
Tetracycline	106	97.25	-	-	3	2.75
Nitrofurantoin	106	97.25	1	0.92	2	1.83
Trimethoprim/ sulfamethoxazole	68	62.39	-	-	41	37.61

Table III. Resistance phenotypes in *Escherichia coli* isolates.

Phenotypes	No.	%
Wild type	33	30.27
High PASE	32	29.35
Low PASE+SXT	3	2.75
Low PASE+Q+SXT	4	3.67
Low PASE+GM+Q+FT	1	0.92
High PASE+Q+SXT	10	9.18
High PASE+GM+Q+SXT	1	0.92
High PASE+GM+Q+FT+SXT	1	0.92
High PASE+AG+Q+SXT	1	0.92
High PASE+TE+SXT	2	1.83
High PASE+Q+TE	1	0.92
High PASE+SXT	4	3.67
CASE+SXT	2	1.83
CASE+Q+SXT	3	2.75
CHN+Q+SXT	3	2.75
ESBL+SXT	1	0.92
ESBL+GM+Q	1	0.92
ESBL+Q+SXT	4	3.67
ESBL+GM+SXT	1	0.92
ESBL+GM+Q+SXT	1	0.92
Total	109	100

Legend: ESBL- extended spectrum beta-lactamase, Low PASE- low level penicillinase, High PASE- high level penicillinase, CASE- cephalosporinase, CHN- high level cephalosporinase, GM- gentamycin, AG- aminoglycosides, Q- fluoroquinolones, SXT-trimethoprim-sulphamethoxazole, FT- nitrofurantoin, Te- tetracycline.

DISCUSSION

In our study, the most frequently isolated germ in the collected urine specimens was *E. coli* (109 strains, representing 68.98%). These data are similar to those found in various studies, in which *E. coli* was the most frequently isolated germ in diabetic and non-diabetic patients diagnosed with UTIs (5, 6).

Studying the antimicrobial resistance and resistance phenotypes in the 109 *E. coli* strains revealed that 8 of 109 strains (7.33%) belonged to the ESBL phenotype. All these strains presented also resistance to other classes of antibiotics.

The ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid or tazobactam. The most common extended-spectrum phenotypes arise from point mutations in the bla_{TEM}, bla_{SHV}, or bla_{CTX} genes resulting in alterations of the primary aminoacid sequence of the enzyme (7). Since these genes are generally found on plasmids, many of the organisms that harbor ESBLs also are resistant to other classes of antibiotics, such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfonamides (8). Many strains are multiresistant, being sensitive only to imipenem (9).

There is a considerable geographical difference in the occurrence of ESBLs in the European countries. In a SENTRY worldwide surveillance program report, ESBL phenotypes were

detected in 1-8% of *E. coli* strains (8).

In the United States, multidrug resistance (≥3 antimicrobial drug classes) in *E. coli* increased from 7.2% during the 1950s to 63.6% during the 2000s (10).

The greatest resistance increase, explored in US outpatients, occured from 2000 to 2010, for ciprofloxacin (3% to 17.1%), trimethoprim-sulfamethoxazole (17.9% to 24.2%), nitrofurantoin (0.8% to 1.6%) and ceftriaxone (0.2% to 2.3%) (11).

The proportion of *E. coli* isolates resistant to ciprofloxacin also increased significantly between 2007 (20.0%) and 2011 (29.2%), in the Canadian hospitals (12).

In 2007, the EARSS (European Antimicrobial Resistance Surveillance System) reported 2.5% *E. coli* strains resistant to 4 classes of antibiotics. Also, in 2011, EARSS reported an increase in the number of XDR (extensively drug-resistant) *E. coli* strains – resistant to all antibiotics, except carbapenems (13).

Of the 109 *E. coli* strains, isolated by us, 31 (28.44%) presented a high level of resistance to quinolones, 41 (37.61%) presented resistance to trimethoprim-sulfamethoxazole, 2 (1.83%) were resistant to nitrofurantoin and 3 (2.75%) were resistant to tetracycline. We did not isolate any strain with imipenem resistance.

CONCLUSIONS

The most frequently isolated germ in the urine specimens of diabetic patients was *E. coli.* 30.27% of our total *E. coli* strains were included in the wild phenotype, while 47.71% were included in the high level penicillinase producing phenotype. The highest percentage of antimicrobial resistance was recorded in β-lactam antibiotics (ampicillin – 68.81%, amoxicillin/clavulanic acid – 52.30%, piperacillin 36.70% and cefazolin 39.44%), fluoroquinolones (ciprofloxacin – 23.85%, norfloxacin – 28.44%) and trimethoprim/sulfamethoxazole (37.61%). Fortunately, only a small percentage of ESBL producing *E.coli* isolates have been recorded.

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IDENTIFICAREA FENOTIPURILOR DE REZISTENTA LA ANTIBIOTICE A TULPINILOR DE *ESCHERICHIA COLI* IZOLATE DIN PROBELE DE URINA ALE PACIENTILOR DIABETICI

REZUMAT

Obiective: Scopul acestui studiu este de a determina rezistenta la preparate antimicrobiene a tulpinilor de *Escherichia coli* implicate in etiologia infectiilor de tract urinar la pacientii diabetici internati intr-o sectie de diabet, in perioada ianuarie - decembrie 2012. Material si metoda: In studiu au fost incluse un numar de 109 tulpini de *Escherichia coli* izolate din 570 uroculturi, recoltate de la 1470 pacienti. Identificarea germenilor s-a realizat pe baza caracterelor culturale si biochimice ale germenilor. Identificarea finala si testele de sensibilitate au fost realizate cu ajutorul analizorului Vitek 2 (Bio Merieux France). Rezultate: Din cele 570 uroculturi, 158 probe (27.71%) au fost pozitive. Dintre ele, 109 tulpini au fost cele de *Escherichia coli*. Incadrarea in fenotipuri de rezistenta a evidentiat un procent scazut de tulpini secretoare de BLSE. Concluzii: Germenul cel mai frecvent izolat din totalul uroculturilor a fost *E. coli.* Un procent de 30.27 % au fost incadrate in fenotipul salbatic, in timp ce 41.47% au apartinut fenotipului producator de penicilinaza de nivel crescut. Cel mai mare procent de rezistenta s-a evidentiat la β-lactamine, fluoroquinolone si trimethoprim/sulfamethoxazole. Din fericire, procentul tulpinilor multirezistente de tip BLSE a fost scazut.

Cuvinte cheie: infectii de tract urinar, *E. coli*, rezistenta la antibiotice, diabet zaharat

TECHNIQUES FOR ENDOTRACHEAL INTUBATION IN RATS

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ABSTRACT

Tracheal intubation is often necessary for experiments involving anesthetized animals. In rats, endotracheal intubation is challenging and more difficult due to the small size of anatomical landmarks and also due to no standard intubation equipment available. This review aims to highlight the techniques available for endotracheal intubation in rats.

Key words: endotracheal intubation, rat, intubation techniques

INTRODUCTION

Airways access is important for ventilation, administration of inhaled anesthetics or instillation of drugs, and maintenance of airway patency is often necessary for survival experiments. On most medium sized experimental animals, endotracheal intubation can easily be performed under general anesthesia using a pediatric laryngoscope and small commercial endotracheal tubes. Instead, on small animals, such as rodents, in which oropharyngeal access is limited, endotracheal intubation is challenging. In rats, endotracheal intubation is more difficult due to the small size of anatomical landmarks (e.g. small palate, larynx and epiglottis). No visualization of epiglottis and vocal cords results in repeated intubation attempts which can cause trauma to the oral cavity and death of the animal due to respiratory failure.

Several methods are available for intubation of the rat; however, not all techniques are easy to perform. In addition, no standard intubation equipment is available for rats.

In rats, tracheal intubation can be achieved with specially designed tubes for this purpose or with an intravenous plastic catheter for human use. Most commonly used for this purpose is 14- or 16-gauge catheter, with length from 45 to 60 mm. Unlike metal tubes, these catheters have the advantage of less injury of the upper airways. A modified Foley catheter having an inflatable cuff near the tip to minimize the leak can be also used as a tracheal tube (1). From vocal cords to bifurcation, the trachea in rats weighing 300–530 g is 3.58 mm on average (range 3.0-4.5 mm) (2). The catheter can be secured to the maxilla with adhesive tape or can be left free, without fixation.

