PREMISES FOR THE THERAPEUTIC USE OF SELENIUM NANOPARTICLES IN OXIDATIVE STRESS-ASSOCIATED DISEASES
PREMISELE UTILIZĂRII TERAPEUTICE A NANOPARTICULELOR DE SELENIU ÎN PATOLOGIILE ASOCIATE CU STRESUL OXIDATIV

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Decrease of the oxidative stress represents a major challenge in current therapy. Among up-to-date approaches, the use of nanoparticles as carriers of antioxidants has been intensively studied. One special case is the use of selenium nanoparticles (SeNPs), as selenium, an essential micronutrient for animals and humans, can act by itself as a good radical scavenger. This paper reviews the current trends for using SeNPs in therapy, describing shortly the major SeNPs preparation and characterisation methods, their in vitro and in vivo effects and their possible therapeutical use. The mechanisms underlying the effects of SeNPs are shortly connected with their beneficial / toxicological risk ratio.

Reducerea stresului oxidativ constituie o provocare majoră în terapia actuală. O abordare recentă, intens studiată, este utilizarea nanoparticulelor ca purtători de antioxidanți. Un caz special este cel al nanoparticulelor de seleniu (SeNPs), deoarece seleniul, un micronutrient esențial pentru animale și oameni, poate el însuși neutraliza radicalii liberi. Această lucrare sintetizează tendințele actuale de utilizare a SeNPs în terapie, descriind succint metodele principale de preparare și caracterizare a SeNPs, efectele lor in vitro și in vivo, precum și posibilile aplicații terapeutice ale acestora. Mecanismele care stau la baza efectelor SeNPs sunt într-o mică măsură corelate cu balanța beneficiu / toxicitate.

Keywords: *selenium, nanoparticles, antioxidant, oxidative stress, effects, toxicity, therapy*

1. Introduction

The reduction of oxidative stress represents a major challenge in current therapy. Oxidative stress, defined as an overproduction of reactive oxygen and nitrogen species (ROS, NOS), can lead to impairment of biomolecules and consequently, to improper metabolic function of the cells, leading to diseases [1]. The oxygen- and nitrogen-reactive species excess act against biomolecules (such as lipids, proteins, DNA, etc.) resulting in a cascade of secondary products, very reactive in their turn; the final products of the chain can induce unwanted changes, and therefore are sometimes used as markers of oxidative stress. Among such final products of protein degradation, a representative oxidative stress responsible class of agents are the advanced glycation end-products (AGEs). Their accumulation drives to structural and functional protein changes, such as decrease in available primary amino groups, protein cross-linking or carbohydrate-protein linking, and increased level of protein carbonyl groups.

An approach in the reduction of oxidative stress is the use of nanoparticles as carriers for antioxidants to cells and tissues. A special case is the use of selenium nanoparticles (SeNPs), which act as a good breaker of the oxidative chain. For example, in the case of oxidative stress associated process of protein glycation, an increase of 40% of the available amino groups was noticed after adding SeNPs along with glucose to bovine serum albumin (BSA). The cross-link between proteins or carbohydrates and proteins, as well as carbonyl content in BSA were diminished progressively by elevating SeNPs level from 0 to 6 mg/mL [2].

Selenium is an essential micronutrient for animals and humans [4].

Se has been routinely quantified in human serum, having levels of tens of µg/L, with differences depending on region, age and sex. For example, the measured serum/plasma concentrations has an European average of 85.19±14.58 µg/L; for subjects below 19 years old, the Se serum/plasma level is 74.21±9.50 µg/L. By comparison, Latin Americans recorded an average level of 91.51±18.78 µg/L in subjects aged 15 and over, and 93. 25 ± 39.20 µg/L in subjects below 15 [5]. Similarly, the physiological level was established at 84.3 ± 11 µg/L for people living in Teheran below 16 years old and 100.6 ± 13 SD µg/L for older [6]. Se can be also assayed in urine, studies mentioning physiological excretion rates of 20-200 µg/day [7].

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The recommended daily intake increases with the age, from 15 to 55 µg in children under 14 years old; above this age, 55 µg Se are recommended for entire life. During pregnancy, the intake should be increased to 60 µg and up to 70 µg during lactation. Food sources rich in Se are Brazil nuts or cooked tuna [8].

The aim of the paper is to review the current trends in using SeNPs in therapy, focusing on their beneficial effects in oxidative stress compared to their toxicity for mammals.

