MODULATION OF THE CAPTOPRIL INTERFERENCE WITH THE ACTIVITY OF SOME ENZYMATIC BIOMOLECULES IN MONKEY KIDNEY VERO CELLS BY DRUG DELIVERY MESOPOROUS SILICA SYSTEM.

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Keywords: mesoporous silica matrix, captopril, nanocomposites, monkey kidney *Vero cells*, membrane bound and intracellular enzymes, oxidative stress, enzymatic activity.

Abstract: The *in vitro* effect of different formulations of captopril on some cellular enzymatic equipments activities of monkey kidney Vero cells was investigated in the present research. The preparation of the samples of the mesoporous silica nanocomposites, loaded or not with captopril, was described and their effect on membranary Na^+-K^+-ATP-ase, cell Mg^{2+}-ATP-ase, LDH, Px, GSH-Px, SOD, CAT, ACP, ALP activities were studied. The Vero cells were incubated, for a period of 144 hours, with growing medium renewed twice. When the cells reached confluence in the monolayer stage, the cultures were divided into control cell cultures and other 4 treated groups. To the 12 hours treated cells were added: Cap H_2, SBA–15, unfunctionalized SBA-15_CapH2_RT and functionalized SBA-15_APTES_CapH2_80°C nanocomposites, each of them in a dose of 0.4µg./flask. As compared with the control Vero cells, which are characterized by a specific level of the enzymatic activities, the cultures treated with SBA-15 have not presented significant alterations of them. The comparative study of captopril interactions with some membrane bound and intracellular enzymatic biomolecules of monkey kidney Vero cells has revealed either an enhancement of membranary Na^+-K^+-ATP-ase, intracell total ATP- ase , LDH, ACP , and GSH-Px activities or a repression of cellular CAT, Px and SOD activities. These variations of the enzymatic activities – which induce modifications of the membranary and metabolic processes – could be due to a direct or indirect interaction of captopril with cellular (plasmalemma) or subcellular (organelles) structures and with intracellular biomolecules (enzymes, DNA, RNA etc.).
The association of captoptil with SBA – 15 or SBA – 15 _ APTES mesoporous silica matrices and treatment of Vero cells with these nanocomposites were correlated with modulation of the captopril interference with the activity of investigated enzymatic biomolecules, its sense (stimulation or inhibition of enzymatic activity) and amplitude (high or small modulation) being dependent of carrier type and peculiarities.

INTRODUCTION

One of the contemporary scientific research priorities is related to the improvement of the currently available formulations of medicines in the direction of enhancement of their effectiveness, maintaining an acceptable safety profile in the same time.

The progress done in this direction has created one of the most dynamic science and technology domains at the confluence of physical sciences, molecular engineering, biology, biotechnology and medicine (Izquierdoet al., 2005; Manzano et al., 2008; Heikkila et al., 2007). This field of nanotechnology has motivated researchers to develop nanostructured materials for biomedical applications, in order to create controlled released drug delivery systems that promise to solve both the challenge of poor biodisponibility of drugs and to maintain the desired release rate throughout the dosage period, in order to safely increase their effectiveness (Vallet-Regi et aql., 2007; Limnell et al., 2007; Trewyn et al., 2008). One of the solutions considered in this direction is the optimization of the effect of drug molecule through the use of rationally designed drug carrier materials. The concept of drug delivery systems has emerged to minimize the toxic side effects of drugs, to broaden their application, to expand modes of their administration and to solve absorption problems (Doadrio et al, 2004).

Characteristics required for a material to be designed as a drug delivery system are as follows: biocompatibility, low toxicity, ability to absorb high loading of drug molecules, lack of susceptibility to premature release, tissue specificity and site orientation, controlled release with a proper rate for an effective local concentration (Slowing et al., 2008). In this context, the ordered mesoporous silica raises a particular interest in nanomedicine because they meet these conditions (Wang, 2009). In general, controlled-release silica systems deliver drugs in the desired dosage for long periods, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble or relatively unstable drugs.

For a couple of years, investigations were performed into evaluating the role of mesoporous silicates in drug delivery, tissue engineering, gene transfection and cell tracking (Doadrio et al., 2004; Slowing et al.,2008; Vallet et al., 2010; Veerapandian et al., 2009; Huang et al., 2010; Bhattarai et al., 2010). As matrix for controlled delivery systems mesoporous silica proved important features: an ordered pore network, very homogeneous in size, which allows fine control of the drug load and release kinetics; a high pore volume (up to 1.2 cm^3/ g) to host the required amount of drugs; a high surface area (up to 1500 m^2/g), which implies high potential for drug adsorption; a silanol-containing surface that can be functionalized to allow better control over drug loading and release (Manzano et al., 2008; Wang, 2009).