The procedure is performed after the animal is previously prepared. Inserting the catheter into the trachea requires adequate general anesthesia of the rat and local anesthesia of tongue, epiglottis and vocal cords with a solution of 4% lidocaine. Local anesthesia is not mandatory; some authors perform intubation without it (3,4). Others found that local anesthesia greatly reduces the difficulty of intubation by reducing glottis spasm if general anesthesia is more superficial (5,6).

Several techniques have been developed for endotracheal intubation in rats:

- Blind intubation technique;
- Transillumination of the neck;
- Direct illumination of the trachea;
- Direct laryngoscopy using an otoscope for human use;
- Direct laryngoscopy using a laryngoscope specially designed for rats; and
- Video-assisted endotracheal intubation.

Blind intubation technique

Stark *et al.* (7) presented a technique for blind oral tracheal intubation using a modified 16-gauge intravenous catheter adapted to the anatomy of the rat's upper airways. This method can be performed much more rapidly than conventional methods, which requires considerable time and skill. The disadvantages for this method are that several attempts were necessary to place the tube correctly. Cheong *et al.* (8) used a blind placement of a 16-gauge intravenous catheter and a modified 18-gauge epidural needle, and the correct placement of the catheter was verified using a rodent ventilator. By this method the authors tried to reduce the number of intubation attempts.

Transillumination of the neck

Tracheal intubation using percutaneous transillumination technique is relatively simple, and requires a strong external light source that penetrates the skin to illuminate the larynx (4,9-13). The light source is placed oblique from caudal to cranial. After immobilization of the upper incisors and tongue retraction, epiglottis and glottis are visualized as a bright red spot. Brown *et al.* (4) used a metal laryngoscope to lift the lower jaw of the mouse and keep the mouth open. The catheter is inserted into the trachea after cleaning pharyngeal secretions with a cotton swab (Figure 1).

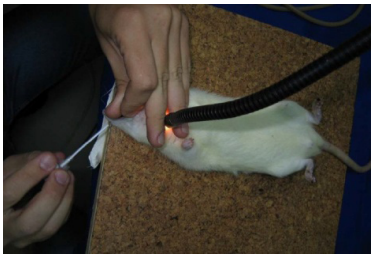

Fig. 1. Transillumination technique (5)

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Direct illumination of the trachea

This method can be performed using a fiber-optic light guide for illumination (14,15) or a surgical operating microscope for magnification and illumination in order to facilitate the procedure (6,16). Jou *et al.* (2) used an intubation wedge made from an obliquely sectioned standard 3-ml plastic syringe and a ordinary lamp for external illumination to facilitate the introduction of the catheter into the trachea under direct vision.

Direct laryngoscopy using an otoscope for human use

Tracheal intubation using an otoscope allows direct visualization of the glottis (17), but is more laborious. It involves the execution of several maneuvers and so the time needed for intubation increase. This fact can have negative consequences in an animal who is insufficient preoxygenated (5).

The otoscope is introduced into the oropharynx and by direct vision, a guide wire is inserted into the trachea and a 16-gauge catheter is glided over the wire. After that, the guide is removed and the rat is connected to the rodent ventilatory. Kastl *et al.* (18) uses a special positioning of the rat in dorsal recumbency and a semisuspended position on an inclined metal plate. This makes the access to the upper airways easier and can facilitate the technique (Figure 2).

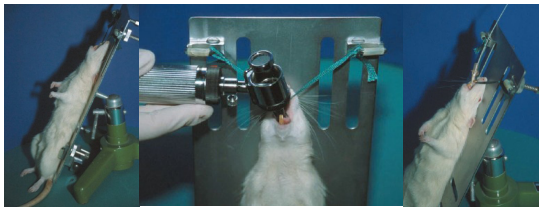

Fig. 2. Direct laryngoscopy with an otoscope (18)

Direct laryngoscopy using a laryngoscope specially designed for rats

This method for intubation in rats allows direct visualization of the glottis and intubation in one time. The technique in rats is the same like in adults. Several devices for this purpose were invented. Direct laryngoscopy with a head-mounted light source was proposed by Alpert *et al.* (19). A laryngoscope, fashioned from a weighing spatula and a glassfibre cable which is connected to a cold light source, was used by Boersma *et al.* (20). The Schaefer's *et al.* (21) intubation method requires no special manufacture of equipment, because it employs the human laryngoscope equipped with an infant blade (size 0). In 1986, a fiberoptic laryngoscope has been developed by Costa *et al.* (22). A rat laryngoscope was also invented by Linden *et al.* (23) and Molthen *et al.* (24). Mothen fashioned a light-carrying laryngoscopic blade (laryngoscope) from readily available acrylic-polymethyl methacrylate tubing.

Ordodi *et al.* (25) developed a simple device for intubation of rats, consisting of the barrel of a standard 2-ml syringe welded to a 6-cm metal spout, and inside the barrel of the syringe, is a light bulb from a flashlight (Figure 3). The intubation can be perform rapidly and relatively simple. The anesthetized rat, positioned in dorsal recumbency, has its head held in position by a flexible plastic tube hooked around the upper incisors. The tong is put aside, after cleaning the oral secretion with a cotton swab. The laryngoscope blade is inserted, and after the visualization of the glottis, the catheter is inserted thru the metal spout into the trachea (4).

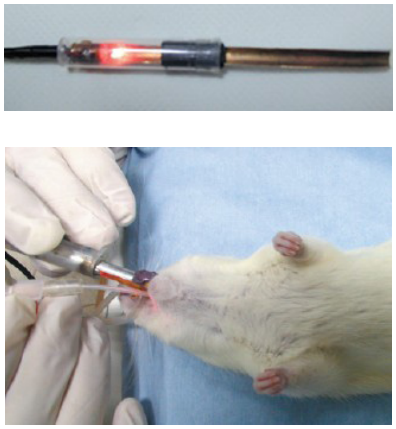

Fig. 3. Direct laryngoscopy using a rat-laryngoscope (25)

Video-assisted endotracheal intubation

This technique was proposed for small animals intubation by Vergardi *et al.* (26), Clary *et al.* (27) and Fuentes *et al.* (2), using equipment commonly found in the endosurgical research setting. This method of direct vision of the trachea uses either a straight, small arthroscope (26,27), or a rigid endoscope (2), connected to a light source and a videocamera. Videocamera is carefully inserted to the left of the incisors and inserted at the base of the tongue to allow visualization of the glottis and the rhythmic opening of the vocal cords (Figure 4). Secretions were removed by a cannula connected to a syringe (26) or using a cotton-tip applicator (27). Under direct videoendoscopic guidance, the catheter is inserted into the trachea directly (26) (Figure 5) or using a guide represented by a stiff wire (27) or a blunted and bent metal needle (2). The catheter is advanced over the guide, and then the guide is removed and the rat is connected to the rodent ventilator.

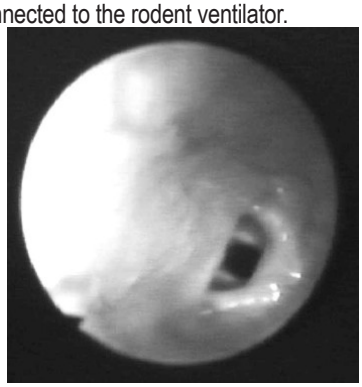

Fig. 4. The rat's larynx on videointubation (26)

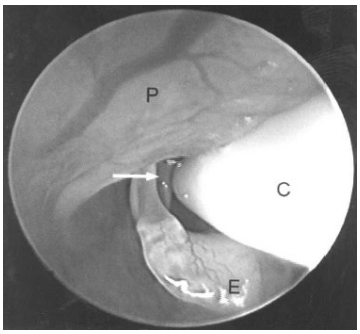

Fig. 5. Correct position of the endotracheal catheter (white arrow) (27); C - endotracheal catheter, E - epiglottis, P - soft palate

DISCUSSION

A variety of methods have been described for endotracheal intubation of the rat. In the majority of studies intubation techniques are very complicated or require expensive equipment. Blind intubation sometimes requires 3 or 4 attempts to position the endotracheal tube correctly in the trachea and may lead to complications or death to the animal. Other non-invasive rat tracheal intubations under direct view often require elaborated animal position and special equipment including a laryngoscope, an otoscope with an incorporated light source, a surgical microscope, a head-mounted mirrorreflected adjustable-focus light, an endoscope, or some other special devices. However, one of these devices may be not available in every laboratory. The laryngeal opening in rats is very small, measuring 1.5 mm in diameter in 200 g specimens (7), and therefore visualization with an untrained eye can be difficult, even with proper illumination. Clearly, the intubation of the trachea under direct vision remains the standard for a safe intubation in rats.