2. Preparation of SeNPs

Selenium nanoparticles (SeNPs) have been prepared since the beginning of the years 2000[9] using many chemical, physical and biological methods. Characterization of the SeNPs was generally performed by means of UV-visible absorption spectroscopy, X-ray diffraction (XRD), electron spin resonance (ESR), differential scanning calorimetry (DSC), atomic force microscopy (AFM), and transmission electron microscopy (TEM).

Although theoretically the admission into the nano-sized particles may be done only if the dimensions are less than 100 nm, agglomerates with up to 500 nm have also been included in the category of nanoparticles [10, 11].

Mainly chemical reactions (such as precipitation, acid decomposition, catalytic reduction with ascorbic acid, sulphur dioxide, glucose or other agents) have been used to obtain SeNPs.

Acids, such as acetic, oxalic and gallic were used as reducing agents for the synthesis of polyvinyl alcohol-stabilized SeNPs using sodium selenosulphate as Se source. The resulted spherical particles had diameters in the range of 35-70 nm, the size increasing with the selenium precursor concentration [12]. A similar procedure had been applied before by Shah et al., but instead of a protic acid, acrylonitrile served as reducing agent [13].

Another chemical method used selenous acid as a Se precursor. This is obtained from selenium dioxide dissolved in water and suspended in a sodium dodecyl sulphate (SDS) solution at pH adjusted at 3.2. The reduction to SeNPs was accomplished by SO_2, generated by adding sodium metabisulphite. During the first two minutes of reaction, nuclei of up to 40 nm were generated, which afterwards grew to 200 nm [11].

Small hollow SeNPs (32 nm particle diameter and about 4nm shell thickness) were obtained by reducing sodium selenite with mercaptoethanol within a protein template [14].

Oxidants, such as H_2O_2, peroxynitrite and singlet oxygen, known as responsible for the induction of oxidative stress, acting upon selenourea under continuous aeration, led to SeNPs, which were stabilized using bovine serum albumin (BSA) or SDS [15].

The major disadvantage of the chemosynthesis consists in the fact that the chemical entities from the reacting media can adhere to the particle's surface during the generation process, and subsequently exhibit their cellular toxicity. The tendency in SeNPs synthesis was then to minimize the particles toxicity provided by the chemical methods. In this line, lyophilized hyperbranched polysaccharides (HBP) were used for capping the SeNPs issued from the reduction of selenous acid with ascorbic acid in an aqueous medium. This new technique prevents the grown SeNPs through aggregation and offers very safe experimental conditions. TEM images showed particles with diameters around 25 nm [16].

Unlike the chemical synthesis, the use of reducing agents originating from herbal preparations (extracts) provided low toxicity for the generated SeNPs. Thus, the herbal extracts content rich in reducing compounds such as phenols, flavonoids and particularly lignans enables a so-called "green synthesis". For example, *Vitis vinifera* fruit dry extract used for reducing of selenous acid led to the formation of 3-18 nm diameter SeNPs, encapsulated in a lignin biopolymer layer [17]. *Capsicum annum* water extract transformed selenous acid, at low pH, into 200-500 nm sized polygonal SeNPs [18] *Terminalia arjuna* leaf extract and sodium selenite resulted into crystalline 10-80 nm diameter nanoparticles [19]. The leaf extract of *Clausena dentata*, a citrus plant, was also able to issue from selenium (powder) spherical SeNPs with diameters of 46-78 nm [20], as well as *Bougainvillea spectabilis,* a decorative plant, whose flowers were used to prepare an infusion which reduced sodium selenite and formed spherical SeNPs having a sharp 25 nm diameter [21]. Some of these methods are synthetically presented in Figure 1.

Among the physical methods, laser ablation, in the presence or not of a hydrothermal procedure, was applied for SeNPs obtaining. For example, the conversion of amorphous selenium into crystalline SeNPs was firstly reported by Quintana et al.[9] SeNPs were synthesized by pulsed laser deposition technique, using the 532 nm harmonic wavelength of a pulsed Nd:YAG laser. AFM and Raman spectroscopy pointed out that particle morphology (around 90 nm diameter) strongly depends on the substrate, both size and population increasing with the laser energy density used for the deposition [9].A similar method was performed in water, with no substrate-active substances; the obtained SeNPs were spherical, with a 60 nm average diameter [22]. A mixture of sodium selenite and hydrazine chloride water solutions was kept in an autoclave at 150 °C for 24 h. The resulted SeNPs had the diameter in the range of 15 to 30 nm [23].