Our interest focused on elaboration of a new formulation for captopril, major cardiovascular drug which acts as an inhibitor of angiotensin converting enzymes (ACEs).. ACEs inhibitors are one of the most important group of drugs that are used for controlling blood pressure, treating heart failure, preventing strokes and preventing kidney damage in people with hypertension or diabetes. Captopril was chosen in this research as loading drug because it is the best documented ACEs inhibitor. It blocks the active sites of an ACE zinc glycoprotein, inhibiting the conversion of angiotensin I to angiotensin II, which triggers the entire cascade of reactions responsible for the clinical symptoms and histological modifications in patients with hypertension (Oscar et al., 2005).

Captopril molecule (Figure 1a) has the dimensions of 0.9 x 0.57 x 0.33 nm and a thiol group, an amide group, an acid carboxilic group and a chiral carbon atom (Figure 1b). Captopril is a zwitterionic drug, with two dissociation constants, pK_{a1} 3.7 and pK_{a2} 9.8, and has an isoelectric point of 6.8 (Veerapandian et al., 2009).

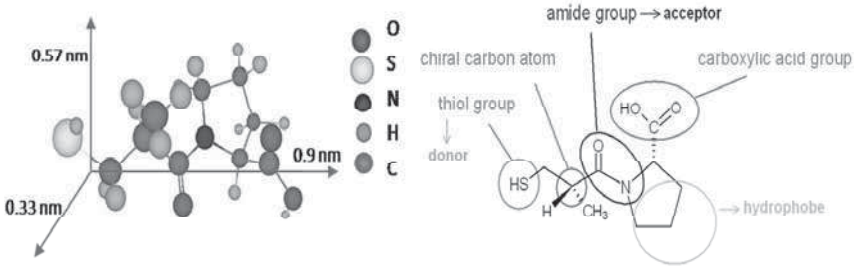

Figure 1: a) *Schematic geometrical representation of captopril molecule; .b.) Chemical formula of captopril (2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid.*

Recent investigations looked into the biocompatibility and cytotoxicity of mesoporous silica nanomaterials (Ukmar et al., 2010; Trewin et al., 2008; Di Pasqua et al., 2008; Hudson et al., 2008; Wang, 2009) and concluded that these materials influence the cell biology by weakening the cell membrane integrity, diminishing cell metabolism and increasing apoptopic signalling. Other research fail to identify a significant cell death of cell culture when treated with the aminated MSNs (Tao et al., 2009).

Knowing the hypotensor pharmacodynamic property of captopril and the mesoporous silicates qualities as a drug delivery matrix and vehiculating system, we considered opportune to investigate the usefulness of captopril loaded onto a mesoporous silica carrier, as a new formulation for him, and also to assess its impact on cell and molecular biology.

Thus, in a first step of the research, the suitable character of the mesoporous silicate matrix as a potential drug delivery system and the impact of the alone and associated captopril on the cell metabolism of healthy monkey kidney *Vero cells* cultures have been evaluated. We have shown that this type of matrix is able to trap the bioactive agents by a soaking procedure and then to release them in conditions mimicking the biological fluids. Also, the impact on cellular metabolism parameters of captopril loaded on functionalized mesoporous silica-matrices was more accentuated than that of the free, unassociated drug. The optimized formulations modulate the metabolic processes in the monkey kidney Vero cells by the enhancing the glycogenolysis, glycolysis, and lipolysis and acting through an inhibitory effect upon protein and nucleic acids biosynthesis (Popovici et al., 2011; Popovici et al., 2011).

In the present study, we have supplementary investigated the *in vitro* modulation of some enzymatic biomolecules activities in healthy monkey kidney *Vero cells* cultures submitted to the action of captopril, used either in free form or loaded on the mesoporous silicate matrices: the SBA-15 (etalon I) and SBA-15_APTES (etalon II). Their interaction with Vero healthy cells enzymatic operation was analysed in the light of some cell transport and metabolic enzymes activities (membrane Na^+-K^+-ATP-ase, cell Mg^{2+}-ATP-ase, lactat dehydrogenase, peroxidase, gluthation-peroxidase, superoxid dismutase, catalase, acid (ACP) and alkaline (ALP) phosphatises, which were biochemically determined.