CONCLUSIONS

Techniques for endotracheal intubation in rats vary according to the experience of authors and to possibilities of laboratory equipment. With the exception of laryngoscopes especially designs for rat intubation, all other techniques uses equipment from human use, adapted to the small size of the animal. The fact that so many different methods for rodent intubation have been described probably indicates that there is no ideal method.

ACKNOWLEDGEMENT

This paper is supported by the Sectorial Operational Programme Human Resources Development (SOP HRD) 2007-2013, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/107/1.5/S/82839.

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TEHNICI UTILIZATE PENTRU INTUBAȚIA ENDOTRAHEALĂ LA ŞOBOLAN

REZUMAT

Intubaţia traheală este deseori necesară pentru experimente pe animale anesteziate. La şobolani, intubaţia endotraheală este provocatoare şi totodată dificilă datorită pe de o parte a mărimii mici a reperelor anatomice, iar pe de altă parte lipsei de standardizare a echipamentului disponibil pentru intubaţie. Acest review işi propune să evidenţieze tehnicile disponibile de intubaţie endotraheală la şobolani.

Cuvinte cheie: intubaţie endotraheală, şobolan, tehnici de intubaţie

CORRELATION BETWEEN PULSE WAVE VELOCITY AND INFLAMMATION MARKERS IN PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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ABSTRACT

In recent years, arterial stiffness expressed by pulse wave velocity and circulating inflammatory markers such as fibrinogen and high sensitive C-reactive protein (hsCRP) were recognized as risk factors for cardiovascular disease (CVD).The purpose of this study was to evaluate the association between pulse wave velocity and inflammatory markers in patients with cardiovascular risk factors. The study included 223 patients with at least 2 risk factors for atherosclerosis, divided considering the presence of coronary artery disease (CAD): 140 patients with angiographically confirmed CAD and 83 patients without CAD. The patients were compared with a control group of 74 healthy age subjects (CON). The following parameters were determined: blood pressure, total cholesterol (TC), triglycerides (TG), LDL-cholesterol, HDL-cholesterol, fasting serum glucose, fibrinogen and hsCRP. Arterial stiffness was quantified by pulse wave velocity (PWV), using Arteriograph device (Tensiomed Ltd., Budapest, Hungary). CAD patients had significantly increased values of inflammatory markers as compared with patients without CAD and controls (all $p<0.001$). The patients with CAD had significant increased values of PWV compared with patients without CAD and CON subjects (12.5±0.7 vs 10.9±0.6 vs 8.5±0.07 m/s, all $p<0.001$). A significant medium correlation was found between PWV and fibrinogen ($r=0.641$, $p<0.001$) and between PWV and hsCRP in all subjects ($r= 0.551$, $p<0.001$). In this study inflammatory markers were significantly correlated with PVW, therefore, inflammation may influence arterial stiffness in patients with cardiovascular risk factors.

Keywords: pulse wave velocity, hsCRP, fibrinogen

INTRODUCTION

Recent investigations of atherosclerosis have focused on arterial stiffness and inflammation, providing new insight into mechanisms of disease (1). An increase in peripheral vascular resistance associated with increased stiffness of central elastic arteries represents hallmarks of this ageing effect on the vasculature, referred to as early vascular ageing (2). Many cardiovascular risk factors such as age, smoking, arterial hypertension, diabetes mellitus, end stage renal disease are associated with increased arterial stiffness (3, 4). Besides these traditional risk factors, chronic inflammation of the cardiovascular system may also affect PWV (5). High-sensitivity C reactive protein (hsCRP) seems to have an important relation to the risk of cardiovascular diseases in a variety of clinical settings including healthy subjects and patients with cardiovascular diseases (6).

Furthermore, the Hisayama Study suggested that brachial-ankle pulse wave velocity is a significant predictive factor for cardiovascular disease in the general Japanese population and substantially improves cardiovascular risk assessment, being included within the framework of large-scale clinical studies (7, 8). Its application as a routine tool for clinical patient evaluation has been hampered until now by the absence of reference values (8).

The aim of this study was to evaluate the association between pulse wave velocity and inflammatory markers in patients with cardiovascular risk factors.

MATERIAL AND METHODS

The study included 223 patients with at least 2 risk factors for atherosclerosis, divided considering the presence of coronary artery disease (CAD): 140 patients with angiographically confirmed CAD and 83 patients without CAD. The patients were compared with a control group of 74 healthy age subjects (CON). We recorded age, sex, smoking habits, hypertension, atherogenic dyslipidemia, and diabetes mellitus as cardiovascular risk factors. We excluded from the study the patients with unavailable data for fibrinogen, hsCRP and PWV measurement and the patients with identified genetic cause of hypertension or secondary hypertension.

Clinical evaluation included blood pressure measurement, physical examination, chest radiograph, 12-lead electrocardiogram. While on their usual diet, a venous blood sample was drawn from an antecubital vein in all subjects after an overnight fast to determine TC, TG and using standard enzymatic method on a COBAS Integra 400 plus analyzer.

Hypertension was diagnosed according to 2013 European Guidelines for the Management of Arterial Hypertension (9). Diabetes was defined by the current intake of oral hypoglycemic treatment or use of insulin.

Assessment of arterial stiffness

Measurement of arterial stiffness indices was done early

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in the morning 1 day before coronary angiography with patients fasted and before the administration of the medications. The assessment of arterial stiffness was noninvasively performed with the Arteriograph (Tensiomed Ltd., Budapest, Hungary) by an operator who was blinded to the results of coronary angiography and other findings. All measurements were taken in the supine position in a quiet, temperature-controlled room (approximately 22°C) after a brief period of rest in the morning.

Informed consent was obtained for each man who agreed to participate in our study. The study protocol was approved by the local ethics committee. The study was conducted according to the Declaration of Helsinki, and the written informed consent was obtained from each subject.

Statistical analysis

Database and processing were performed using statistical software SPSS Statistical Software Package, version 15.0 (SPSS Inc, Chicago, Illinois, USA). Continuous data are presented as mean ± standard deviation for continuous variables and number of subjects (*n*) and percentage (%), respectively, for categorical variables. The independent sample *t* test was used to reveal significant differences in parameters between patients with and without CAD and controls. The statistically analysis was carried out with Pearson's test for correlation. $p < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the subjects, the mean values of lipid profile parameters, renal parameters (serum creatinine, blood urea nitrogen, uric acid levels) inflammatory markers (fibrinogen and hsCRP) and PVW are summarized in Table I. No significant differences were determined between groups referring gender, and smoking habits.

Table I. Characteristics of the studied group (mean ± SD values)

Parameters	CAD group (*n*=140)	Without CAD group (*n*=83)	CON (*n*=74)	*p-value*
Age	60.5±10.8	56.63±8.9	55.8±5.2	<0.001
Gender females/ males (%)	51.4/48.6	51.8/48.2	54.1/45.9	0.93
Family history for CAD (%)	42.9	67.5	44.6	0.001
Smoking (%)	37.1	47	35.1	0.23
SBP (mmHg)	154.9±20.9	157.8 ± 13.5	123.7± 6.3	<0.001
DBP (mmHg)	91.1±12.3	93.67±7.4	72.6±5.3	<0.001
Glucose (mg/dL)	117.1±44	98.7±12.1	90.9±8.7	<0.001
Total cholesterol (mg/dL)	262±39.5	268.1±35.8	181.27±13.044	<0.001
Triglycerides (mg/ dL)	168.3±79.2	147.3±49.6	111.9±27.8	<0.001
LDL-C (mg/dL)	145±26	126.3±23.8	115.6±18.5	<0.001
HDL - C (mg/dL)	35.9±4.3	40.5±7.5	46.7±6.4	<0.001
Serum creatinine (mg/dL)	0.9±0.3	0.8±0.1	0.7±0.08	<0.001
Blood urea nitrogen (mg/dl)	37±11.4	38.3±6.9	30.1±7	0.001
Uric acid levels (mg/dl)	6.3±1.8	5.7±1.7	4.7±1.1	<0.001
Fibrinogen (mg/dl)	513.2±110.3	357.6±57.2	301.8±21.5	<0.001
hsCRP (mg/l)	3.6±1.1	3.2±1.4	1.06±1.2	<0.001
PVW (m/s)	12.5±0.7	10.9±0.6	8.5±0.07	<0.001

SBP: systolic blood pressure, DBP: diastolic blood pressure, PVW: pulse wave velocity; high sensitive C-reactive protein: hsCRP

Table I shows that the parameters of lipid profile (TC, TG, LDL-C and HDL-C), fibrinogen and hsCRP were significantly higher in patients than in controls (all $p < 0.001$). Mean levels of fibrinogen in serum were significantly higher in CAD patients than in those without CAD and than in CON group (513.2 ± 110.3 mg/dl versus 357.6 ± 57.2 mg/dl versus 301.8 ± 21.5, all $p < 0.001$).