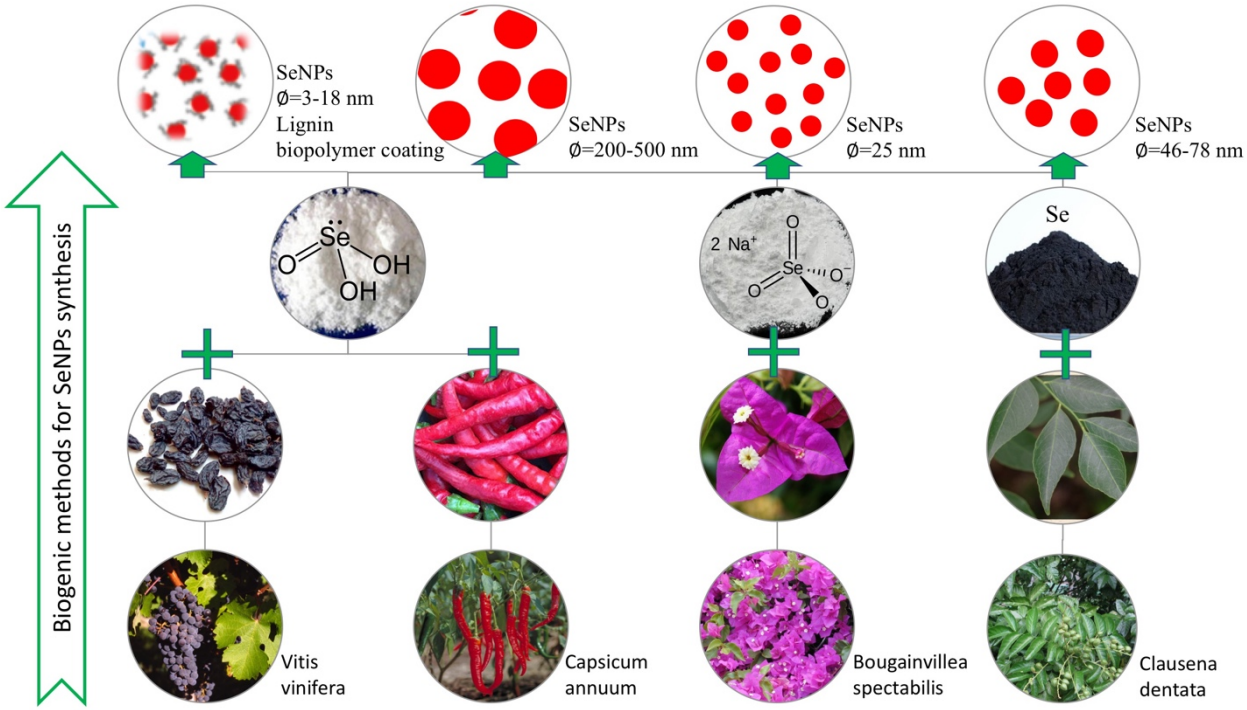

Fig. 1 - SeNPs synthesis methods using herbal preparations / *Metode de sinteză a SeNPs folosind preparate vegetale* [17,18,20,21]

It seems that the less toxic nanoparticles are obtained by biosynthesis, which requires a biological substrate (generally bacteria or fungi). Bacteria often exhibit Se resistance, and produce SeNPs as a defence mechanism against its toxicity. Bacteria transform selenate into red allotropes of elemental Se, that usually accumulate in a certain zone of the cell, depending on the bacteria type. All SeNPs obtained from bacteria synthesis were spherical [10].

For example, *Shewanella sp.* produces some of the smallest SeNPs, having a diameter of 11-20 nm. If a variety of sizes is desired, *Pseudomonas alcaliphila* is a good option, leading to SeNPs of 50-500 nm diameter. A popular microbial strain useful for this purpose is *Klebsiella pneumoniae*, while easily available bacteria for generating SeNPs are *Pantoea agglomerans* (in rivers) or *Bacillus* sp. MSh-1 (in fresh sea water) [10] In order to recover elemental selenium from the bacterial culture, wet heat sterilization can be used, with very satisfactory yields [24].