MATERIAL AND METHODS

Mesoporous silica matrix was synthesized by self-assembly from a silica-surfactant liquid-crystal phase by the hydrothermal method using the method reported by Zhao et al., 1998, using the following molar composition of the mixture: TEOS:5.87HCl:194H_2O:0.017P123. The obtained product was denoted as SBA-15 (etalon I).

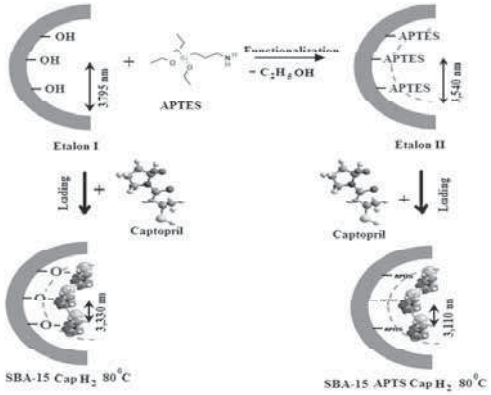

Figure 2. Schematic representation of samples preparation.

The surface modification was realized (Bruhwiler, 2010) via silylation with 3-aminopropyl-triethoxysilane (APTES) . The obtained product was denoted as SBA-15_APTES (etalon II).

The following materials were used for the preparation of un-modified and amino-modified mesoporous SBA-15: tetraethylorthosilicate (TEOS 98%, Acros Organics) as silica source, HCl, triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, Aldrich) as polymeric template and 3-aminopropyl-triethoxysilane (APTES, Aldrich), as surface functionalizing reagent, respectively. Captopril powder was purchased from the Company Sigma-Aldrich (product number C4042).

The loading experiments were conducted at room temperature (RT) and at 80°C. The samples were denoted as SBA-15_CapH2_T when captopril was loaded the on un-functionalized SBA-15 (etalon I) and as SBA-15_APTES_CapH2_T when captopril was loaded on the etalon II, the functionalized SBA-15 sample, respectively (T stands for the working temperature).

The structure of the mesoporous support was characterized using powder X-ray diffraction (TUR M-62 diffractometer under the following conditions: Ni filtered radiation Cu – K_α = 0.1518 A°, a voltage of 36 kV, a current of 20 mA, and a goniometer speed of 0.5°/mi), nitrogen adsorption and desorption isotherms (NOVA 2200e system using nitrogen as the absorbate at liquid nitrogen temperature), Scanning Electron Microscopy (VEGA//TESCAN instrument, operating at an accelerating voltage of 20 kV).

The biological material used for the *in vitro* experiments was represented by mycoplasm-negative, stabilized, normal renal Vero cells – epithelial renal cells are involved in the control of hypertension and main place of action of ACE inhibitors – obtained from Cercopithecus aethiops monkey kidneys and cultured in DMEM growing medium (Dulbeco’s Modified Eagle’s Medium, Biochrom AG, Germany, FG 0415), supplemented with 10.0% fetal bovine serum (Sigma, Germany, F9665), 100 µg/mL streptomycin (Biochrom AG, Germany,A 331- 26), 100 IU/mL penicillin (Biochrom AG, Germany, A 321-44) and 50 µg/mL antimycotic amphotericin B (Biochrom AG, Germany, A 2612), at a density of 2 x 10^6 cells / 300 cm^2 flask, in a humidified 5% CO_2 atmosphere at 37°C. (Abbro et al.,2004).The cells were incubated for a period of 144 hours, with growing medium renewed twice. When the cells reached confluence in the monolayer stage, the cultures were divided into control and 4 groups of treated cell cultures. In each experiment a dose of 0.4 µg/flask of the studied samples was used.

The treated cultures were incubated another 12 hours, under the same conditions. After this long time treatment, the medium was discarded from the test flasks, the layers of cells were washed with PBS (Saline Phosphate Buffer, Biochrom AG, Germany,L 1825) and then detached from the flasks with 0.25% trypsin + 0.02% EDTA (ethylenediaminetetraacetic acid, Biochrom AG, Germany, L 2163). The cells were pelleted in cilindrical glass tubes by centrifugation at 1.800 rpm for 2 minutes and were weighted for setting the cellular mass/flask representing diffent experimental groups.

The cell pellets of control and treated cultures were resuspended in an adequate volume of 0.88 M sucrose – 0.2 M

Tris – HCl buffer, omogenized by ultrasonication on ice with Bandelin – Germany Sonopuls device (2 minutes, 80 cycles/sec/75% power) and centrifuged for 15 minutes at 5.500 rpm, in a swing-out rotor, for obtaining the cell clarified lysates. All operations were performed at 4° C. Adequate aliquots were used for the biochemical determination of some cell enzymes: Na^+-K^+-ATP-ase, Mg^{2+}-ATP-ase, lactat dehydrogenase (LDH), peroxidase (Px), gluthation-peroxidase (GSH-Px), superoxid dismutase (SOD), catalase (CAT), acid (ACP) and alkaline (ALP) phosphatase activities (Artenie et al., 2008).