The patients with CAD had significantly increased PWV values compared with patients without CAD ($p < 0.001$) and CON group ($p < 0.001$) (Figure 1).

The correlation between PWV and fibrinogen was moderate and significant and is showed in Figure 2 ($r = 0.641$, $p < 0.001$).

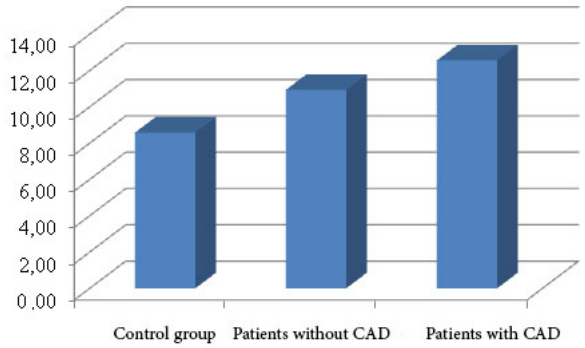

Fig.1. The PVW values in three studied groups

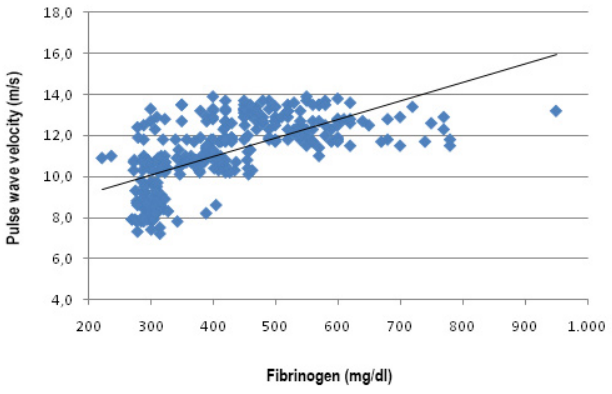

Fig.2. Correlation between PVW and fibrinogen

It was found a correlation moderate and significant between PWV and hsCRP (r=0.551, p<0.001) (Figure 3).

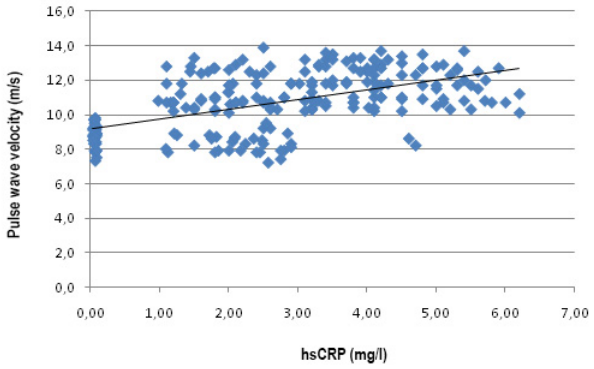

Fig.3. Correlation between PVW and hsCRP

DISCUSSION

It seems that, arterial stiffness measured by carotid-femoral PWV may reflect the sclerotic rather than atherotic component of atherosclerosis and it is more closely correlated to age, blood pressure, plasma glucose and arterial thickness (10, 11). However, findings with regard to its relation with other risk factors have been inconsistent.

This prospective observational study could reveal risk associations but could not establish causal relationships or explore potential mechanisms. Similar with the results of others studies (12, 13), in this study was found that PVW was significantly associated with serum levels of hsCRP and fibrinogen, suggesting that inflammation is involved in arterial stiffening. On the contrary, a recent study showed that inflammation does not influence arterial stiffness and pulse-wave velocity in patients with coronary artery disease (14). Another study observed that tumor necrosis factor-α and C-reactive protein appear to be critical inflammatory cytokines associated with reductions in arterial elasticity in older women (15). Studies have had conflicting result until now because techniques based on the pulse wave lack precision, assume that arterial stiffness is uniform throughout the path of the pulse wave, and that it is constant throughout the cardiac cycle (16).

A study that explored the association between PWV and the extent of angiographically proved CAD in patients with angina and without any previous history of heart disease concluded that PWV is an independent predictor of significant CAD, but was neither associated significantly with the extent of CAD nor with the risk of revascularization (17). In a recent study, a higher dairy food intake was associated with lower PWV and accompanying reductions of pulse pressure and SBP (18).

Early diagnosis and prevention of CAD have stimulated a search for reliable noninvasive methods through detection of changes in the endothelium by determining PWV and inflammatory markers as hsCRP and fibrinogen (19). PWV has been becoming a widely adopted index of arterial stiffness because of its simplicity, reproducibility, and inexpensiveness. PVW and associated inflammatory markers may be of clinical use for early diagnosis of subclinical atherosclerosis, in the development of multimarker strategies to reduce the rate of subsequent cardiovascular events.

CONCLUSIONS

In this study inflammatory markers were significantly correlated with PVW, therefore, inflammation may influence arterial stiffness in patients with cardiovascular risk factors. PVW and associated inflammatory markers may be of clinical use for early diagnosis of subclinical atherosclerosis, in the development of multimarker strategies to reduce the rate of subsequent cardiovascular events.

Competing interests

The authors declare that they have no competing interests.

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CORELAȚIA DINTRE VITEZA DE PROPAGARE A UNDEI DE PULS ŞI MARKERII INFLAMATORI LA PACIENȚI CU FACTORI DE RISC CARDIOVASCULAR

REZUMAT

În ultimii ani, rigiditatea arterială exprimată prin viteza de propagare a undei pulsului şi markerii inflamatori precum fibrinogenul şi proteina C reactivă înalt sensibilă (hsCRP) sunt recunoscute ca factori de risc pentru bolile cardiovasculare (BCV). Scopul acestui studiu a fost evaluarea relaţiei dintre viteza de propagare a undei de puls şi markerii inflamtori la pacienţi cu factori de risc cardiovasculari. Studiul a inclus 223 de pacienţi cu cel puţin 2 factori de risc CV pentru ateroscleroză, împărţiţi în funcţie de prezenţa bolii coronariene: 140 de pacienţi cu boală coronariană confirmată angiografic şi 83 de pacienţi fără boală coronariană. Pacienţii au fost comparaţi cu un grup de control (CON) de 74 de subiecţi sănătoşi. Au fost determinaţi următorii parametri: tensiunea arterială, colesterolul total (CT), trigliceridele (TG), LDL-colesterolul, HDL-colesterolul, glicemia serică, fibrinogenul şi hsCRP. Rigiditatea arterială a fost cunatificată prin viteza de propagare a undei de puls (PWV) folsind aparatul Arteriograf (Tensiomed Ltd., Budapest, Hungary). Pacineţii cu boală coronariană au avut valori semnificativ crescute ale markerilor inflamatori comparativ cu pacienţii fără boală coronariană şi grupul de control (toate $p<0.001$). The patients with CAD had significant increased values of PWV compared with patients without CAD and CON subjects (12.5±0.7 vs 10.9±0.6 vs 8.5±0.07 m/s, all $p<0.001$). A fost obţinută o corelaţie medie semnificativă între PWV şi fibrinogen ($r= 0.641$, $p<0.001$) şi între PWV şi hsCRP la toţi subiecţii ($r= 0.551$, $p<0.001$). În acest studiu markerii inflamatori au fost semnificativ corelaţi cu PVW, aşadar, inflamaţia ar putea influenţa rigiditatea arterial la pacienţii cu factori de risc cardiovasculari.

Cuvinte cheie: viteza de propagare a undei de puls, fibrinogen, hsCRP

TAURINE EFFECT ON WORKING MEMORY IN HEALTHY ELDERLY

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ABSTRACT

Demographic aging is relevant for health field objectives. In normal aging occurs a decline in executive processes, as manipulation of information in working memory, coordination of multiple simultaneous operations. In nervous system, taurine acts as neuroprotective agent. To assess the working memory performance we used the Stroop test. Plasma concentration of homovanillic acid have been used as an indicator of dopaminergic activity of the central nervous system. We tested two groups, one test and one control, each consisting of ten human subjects over sixty five years old, female and male. We recorded reaction times and we determined the concentration of the homovanillic acid before the administration of taurine, 1500 mg/ day, at one, at two and at three months of taurine administration. Working memory activation is related with dopamine release in the prefrontal cortex. Age-related decline in dopamine system and in working memory is observed in humans. Results obtained indicate the decrease of the reaction times and of the interference index and the increase of homovanillic acid plasma concentration after taurine administration.