Extracellularly produced particles are easier to isolate and purify. The well-known *Pseudomonas aeruginosa* reduces selenite to SeNPs, visible by SEM around or on the surface of the rod-shaped bacteria. This capacity of the bacteria was seen as a clearing method for waters contaminated with soluble toxic selenite as well as a green method for aerobic synthesis of SeNPs [25]. *Bacillus mycoides* is a handy soil bacterium that leads to formation of SeNPs sized from 50 nm up to 400 nm [10] Certain natural environment living bacteria that can synthesise SeNPs congregate, thus making easier the collection and purification of the nanoparticles. Among them, *Duganella sp.* (soil) generates extracellular SeNPs and *Bacillus cereus* (coalmine soil) deposits the particles inside the cell. In some cases, SeNPs might also be placed near the cell surface or between cell wall and membrane [10].

In 2016, Wadhwani counted five types of fungi and two strains of *Streptomyces* (actinomycetes), strains for which SeNPs production had been reported. *Saccharomyces cerevisiae*, *Aspergillus terreus* (from soil) and *Alternaria alternate* stock the particles extracellularly. The last two produce spherical SeNPs, with diameters of precisely 47 nm and in the range of 30-150 nm, respectively [10]. Besides these, *Streptomyces minutiscleroticus* also proved the ability to synthesise SeNPs, and the biogenic Se was evaluated with positive results for antioxidant, antiviral, cytotoxic activities, and even for wound healing [26]. Same functionality regarding Se transformation has been observed for the strictly aerobic *Streptomyces* sp. ES2-5, found in a Se mining soil in China [27].
Representatives from *Basidiomycetes* were also stated useful reducing agents for SeNPs obtaining. *Ganoderma (G.) lucidum* produces extracellular SeNPs having a 20-50 nm diameter, while *Pleurotus ostreatus* leads to 50-320 nm particles. *Grifila frondosa* and *Lentinus edodes* produce intracellular SeNPs with the same size domain as *P. ostreatus*. Thus, *G. lucidum* is considered a valuable SeNPs source, being known that small particles have remarkable antioxidant activity and low toxicity [28].

All data above are indicating the great interest of the scientific community in producing SeNPs. While the interest for Se in semiconductors industry is still important, SeNPs relevance for health and environmental science is steadily increasing. Only the potential use of SeNPs in health will be considered in the following section.

3. Beneficial effects and possible uses of SeNPs in therapy

3.1. Anti-inflammatory

SeNPs were assessed for their anti-inflammatory effects in different types of diseases. On rats with chronic inflammation, Se nanoparticles at dose of 500 µg/kg rat b.w. exhibited an important anti-arthritic effect, restoring the normal enzymatic antioxidant levels in main organs (spleen, liver, and kidney). Also, at 250 µg/kg b.w. SeNPs coated with dextrin significantly reduced the arthritis-induced parameters, at this dose acting like a potent anti-inflammatory drug. C-reactive protein was re-established at its regular level with only 100 µg/kg SeNPs [29].

In vivo studies on 6 Gy gamma (γ)-irradiated and non-irradiated mice revealed that paw volume and other inflammation parameters, such as leukocytes count, TNF-α, PGE_2, were reduced by oral administration of 2.55 mg SeNPs /kg b.w. once or twice a day, depending on the animal model. The nociceptive threshold was not modulated in any model used [30].

Both skeletal and visceral inflammations might be modulated by SeNPs. Regarding acute colitis-associated inflammation at mice, SeNPs stabilized with *Ulva lactuca* polysaccharide exhibited a lowering effect on the inflammatory process, inhibiting the hyperactivation of NF-kB by supressing its nuclear translocation, in colonic tissues and macrophages. This way, cytokines such as IL-6 or TNF-α were down-regulated [31].

3.2. Wound-healing

Wound healing effect of SeNPs has been studied using excision wound model at rats. Comparing to the standard ointment with gentamycin 0.1%, the product containing 10% SeNPs exhibited better healing properties, leading to the treatment duration. The efficacy of SeNPs was superior to that of gentamycin, as the resemblance after the treatment to normal skin was better and the scars were less hypertrophic.[26]

Nevertheless, the immunomodulatory effect of SeNPs is better played by the biogenic than by the chemically produced ones, probably because of the adherent residual molecules existing in the last case, which can induce several pathologies.[32]

3.3. Antibacterial

3.3.1. Applications

The mechanism of SeNPs antibacterial effect is supposed to be related to their capacity of depleting the antioxidant glutathione inside bacteria, resulting a great increase of intracellular bacterial ROS [33].