The estimation of the total Mg^{2+}- Na^+ - K^+ and respectively membranary Na^+-K^+-ATP-ase (tATP and mATP) activities, expressed in mg inorganic phosphate/minute/g cellular mass (mg Pi/min/gcm) was based on the amount of inorganic phosphorous released after ATP hydrolysation by ATP-ases present in the cellular homogenate. Lactate dehydrogenase activity (µM/min/gcm) was determined through the measurement of NADH oxidation velocity in the case of transformation reaction of pyruvic acid in lactic acid. Peroxidase activity (peroxidase unit, UP, /min/gcm) was estimated by orto-dianisidine method, which measures the intensity of the o-dianisidine oxidation product colour.

Glutathione peroxidase activity (µM GSH/ml/min/gcm) was measured on the basis of the reaction of unconsumed reduced glutathione with 2, 2'- dinitro-5,5'- dithio-dibenzoic acid (Merck), which drives to a yellow, photometrable complex.

The evaluation of superoxid dismutase activity (superoxid dismutase unit, USOD, /ml/min/gcm) is based on the enzyme capacity to inhibit the nitroblue tetrazolium reduction by the superoxid radicals generated in reaction medium through riboflavin reduction.

Catalase activity was estimated through spectophotometric registration of the hydrogen peroxide consumed quantity, being expressed in enzymatic unit (UE/gcm).

Alkaline and acid phosphatases activities (international unit,U.I./gcm) were determined with para-nitrophenol, which is converted in a spectrophotometrable product, p-nitrophenolat, under the action of phosphatases.

Five flasks of cultures have been used for each culture type, the results being analyzed statistically by means of Student „t" test (Cann, 2002). The level of significance in all statistical analyses was set at minimum $p< 0.05$.

RESULTS AND DISCUSSIONS

The investigation of the captoprilic treatment effect upon the membranary Na^+-K^+-ATP-ase and cellular Mg^{2+}-ATP-ase activities of Vero cell cultures has been correlated with a set of data presented in table 1 and graphically represented in figure 3.

Table 1. M*embranary Na^+-K^+-ATP-ase and cell Mg^{2+}-ATP-ase activities in Vero cell cultures treated with captopril(0.4 µg/flask), associated or not with mesoporous silica delivery systems. Figures in brackets indicate the number of experimental cultures for each type. Statistical significance was calculated versus control group. ES= standard error.*

Experimental group	Mg-ATP-ase (mM Pi/min/g cell culture)		Na-K-ATP-ase (mM Pi/min/g cell culture)	
Control	1.883±0.04 (5)	p<	1.909±0.05 (5)	p<
Cap H2	3.143±0.1 (5)	0.001	3.144±0.15 (5)	0.001
SBA-15	1.870±0.06 (5)	NS	1.856±0.09 (5)	N.S.
SBA-15/CapH2	2.405±0.09 (5)	0.001	3.093±0.36 (5)	0.02
SBA-15/APTES/CapH2	5.384±0.23 (5)	0.001	4.654±0.93 (5)	0.02

It can be seen that, in the case of control Vero cell cultures, the enzymatic activities for cellular Mg^{2+}-ATP-ase and membrane Na^+-K^+-ATP-ase, respectively, were of 1.883 and respectively 1.909 mM/min/cell mass g, these reference values being necessary for the

interpretation of the chemical compounds impact signification upon the activitiy of these biomolecules.

The Vero cultures incubated only in the presence of SBA- 15 mesoporous silica matrix, have been characterized, at the end of the experiment, by an enzymatic activity for both ATP-ases similar to the control one. The lack of a statistical significant difference, comparatively with control value, suggested that this carrier was biologically inert.

Comparing to the control group, the development of the cell cultures in the growth medium containing captopril loaded on the unfunctionalized mesoporous silica matrix was correlated with a significant enhancement of both enzymatic biomolecules. Finally, the Vero cells treated with functionalized SBA-15_APTES_CapH2_RT captopril nanocomposite were characterized by a very high stimulation of membranary Na^{+}-K^{+}-ATP-ase and cell Mg^{2+}-ATP-ase activities.