Keywords: aging brain, Stroop test, working memory, taurine, dopamine, homovanillic acid

INTRODUCTION

Characteristic for our century is the demographic aging that determined WHO to include aging problem among the five health problems of the world. WHO showed that the percentage of people with age equal or greater than 65 is expected to increase from 14% in 2010 to 25% in 2050. Aging is associated with structural and cognitive brain changes that limit functional capacity. The primary cognitive functions most affected by age are attention and memory. The purpose of this article is to demonstrate taurine effects to improve working memory, function whose parameters are altered in aging. To assess the working memory performance we used the Stroop test.

Brain aging and working memory

With aging, the volume of the brain reduces, especially in the frontal cortex. It was found that the volume of the brain decreases with about 5% per decade, after 40 years (1). The most obvious changes are gyri atrophy, sulci expansion and increase in ventricles volume. At microscopic level, it was observed loss of neurons in neocortex, hippocampus and cerebellum, a decrease in size and shape of neurons and a reduced cerebral vascularization. Nimchinsky et al. reported a loss of 50% in spines on apical dendrites of pyramidal cells in the prefrontal cortex of old animals (2). Other changes include decreased levels of serotonin (3), GABA (4) dopamine, somatostatin (5) and of metabolites of acetylcholine, dopamine and norepinephrine (6). There are also, intracellular calcium homeostasis disturbed (7), mitochondrial dysfunction and increased production of reactive oxygen species (8). Gene transcription is affected (9), kinases and phosphatases are altered (10) and protein changes occur such as formation of carbonyl groups (11) covalent modification of cysteine residues, lysine and histidine, nitration of tyrosine residues (12, 13) and glycation (14).

Studies with MRI, and PET MRIf showed that the most important structural changes in aging occur in the prefrontal cortex, where it produces both white and grey matter atrophy (15) and especially in the lateral prefrontal cortex (16). There is evidence that cognitive deficits of older adults correspond to neuropsychological profile associated with lateral frontal lesions (17). Executive functions supported by prefrontal cortex (FPC) systems can be conceived in terms of control and planning mechanisms that mediate and guide goal - directed behavior. These processes include cognitive functions such as attention, working memory, cognitive flexibility (e.g. switch between sets of attention) and making decisions.

Working memory term was proposed by Baddeley (18, 19) and indicates a space with limited storage capacity, where the information is kept active through repetition for 3 up to 30 s and manipulated to complete task. Ricks and Wiley (20) suggest that working memory is focused on complex cognitive tasks including reasoning, planning, handling and coordination of precepts and actions. Connection between the frontal lobes and manipulating information in working memory is supported by numerous neuroimaging studies (21) including the discovery that dorso – lateral prefrontal cortex indicate greater activity when working memory content is rearranged but it is maintained in its original form (22, 23). Maintaining the information in front of interference is the

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critical function of working memory capacity (24, 25, 26). Working memory also keeps action plans, representations of goals and relevant information for task in an accessible form, allowing the choice of a correct response (27).

In normal aging occurs a decline in executive processes, as inhibition of irrelevant information, coordination of multiple simultaneous operations, manipulation of information in working memory and control of episodic memory operations (28). Hasher and Zacks (24) showed that cognitive deficits of elderly are related to the inability to ignore the irrelevant information from the environment while focusing on information relevant to the objective. Inhibition deficits occur in tasks that require the selection of the information from the environment or inhibition of a strong association or a common response. If elderly distinguish with difficulty the relevant information from irrelevant one, means that they store information irrelevant to the task, reducing storage capacity available for information relevant to the task.

Stroop test

In 1886 Cattell (29) showed it that lasts longer to name objects and properties of objects (colors) than to read the corresponding words. The explanation was that reading words is more practiced than naming images or words and thus he introduced the concept of automaticity in psychology (30,31). Almost 50 years later, Stroop (32) combined the words and colors, creating a conflict situation and the test that today bears his name. Classic Stroop test consists of three types of stimuli: neutral (a string of "XXXX" printed in different colors), congruent or without interference (the meaning of the word and the printing color are the same, e.g.: yellow written in yellow) and incongruent or with interference (the meaning of the word and the printing color are different, e.g.: yellow written in blue). Solving test requires naming the printing color of the words and ignoring the meaning of those. It is recorded the reaction time (latency), which is the time from the appearance of the stimulus until the participants' answer, given verbally or on computer. Stroop interference effect refers to increase reaction time to interference stimuli compared to reaction time for neutral stimuli.

PET and MRIf results showed the strongest activated cerebral areas during color – word Stroop test are anterior cingulate cortex (33, 34) and lateral prefrontal cortex , especially dorso - lateral prefrontal cortex. Anterior cingulate cortex is considered susceptible to conflict and dorso - lateral prefrontal cortex is associated with the implementation of cognitive control (35, 36).

Interference Stroop effect was explained in terms of response to competition. Greater reaction time and greater interference are present when irrelevant stimulus attributes (word meaning) are analyzed fastest than relevant attributes (color) and thus undesirable response is therefore available.

Automatic processing can be defined as implicit or intuitive thinking, that occurs unconsciously and effortlessly, while controlled processing is conscious, intentional and accompanied by effort. One explanation is that during a lifetime of practice, reading became automated. In theory developed by Cohen et al. (37), evidence accumulates separately for meaning and color so that the total activation received by output units (meaning, color) will determine who will reach first the threshold in order to answer. Resolving a conflict created by incongruent tests can be done either by increasing relevant response activation and/ or by increasing irrelevant response inhibition (38, 39, 40, 41). Inhibition of the response is a function of executive control (42). The advantage of congruent stimuli versus incongruent ones is explained by the fact that for the first category both attributes (meaning and color) counts for the correct answer.

Interference Stroop effect is greater with age and could be explained by decreased efficiency of inhibition processes (43) by slowing that occurs with age (44) and by decreasing in processing resources (45). These changes are based on morphological modifications from anterior cingulate cortex that is involved in conflict monitoring and from the lateral prefrontal cortex (46), which is involved in maintaining the aim.

Taurine – a short overview

Taurine or 2 - aminoetansulfonic acid was first isolated from ox bile in 1827 by Tiedemann and Gmelin (47). It is a nonessential amino acid in rodents, an essential one in cats and conditional essential one in humans. Taurine is a neutral amino acid, with both groups, amino and sulfonic, ionized, dissociation of the last one giving the biological and functional specificity (48). It is one of the most abundant intracellular free amino acids.

Methionine and cysteine are the precursors of taurine. Its average daily rate of synthesis is between 0.4 - 1.0 nmol (50-125 mg). Taurine biosynthesis involves the sequential oxidation of cysteine to cysteine - sulfinic acid, catalyzed by cysteine dioxygenase, decarboxylation by the cysteine - sulfinic acid decarboxylase (CSAD) to hypotaurine and its transformation into taurine by hypotaurin dehydrogenase. An alternative path for taurine synthesis, involves cysteamine production (49).

Taurine is found in neurons and astrocytes (50, 51) and its biosynthesis involves metabolic cooperation between astrocytes and neurons. CSAD is rate limiting enzyme in taurine synthesis and is found in astrocytes (52, 53). CSAD is activated in phosphorylated state and inhibited in dephosphorylated state. Protein kinase C is the enzyme responsible for CSAD phosphorylation and phosphatase 2C is responsible for CSAD dephosphorylation. Neurons do not have CSAD (54, 53) and they are based on astrocytes deposits of hypotaurine (55) and on taurine acquisition. Cellular content of taurine is mainly determined by the sum of three processes: synthesis from methionine / cysteine, active uptake by taurine transporters and its release via flow volume – sensitive pathways. An adult of 70 kg contains about 70g of taurine, with an intracellular concentration of taurine in the range from 5 to 50 mM, while plasma concentration is about 100 microM. Taurine concentration is different in different tissues. In the brain, it is between 0.8 - 5.3 mmol / g (48).

Taurine is transported by a specific transporter TAUT, whose sequence places it in family of transporters Na and Cl dependent (56, 57). In the brain there are two types of transporters, TAUT1 and TAUT2, TAUT2 being located mostly in glial cells (58, 59). TAUT is regulated by activation of two enzymes Ca dependent:

PKC (transporter inhibitor) and calmodulin (transporter stimulator) (60, 61). Taurine increases chloride conductance in the cerebellum, substantia nigra and olfactory bulb (62). Taurine is excreted in urine and bile. In kidney, taurine is filtered at the glomerular level and reabsorbed partially in nephron tubes through a Na-dependent transport system specific for alpha amino acids. The amount of taurine excreted daily varies between 0.22 - 1.85 mmol and is influenced by genetic factors, age, dietary intake, renal function and clinical conditions (63).

Mechanisms of action of taurine are complex, with important cardio - vascular, nervous and digestive effects. In the liver is involved in bile acid conjugation (64). On cardio - vascular system taurine has antiarrhythmic, positive chronotropic and inotropic effects (65, 66). It is hypotensive (67), hypocholesterolemiat (68) and antiatherogenic (69). In retina is the most abundant amino acid and is involved in normal vision. In nervous system taurine controls cell growth and differentiation (70), acts as trophic factor (71) affects cell migration and modulate neurotransmission (72, 73). Taurine is involved in maintaining the structural integrity of the membrane (74) in modulating protein phosphorylation (75) It acts as osmoregulator (76, 77, 78, 79) and neuroprotective agent in ischemia (80) and in neurotoxicity induced by L - glutamate (81). Taurine interferes with calcium equilibrium (82). It is known that intracellular free Ca levels are maintained at low values through its sequestration in intracellular calcium stores, such as endoplasmic reticulum, mitochondria and by pumping it through Ca ATP - ase in the extracellular space. In the presence of taurine, glutamate-induced increase in intracellular Ca is reduced by reversing Na / Ca carrier. At resting membrane potential, Na / Ca carrier works to remove Ca from the cell. At depolarization conditions such as stimulation by glutamate, it works in reverse and makes Ca influx. Taurine promotes the Ca efflux through Na / Ca exchanger by increasing Ca concentration near the transporter (83). Taurine inhibits Ca release from internal deposits and various Ca voltage-dependent channels such as L- , N - and P / Q type (84 , 85).

Increased oxidative stress and accumulation of oxidative modified molecules (proteins, nucleic acids and lipids) promotes dysfunction of various metabolic and signaling pathways (86). Cell toxicity induced by oxidative stress is reduced by taurine. It does not directly bind superoxide, hydrogen peroxide and hydroxyl radical (87) but couple directly HOCl in the presence of myeloperoxidase. Neurons may face energy deficits resulting from alterations in cerebral vascularisation and mitochondrial function (88). Major source of superoxide generation in mitochondria are complexes I and III. Slowing the flow of electrons through the respiratory chain determines electron transfer from complexes I and III to an alternative receiver, such as oxygen (89). Taurine prevents diversion of electrons to superoxide generation by improving electron transport function (90).

Taurine concentration decreases in aging rodent brain (91, 92). Taurine supplementation in the diet restored the tissue concentrations of taurine (93, 94).

MATERIALS AND METHODS

Stroop test was administered to two groups, one test and one control. The test group was formed of six women and four men with age between sixty five and seventy years. The control group was formed of six women and four men with age between sixty five and seventy years. Test participation was unconstrained, each subject signing an informed consent before being admitted to the study. Initially, each participant was presented information on the purpose and progress of the study and about taurine. Subjects in the study were patients of "Sf. Luca" Chronics Pathologies Hospital, Bucharest and had the right to withdraw from the study at any time.

Groups' inclusion criteria were: age over 65 years, absence of associated pathology, absence of a current treatment, absence of a history of psychiatric or neurological disorders or brain trauma with loss conscience. Volunteers completed a Mini Mental State Examination, the minimum score for participation was 25 (95, 96). Subjects were tested for color vision by Ishihara tests. The Stroop test followed the model proposed by MacLeod (97) in the chapter entitled "The Stroop task in cognitive research" and consisted of three subsets of stimuli: neutral (a string of "XXXX" printed in colors), incongruent or with interference (the meaning of the word and the printing color were different, e.g.: yellow written in blue) and congruent or without interference (the meaning of the word and the printing color are the same, e.g.: yellow written in yellow). We used the four standard colors: green, red, yellow and blue. Each subset had a total of 36 slides. The test was administered on the computer. Subjects were seated in a chair with monitor (NEC MultiSync FC color monitor, 17 inch) placed at 40 cm away from the eye. All tests were printed in large bold size 24 Helvetica font, in the center of the screen, on a black background. The order of presentation of the trials was randomized. Responses were given via a keyboard with keys in four colors already named. Using the keyboard has the advantage that no answers were lost. Comparison from literature between the reaction times obtained from verbal and manual response mode showed higher reaction times for manual version. Subjects were advised to respond as fast as they could. Test slides remained on the screen until each subject responded by pressing a key. Test slides were separated with white slides that were displayed 250ms.

We recorded reaction times on Stroop Test before taurine administration (control), at one month, at two months and at three months, after the beginning of taurine administration. Response times were registered using SuperLab 4.0 software. The most used performance index for Stroop test is the interference index, defined as the difference between reaction time to incongruent stimuli and reaction time to neutral stimuli (97). The results were compared with the control group.

We determined the concentration of the homovanillic acid, the major metabolite of the dopamine, in the plasma. We collected blood samples from participants at 9.00 am, before administration of the taurine, at one month, at two months and at three months from taurine administration. Plasma was separated by

centrifugation and samples were stored at - 70°C. Homovanillic acid concentration was determined by high performance liquid chromatography.

We choose this testing period because The European Food Safety Authority (98) has studied the effects of taurine on lots of rats for periods of about three months. The Scientific Committee on Food (99) admitted that oral administration of taurine at doses between 600 and 1000 mg / kg / day was well tolerated by male and female rats and did not lead to behavioral changes. Studies revealed no genotoxic, carcinogenic or teratogenic effect of taurine. Also, theScientific Committee on Food (100) studies showed that taurine levels return to initial value approximately 8 hours after the administration. Taurine was administrated at dose of 500 mg x 3/day. Taurine was manufactured by Nutritech, the product being notified by the Institute of Food Bioresources, Bucharest.

RESULTS

The recorded times were neuter time, time with interference and time without interference. Even if the two groups contain only ten participants each group, the p is below 0.05 for the data analyzed. Mean reaction times recorded are presented in Table I.

Table I. Mean reaction times before taurine administration(control), at one month, at two months and at three months

	Neuter time	Times without interference	Times with interference	Interference index
	Control			
Control group	2026.5625	1867.375	2240.62575	214.06325
Test group	1905.5	1802.725	2159.0483	253.9983
	One month			
Control group	1940.65	1808.9334	2173.7944	233.1444
Test group	1430.808	1354.175	1684.0602	253.2522
	Two months			
Control group	2056.833	1947.333	2275.875	219.0413
Test group	1339.95	1298.35	1467.628	127.678
	Three months			
Control group	1946.583	1824.333	2155.033	208.45
Test group	1132.417	1063.75	1228.586	96.16888

We have used one-way ANOVA in order to test whether there are statistically significant differences between the means of the values recorded initially, at one month, at two months and at three months. In other words, we wanted to test if the decrease of the reaction times along the three-month period is statistically significant.

Considering that there are more than three groups, ANOVA is a suitable analysis. Also, in case of violation of the presumption of normality of the sample, the analysis is robust enough. We have also used contrasts analysis, comparing more in-depth the differences between the groups, and also Dunnett test, as post-hoc analysis.

We have first analyzed the variance of means for the control group. Our assumption is that, in the case of the group witch had not received taurine, the means will not decrease significantly, between the initial moment and the successive recordings at one month, two months and three months. The one-way ANOVA for the control group for neuter time is presented in Table II.

Tabel II. One-way ANOVA for control group for neuter time**.** As expected, the significance value for the F-test is above 0.05, meaning that there is no significant variation in means between the four recordings: initially, after one month, after two months, and after three months.

Reaction times					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	162847.876	3	54282.625	0.359	0.783
Within Groups	2419058.455	16	151191.153		
Total	2581906.331	19			

Reaction times do not vary by themselves over time, only if they are influenced by the administration of taurine. For neuter times, with interference times and without interference times, for the test group the variation between means has been tested with ANOVA. For the neuter time, there is a significant variation between the means, that is, reaction times decrease significantly between the initial moment, the one month recording, the two months recording, and the three months recording. In order to better describe the structure of this variance, we have built a means plot, shown in Figure 1.