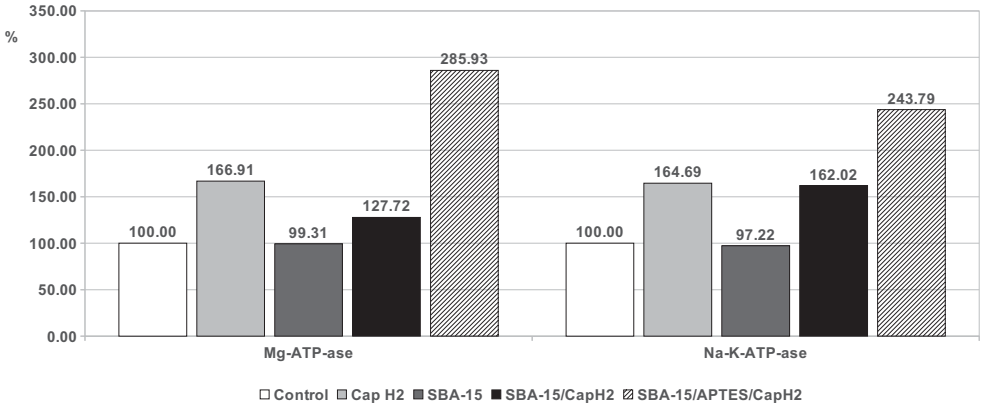

Fig.3.Modulation of the membranary Na^{+}-K^{+}-ATP-ase (mM/min/g cell mass) and cell Mg^{2+}-ATP-ase (mM/min/g cell mass) activities of Vero cell cultures by the treatment with 0.4 µg/flask captopril, SBA-15, SBA-15/CapH2 and SBA-15/APTES/CapH2.

In Figure 3, we have traced the ATP-ases activity profile of the Vero cultures submitted to the free and associated captopril action . As compared to the control group, the interference of Cap H2, SBA-15, SBA-15/CapH2 and SBA-15/APTES/CapH2 with the enzymatic activity has determined significant functional and statistical modifications of these membranary and intracellular enzymatic biomolecules. Thus, the activity of membranary Na^{+}-K^{+}-ATP-ase has been amplified with 64.7*%* (by Cap H2), 61.9% (by SBA-15/CapH2*)* and 143.7% (SBA-15/APTES/CapH2), while the functioning level of cellular Mg^{2+}-ATP-ase increased with 66.9% (Cap H2), 27.7% (SBA-15/CapH2) and 143.8% (SBA-15/APTES/CapH2).

Other investigated enzymatic biomolecules were the phosphatases – implied in the phosphorilation and dephosphorilation of different metabolic substrates – and lactate dehydrogenase – a key-enzyme of the glucidic intermediary metabolism – the pattern of unfolding of their activity in the monkey kidney *Vero cells*, treated with free or carriers associated captopril, being illustrated by table 2 and Figure 4.

As compared to the control and SBA-15 values – which were almost identically – in the Vero cultures incubated with free and associated captopril, we have registered a significant stimulation

of the acid phosphatase and lactate dehydrogenase activities, as well as a very small one in the case of alkaline phosphatase.

The influence of captopril, of SBA-15 mesoporous structure or of the captopril included in

Table 2. Interference of the free and associated captopril (0.4 μg/flask) with phosphatases and lactate dehydrogenase activities in treated Vero cell cultures. Figures in brackets indicate the number of experimental cultures for each type. Statistical significance was calculated versus control group. ES= standard error

Experimental group	ACP (U.I./gcm)		ALP (U.I./gcm)		LDH (μM/min/gcm)	
	X±ES	p<	X±ES	p<	X±ES	p<
Control	0.250±0.020 (5)	-	1.403±0.02 (5)	-	146.408±5.5 (5)	-
Cap H2	0.344±0.013 (5)	0.02	1.407±0.05 (5)	NS	176.329±9.0 (5)	0.05
SBA-15	0.264±0.020 (5)	NS	1.409±0.03 (5)	NS	149.828±11.6 (5)	NS
SBA-15/CapH2	0.408±0.020 (5)	0.001	1.450±0.04 (5)	NS	165.289±4.3 (5)	0.05
SBA-15/APTES/CapH2	0.339±0.030 (5)	0.05	1.420±0.02 (5)	NS	158.337±6.3 (5)	NS

mesoporous structures SBA-15/CapH2 and SBA-15/APTES upon the activity of alkaline phosphatase was negligible, the differences between control and treated values being in the range of 0.3% - 3.4%. Contrary, the activity of acid phosphatase was increased by the tested substances. Thus, the captropil determined a stimulation of the acid phosphatase with 37, 9%, SBA-15 with 17.7%, SBA-15/CapH2 with 35.8% and SBA-15/APTES/CapH2 with 63.6%.