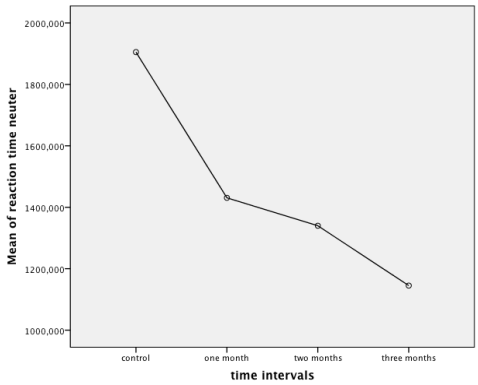

Fig.1. Means plot for test group for neuter time. It may be seen that the most visible decrease in the reaction time is between the initial recording (control) and the recording at one month. However, between the initial moment and the three-months recording the reduction in reaction time is significant.

In order to better analyze the structure of the differences between the means of the considered groups, we have designed a two-layer contrast analysis. The contrast coefficients are shown in Table III.

Table III. Contrast coefficients for test group for neuter time. In the first layer, we have contrasted the first and the second group, while in the second layer we have contrasted the pair formed by the first and second group with the pair formed by the third and forth group.

Contrast	Time intervals			
	Initial	At one month	At two months	At three months
1	1	-1	0	0
2	0.5	0.5	-0.5	-0.5

The contrast analysis for test group for neuter time is shown in Table IV.

Table IV. Contrast analysis for test group for neuter time. In the case of equal variances assumed, we may see that both group 1 (initially recorded values) differs significantly (p lower than 0.05) from group 2 (values recorded after one month), and groups 1 and 2 together differ from groups 3 and 4 (values recorded after two months and three months, respectively).

		Contrast	Value of Contrast	Std. Error	t	df	Sig. (2-tailed)
Neuter time	Assume equal variances	1	474.242	167.446	2.832	36	0.008
		2	425.399	118.402	3.593	36	0.001
	Does not assume equal variances	1	474.242	223.101	2.126	10.706	0.058
		2	425.399	118.402	3.593	13.450	0.003

The ANOVA for test group for reaction times with interference is presented in Table V.

Table V. ANOVA for test group for reaction times with interference. It may be seen that the significance is below 0.05, meaning that in this case, of interference, the means vary significantly between the four moments recorded.

Times with interference					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4643039.696	3	1547679.899	6.686	0.001
Within Groups	8333227.853	36	231478.551		
Total	12976267.550	39			

We have used the post-hoc Dunnett test to analyze multiple comparisons Table VI.

Table VI. Multiple comparisons for test group for time with interference. It may be seen that, in the case of interference, the most significant difference appears between the values recorded initially and the values recorded at three months.

Dependent Variable: reaction times with interference						
Dunnett T3						
(I) time intervals	(J) time intervals	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	At one month	474.988	288.500	0.499	-390.112	1340.088
	At two months	691.420	258.963	0.112	-124.738	1507.578
	At three months	923.132*	253.082	0.024	112.401	1733.863
At one month	Control	-474.988	288.500	0.499	-1340.088	390.112
	At two months	216.432	168.940	0.737	-296.133	728.998
	At three months	448.144	159.778	0.086	-49.836	946.124
At two months	Control	-691.420	258.963	0.112	-1507.578	124.738
	At one month	-216.432	168.940	0.737	-728.998	296.133
	At three months	231.712	96.740	0.150	-54.880	518.304
At three months	Control	-923.132*	253.082	0.024	-1733.863	-112.401
	At one month	-448.144	159.778	0.086	-946.124	49.836
	At two months	-231.712	96.740	0.150	-518.304	54.880
*. The mean difference is significant at the 0.05 level.						

We have analyzed the means plot for reaction times with interference, presented in Figure 2, in order to better interpret the differences between the groups.

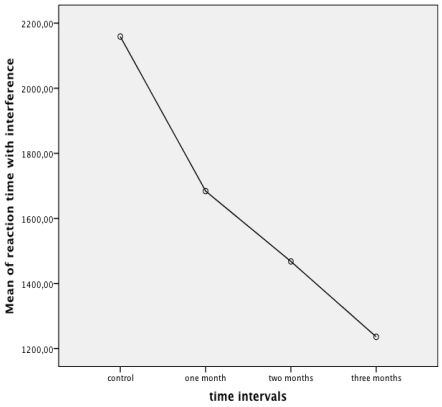

Fig.2. Means plot for test group for the time with interference. It may be seen that the pattern is similar to the one obtained for the neuter time.

We have further investigated the differences between the groups by contrast analysis in Table VII.

Table VII. Contrast analysis for test group for the time with interference. With equal variances assumed, differences are significant, at 0.05 level, both between group 1 and group 2, and between groups 1 and 2 and groups 3 and 4. The reaction times decrease more in the beginning of the experiment and then more smoothly.

		Contrast	Value of Contrast	Std. Error	t	df	Sig. (2-tailed)
Time with interference	Assume equal variances	1	474.988	215.164	2.208	36	0.034
		2	569.782	152.144	3.745	36	0.001
	Does not assume equal variances	1	474.988	288.500	1.646	14.825	0.121
		2	569.782	152.144	3.745	18.137	0.001

The ANOVA for reaction times without interference is presented in Table VIII.

Table VIII. ANOVA for the test group for time without interference. It may be seen that there are significant variances in means between the four moments when the results were recorded. In other words, reaction times without interference improve over time.

Reaction times without interference					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2785985,931	3	928661,977	6,971	0,001
Within Groups	4795868,063	36	133218,557		
Total	7581853,994	39			

The means plot for reaction time without interference is shown in Figure 3.

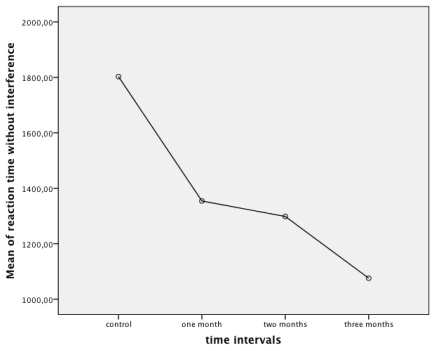

Fig.3. Means plot for test group for time without interference. It may be seen for time without interference, there is a obvious decrease between control and first month and between the second month and the third month.

The contrast analysis for test group for the time without interference is presented in Table IX.

Table IX. Contrast analysis for test group for the time without interference. With equal variances assumed, we notice a significant difference between group 1 and group 2, as well as between groups 1 and 2 and groups 3 and 4.

		Contrast	Value of Contrast	Std. Error	t	df	Sig. (2-tailed)
Reaction times without interference	Assume equal variances	1	448.550	163.229	2.748	36	0.009
		2	391.425	115.420	3.391	36	0.002
	Does not assume equal variances	1	448.550	220.792	2.032	10.691	0.068
		2	391.425	115.420	3.391	12.700	0.005

A conclusion that may be drawn from this analysis is that, the pattern of evolution is preserved across the three instances – neuter time, time with interference and time without interference.

We analyzed the interference index with the aid of the T – test. For the test group, Tables X, the p is 0,036 and it is included in the accepted interval.

Table X. One-Sample T – Test. The values of the interference index decrease significantly between recordings.

	Test Value = 253					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Intf_index_test	-2.591	7	0.036	-70.228	-134.327	-6.129

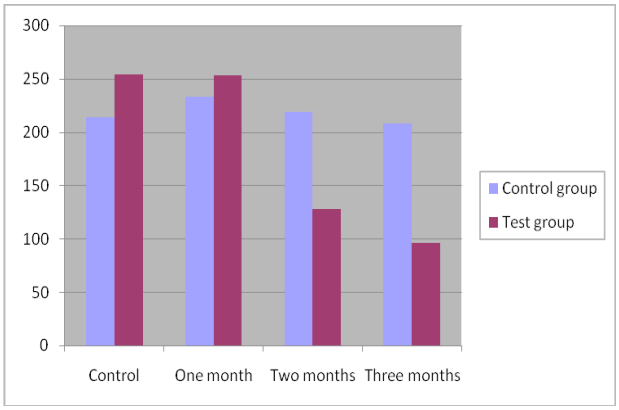

Fig.4. The evolution of the index of interference. It may be seen that, while for the control group it remains fairly constant over time, for the test group the interference index decreases gradually.

The comparative graphs, made with Excell software from Microsoft Office, present the evolution of the neuter time, time with interference and time without interference for the two groups, at the four moments considered.