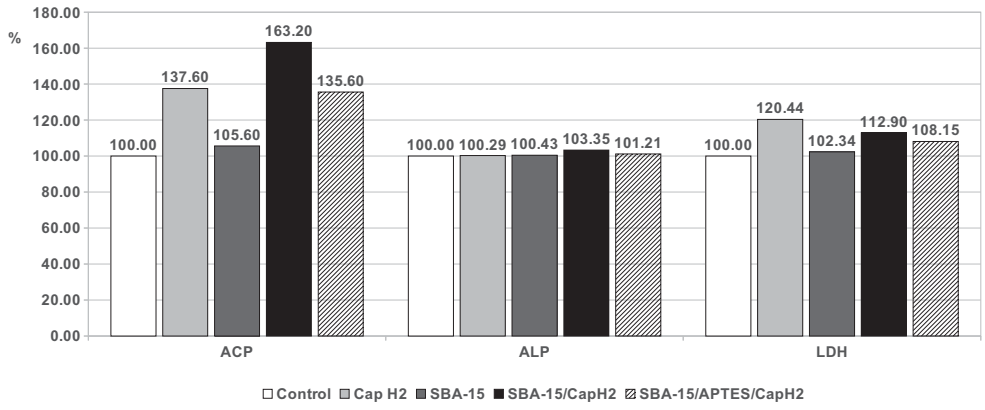

Fig. 4. The impact of CapH2, SBA-15, SBA-15/CapH2, SBA-15/APTES/CapH2 upon the activities of ACP(U.I.), ALP(U.I.) and LDH (μM/min) enzyme of Vero renal monkey cells.

Also, it can be observed that the activity of lactate dehydrogenase was modulated by the treatment of Vero cells with captopril, mesoporous structures, and captopril-mesoporous

complexes. Captopril, alone or included in mesoporous structure SBA-15/APTES, has increased activity of LDH with 20.4% or with 12.9%, the differences being statistically nonsignificant. On the contrary, SBA-15 and respectively SBA-15/CapH2 have reduced LDH activity with 11.3% and respectively 12.3%, these variations being also without statistical significance.

The last enzymatic system, studied in the conditions of the free or loaded captopril action, was that of biocatalysers (catalase, peroxidase, glutathione peroxidase = the reduction of lipid hydroperoxides to their corresponding alcohols and to the reduction of free hydrogen peroxide to water; superoxid dismutase = dismutation of superoxide free radical into oxygen and hydrogen peroxide) implied in scavenging of reactive oxygen species (hydrogen peroxide, superoxide anions, hydroxyl radicals, lipidic peroxides), issued from oxygen normal cell metabolism.

Table 3.Catalasic, peroxidasic, glutathione peroxidasic and superoxid dismutasic activities in Vero cell cultures, of 144 hours old, in vitro treated with captopril(0.4 µg/flask) associated or not with mesoporous silica delivery systems. Figures in brackets indicate the number of experimental cultures for each type. Statistical significance was calculated versus control group. ES= standard error.

Experimental group	CAT (UE/gcm)		Px (UP/gcm/min.)		GSH-Px (nM GSH/ml/min/gcm)		SOD (USOD/ml/min/gcm)	
	X±ES	p<	X±ES	p<	X±ES	p<	X±ES	p<
Control	156.3±10.60 (5)	-	7.05±0.98 (5)	-	0.72±0.020 (5)	-	7.2700±1.05 (5)	-
Cap H2	128.32±9.56 (5)	NS	6.12±0.65 (5)	NS	0.99±0.012 (5)	0.001	5.6187±1.32 (5)	NS
SBA-15	244.3±11.36 (5)	0.001	7.18±1.20 (5)	NS	0.51±0.014 (5)	0.001	3.9636±0.40 (5)	0,02
SBA-15/CapH2	134.26±9.01 (5)	NS	6.19±0.96 (5)	NS	0.62±0.021 (5)	0.01	5.0203±1.10 (5)	NS
SBA-15/ APTES/CapH2	120.04±8.95 (5)	0.05	5.21±1.30 (5)	NS	0.45±0.010 (5)	0.001	3.0178±0.93 (5)	0,02

Generally, it can be seen from table 3 and in figure 5, which presents and represents the behaviour of ROS scavenging enzymes, that the effects of tested substances upon their activity are dependent by the used substance and enzyme type.

Thus, the captopril has inhibited the catalasic, peroxidasic and superoxid dismutasic activities, being devoid of statistical significance, while the reactivity of glutathione peroxidase has revealed its significant stimulation by this medicine. The effects of SBA-15mesoporous structure upon the activity of scavenging enzymatic system of reactive oxygen species were either statistical non-significant (peroxidase) or significant (catalasic stimulation, glutathione peroxidasic and superoxid dismutasic inhibition). The pattern of scavenging enzymes reactivity to the treatment with captopril included into the unfunctionalized SBA-15 mesoporous matrix was similar with the one of alone captopril (attenuation their function), with exception of GSH-Px (its activity is exacerbated). The functionalization of CapH2/SBA-15 with APTES has potentiated the effect of captopril integrated into the mesoporous structure.

In comparison with the control enzymatic activities, the impact of CapH2, SBA-15, SBA-15/CapH2 and SBA-15/APTES/CapH2 upon Vero cells has conditioned a modulation of the enzyme’s function. Thus, the captopril has induced a repression of catalase (with 17.9%),

peroxidase (with 13.3%) and superoxid dismutase (with 22.7%) activities, as well as an intensification of the glutathion peroxidase activity (with 36.4%). The treatment of Vero cells

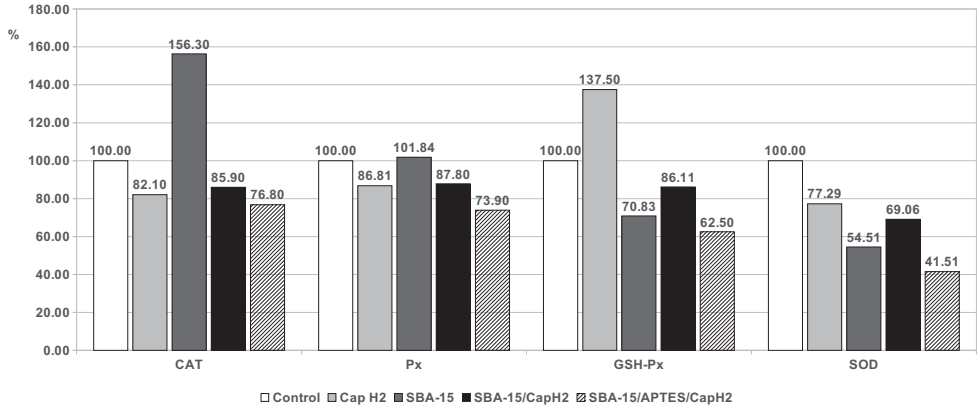

Fig.5. The reactivity of catalase, peroxidase, glutathion peroxidase and superoxid dismutase to the treatment of Vero cells with captopril, SBA-15, CapH2/SBA-15 and CapH2/APTES/SBA-15.

with SBA-15 mesoporous structure has determined, on the one hand, an activation of the catalase and peroxidase, or on the other hand,an attenuation of the glutathion peroxidasic and superoxid dismutasic activities. The procentual difference between enzymatic activities of the treated and control groups was of + 11.3% (CAT), + 1.8% (Px), - 29.1% (GSH-Px) and - 45.5% (SOD). The CapH2/SBA-15 compound has induced an inhibitory effect – with 23.2% (CAT), 26.1% (Px), 28.2% (GSH-Px) and 58.5% (SOD) upon these biocatalysers. The reactivity of studied enzymes to SBA-15/APTES/CapH2 action has materialized by reduction of the catalase (14.1%), peroxidase (12.2%), glutathion peroxidase (14.5%) and superoxid dismutase (31.0%) activities.

The activity intensification of some key intracellular anti-oxidative stress enzymes under the action of captopril and the repression of these by SBA-15 matrix suggests a stressful effect or a stimulatory effect on the metabolic processes implied in production of different free radicals of captopril medicine and a diminution of the intensity of metabolic processes generating free radicals by SBA-15 matrix. This protective propriety of mesoporous structure could be useful in the design of new vectorizing structures with a high affinity for the target and with low interference upon the normal cells.

The bulk of the above presented results highlights reactivity of some enzymatic biomolecules (Na^+-K^+-ATP-ase, Mg^{2+}-ATP-ase, lactat dehydrogenase, peroxidase, gluthation-peroxidase, superoxid dismutase, catalase, acid and alkaline phosphatise) in the monkey renal Vero cells submitted to the 12 hours treatment with captopril, unfunctionalized mesoporous silica matrix, unfunctionalized SBA-15_CapH2_RT complex and functionalized SBA-15_APTES_CapH2_RT nanocomposites, each of them in a dose of 0.4 μg/flask.