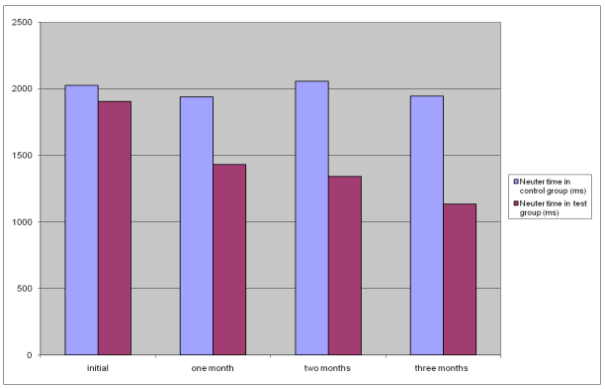

Fig.5. Evolution of time with interference. It may be seen the decrease of the neuter times in each month with taurine administration in test group.

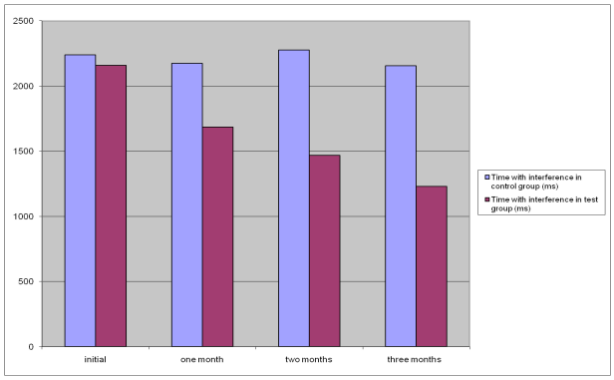

Fig.6. Evolution of time with interference. The values of the times with interference reduce every month with taurine administration in test group.

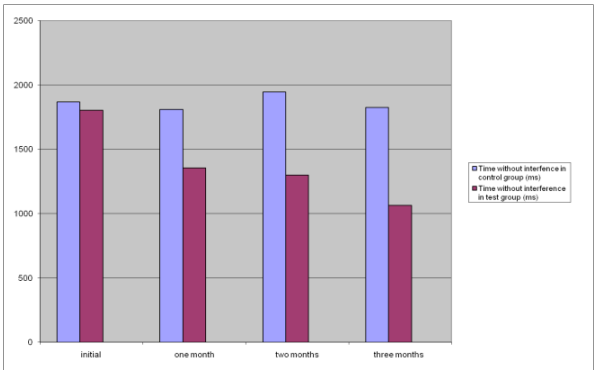

Fig.7. Evolution of time with interference. Times without interference decrease each month with taurine administration in test group.

It may be seen that the two groups start from approximately the same point and are gradually set apart by the administration of the taurine, the gap becoming visible after one month, and also that, while the control group does not suffer significant changes, the response times of the test group are modified in time.

The homovanillic analysis indicate progressive increase of its levels at one month, at two months and at three months for the test group. For the control group there were not significant changes in levels of homovanillic acid. For the test group, the p value at one month was 0.004. At the three months the p value was 0.002. We present results of the mean concentration of homovanillic acid as percentage reported at its value before taurine administration. The statistical analysis was made with the aid of the Kingsoft Spreadsheets from Kingsoft Office Software Corporation Limited.

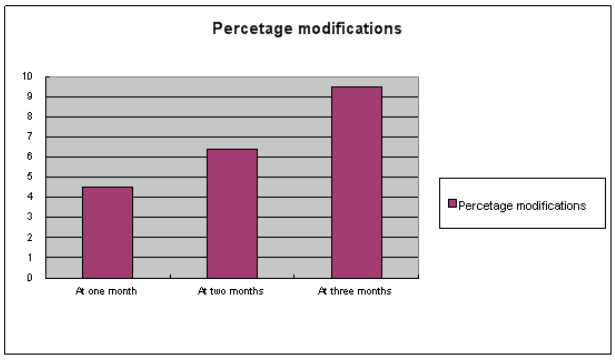

Fig.8. Percentage modifications in the test group for homovanillic acid levels. The most important statistically increases were at one moth and at three months.

DISCUSSION

There are four dopaminergic pathways in the brain, namely mesocortical, the mesolimbic, striatal and tubero - infundibular system (101). Mesocortical dopamine system modulates the activity of working memory in dorsolateral prefrontal cortex. Alto et al demonstrated that during working memory activation there is dopamine release in the prefrontal cortex (102).

By combining dopamine receptor analysis with cell recordings in monkeys it was demonstrated that D1 receptors selectively modulate memory fields, in dorso - lateral prefrontal cortex (103). Improvement of working memory performance and predominant effects of D1 receptors over D2 ones in this mechanism was demonstrated by simultaneous testes with pergolide (D1 and D2 receptor agonist) and bromocriptine (D2 receptor agonist) on working memory tasks. The performance was improved by pergolide and not by bromocriptine (104).

Dopamine levels decrease by 10% per decade starting in adulthood and are associated with the decline of cognitive and motor performance. Age-related decline in dopamine system and in working memory is observed in humans and primates (105, 106, 107). Decreased dopaminergic function in aging include decreased dopamine synthesis, demonstrated with PET studies in humans (108), reduced dopamine concentration, receptor density and availability of carrier (109). Diminished dopaminergic activity in aging is associated with functional changes especially in the prefrontal cortex, striatum and medial temporal lobes (110, 111, 112, 113). It was shown that decreased dopaminergic projections and dopamine availability in prefrontal cortex with aging mediates age-related cognitive deficits, including difficulties of contextual processing and of working memory (114).

An improvement of working memory performance was observed at low doses of D1 receptor agonist (0.0001 mg / kg) in old monkeys that have a natural reduction in extracellular levels of dopamine in prefrontal cortex (115, 105, 116). Extracellular dopamine levels were significantly incr eased by taurine administration intracerebroventricular (117) or intraperitoneal (118) or directly in the striatum (119). Ericson et al. (120) showed that local infusion of taurine (1, 10 or 100 mM) increases dopamine levels in the nucleus accumbens.

Recorded results indicate progressive decrease reaction times for each subset of the test during the three months of taurine administration and a decreased in interference.

Homovanillic acid (HVA) is the main product of metabolism of dopamine, resulting from both 3-methoxytyramine deamination and 3,4-dihydroxyphenylacetic acid methylation (121). Plasma concentration of homovanillic acid have been used as a peripheric indicator of dopaminergic activity of the central nervous system (122). This aminoacid levels decrease in the aging brain (123). The determined levels of homovanillic acid indicate that taurine administration generate progressive increase of homovanillic acid concentration in plasma, in healthy elderly.

CONCLUSION

Taking into account the results obtained by us, the decrease of the reaction times and of the interference index and the increase of homovanillic acid plasma concentration after taurine administration, the information from literature that taurine increase dopamine levels that decreased with aging, that plasma concentration of homovanillic acid was used as an indicator of

dopaminergic activity of the central nervous system, the implication of dopamine in working memory, we can say that taurine improve the working memory through the increase of dopamine concentration.

ACKNOWLEDGEMENTS

The presented results are part of the research grant TD No. 449/2007 offered by the Board of the National Council of Scientific Research in Higher Education of Romania and was approved by the Board of the University of Medicine and Pharmacy „Carol Davila", Bucharest.

DISCLOSURE STATEMENT

There are no conflicts of interest or financial disclosure. The manuscript is not considered for publication by any other journal.

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EFECTUL TAURINEI ASUPRA MEMORIEI DE LUCRU LA VARSTNICII SANATOSI

REZUMAT

Imbatranirea demografica este importanta pentru obiectivele domeniului sanatatii. In imbatranirea normala se produce un declin al proceselor executive, cum ar fi manipularea informatiei in memoria de lucru, coordonarea de operatii multiple simultan. In sistemul nervos, taurina actioneaza ca agent neuroprotector. Pentru a evalua performanta memoriei de lucru am folosit testul Stroop. Concentratia plasmatica a acidului homovanilic a fost folosita ca un indicator al activitatii dopaminergice din sistemului nervos central. Am testat doua grupuri, unul test si unul control, fiecare fiind alcatuit din zece subiecti umani, barbati si femei peste saizeci si cinci de ani. Am inregistrat timpii de reactie si am determinat concentratia acidului homovanilic inainte de administrarea taurinei, 1500 mg/day, la o luna, la doua luni si la trei luni de administrare a taurinei. Activarea memoriei lucru este corelata cu eliberarea dopaminei la nivelul cortexului prefrontal. Declinul legat de varsta in sistemul dopaminergic si la nivelul memoriei de lucru a fost observat la subiectii umani. Rezultatele obtinute indica scaderea timpilor de reactie si a indexului de interferenta si cresterea concentratiei plasmatice a acidului homovanilic dupa administrarea taurinei.

Cuvinte cheie: imbatranire cerebrala, test Stroop, memoria de lucru, taurina, dopamina, acidul homovanilic