The action of the free or loaded captopril upon different enzymatic systems has been analyzed in the light of the control cultures enzymatic activity. Thus, the quantitative variations of these cell enzymes activities, have revealed their modulation by captopril associated or not with diverse delivery systems. The sense and intensity of the enzymatic reactivity to the impact of

alone or associated captopril are different from an experimental group to another, the studied enzymes characterizing through new operating levels. Therefore, we have assisted either to an enhancement or to a repression of the enzymatic biomolecules activities by captopril, a hypotensor medicin. The significance or nonsignificance of the modulatory effect induced by captopril seems to be a variable related to its free or associated state and to the structural and functional peculiarities of its carriers.

The present research focused on evaluation of captopril interaction with some cell enzymatic systems of the Vero cells cultures, in the conditions of its singular action or of its association with mesoporous silica matrices. The selected cardiovascular therapeutical medication, captopril, was chosen in this research because it is the best documented ACE inhibitor in terms of clinical and metabolic effect and it is used to treat hypertension, congestive heart failure and renal nephropathy.

Reviewing our date, it can emphasize that the *in vitro* treatment of the monkey renal cell cultures with captopril, in the usual formulation, has induces at cellular level either a stimulatory effect upon the activity of some enzymatic biomolecules, such as: ATP-ases system, acide phosphatase, lactate dehydrogenase, or an inhibitory one upon catalase and superoxide dismutase enzymes. The same drog did not interfere with alkaline phosphatase and the gluthation-peroxidasic activities.

SBA-15/ CapH2 and SBA-15/APTES/CapH2 mesoporous silica nanomaterials**,** new modified formulation of captopril performed by its loading in unfunctionalized SBA-15/ and respectively functionalized SBA-15/APTES matrices, have modulated the enzymatic reactivity of the Vero cells, optimizing (in the case of ATP-ases, acide phophatase, LDH, catalase, SOD) or repressing (GSH-Px) the specific enzymatic .effects of captopril.

This interference of the used bioactive agents with the activity of diverse cell enzyme systems will lead to positive or negative modifications of the membrane permeability, cell signaling transmembranary ionic fluxes, ionic equilibrium, extra- and intracellular ionic ratios and respectively of the unfolding of the metabolic events, which will express by potentiation or attenuation of the effects of the captopril, vehiculated and progressively delivered by the mesoporous silica carriers from componence of our nanocomposites.

Interestingly, the enzymatic effects observed with captopril on silica mesoporous matrix were greater than those with free captopril, while the quantity of captopril in the matrix 2 sample was approximately 4 times less than the captopril free. This potential dose sparing of the active substance carried on the functionalized drug delivery system was noticed only for functionalization with APTES, with less drug used, but stronger enzyme impact. An explanation for the overall findings might be related to the structural and functional differences of the two formulations used for the captopril transport, from which the bioactive agent is progressively released in the medium.

The amplification of the impact of CapH2 upon the Vero cells, assured by association with several forms of SBA-15 mezoporous silica matrix, could be explained by its protector effect upon captopril from the enzymatic degrading, by prolongation of captopril life span or action and by progressively drog delivering, as well as by SBA-15 functionalization with APTES,

Data accumulated in this study argue the possibility to improve the effect of captopril by its integration into a functionalized or unfunctionalized mesoporous matrix, the efficiency of this association being superior to either component of the performed nanocomposites.

The interference of the diverse captopril formulations with different enzymatic activities of the Vero cell could be probably mediated by membrane or intracell (DNA, RNA, proteins or

enzymes) receptors. From these primary sites of its action. will trigger a cascade of cell events with modulated enzymatic and metabolic expression.

CONCLUSIONS

The integration of captopril into the unfunctionalized or functionalized mesoporous silica-captopril nanocomposites has correlated either with enhancement of its effects upon some enzymatic activities (ATP-ases, acid phosphatase) or with repression of the activity of oxidative antistress enzymes of Vero cells cultures.

The enzymatic effects' intensity, observed with captopril loaded on silica mesoporous matrix, were greater than that one of free captopril, while the quantity of captopril in the matrix 2 sample was approximately 4 times less than the one used in the singular captoprilic treatment.

The enzymatic effect improvement of captopril by its association with functionalized or unfunctionalized SBA-15 recommends mesoporous silica matrices as controlled released drug delivery systems, usefull for the development of new drug formulations with high pharmacodynamic potential and low cytotoxicity.

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