Therefore, the SeNPs activity on bacteria was applied in different domains (Figure 2). For instance, nano-selenium coated paper towels were proved great antibacterial effectiveness, the bacteria colony count remaining unchanged up to 72 h [34]. Electrospun silk combined with biogenic SeNPs as a nanocomposite scaffold with antibacterial properties. Further research is needed to establish the antibacterial mechanism [35]. Also, having in view the integration of SeNPs to a topical antibacterial remedy, clinical samples from hard-to-heal wounds were collected and SeNPs inhibitory ability over the present bacterial strains was assessed. Inhibitory effect over the tested bacteria was noticed [36].

S. aureus frequently contaminates medical devices, causing life-threatening infections. Ventilator-associated pneumonia alters more than one of four mechanical ventilated patients, and it is triggered by infected PVC ventilation tubes. As

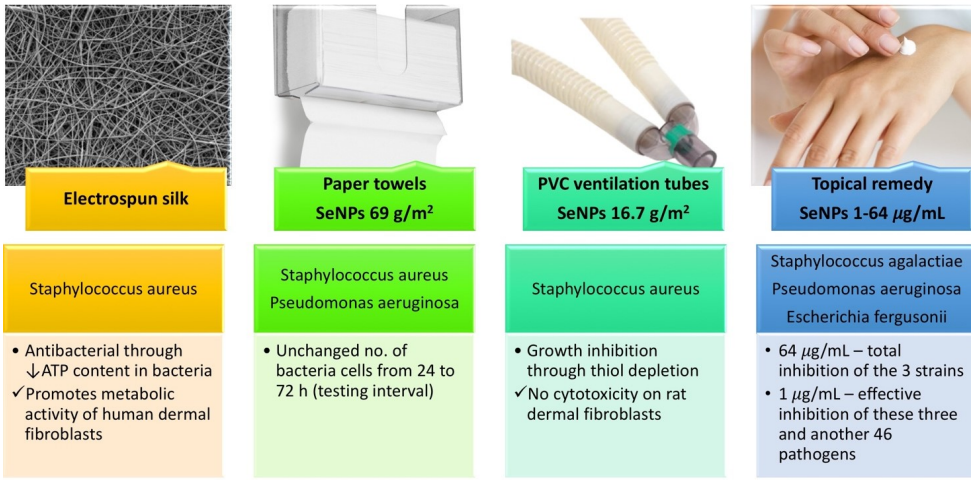

Fig. 2 - Applications of the antibacterial effect of SeNPs (Electrospun silk [35], Paper towels [34], PVC ventilation tubes [40,41], Topical remedy [36]) / *Aplicații ale efectului antibacterian al SeNPs (Mătase sintetică [35], Prosoape de hârtie [34], Tuburi de ventilație din PVC40, Produse topice [36])*.

Table 1

Antibacterial effect of SeNPs on bacteria generating nosocomial infections /
Efectul antibacterian al SeNPs asupra bacteriilor generatoare de infecții nosocomiale

Tested bacteria strains	Parameter	Results	Ref.
Gram positive: • **Staphylococcus aureus** • **Bacillus subtilis** **Gram negative:** • **Escherichia coli** • **Pseudomonas aeruginosa** • **Salmonella typhymurium** • **Klebsiella pneumoniae**	Area of inhibition	Largest areas of inhibition for: • Staphylococcus aureus • Escherichia coli • Pseudomonas aeruginosa • Salmonella typhymurium	37
Staphylococcus aureus	Living bacteria	7.8 µg/mL SeNPs reduced living bacteria with 40% after 3 h	38
Staphylococcus aureus	Complete inhibition	5 µg/mL SeNPs – complete inhibition 23.7 µg/mL SeNPs – complete inhibition during 24 h	39
Biofilm-associated pathogens **Gram positive:** • **Staphylococcus aureus** • **Bacillus cereus** • **Enterococcus faecalis**	Biofilm eradication	75 µg/mL SeNPs partially removed the existing biofilm	42
Gram negative: • **Escherichia coli** • **Salmonella enterica (2 serovars)**		60 µg/mL SeNPs completely eradicated E. coli biofilm and eradicated most part of P. aeruginosa and S. aureus biofilms	43

silver and silver compounds were unable to exhibit a satisfactory antibacterial effect, SeNPs were tested. The results suggest that SeNPs-coated PVC is a suitable and safe material for medical devices [40, 41].

3.3.2. Effect on nosocomial infection

Also, a selection of nosocomial infection caused by Gram negative and Gram positive strains were considered for testing the antibacterial effect of SeNPs. The results qualified SeNPs as an effective antimicrobial agent against life threatening microbes [37]. Afterwards, it was shown that SeNPs induced a decrease in the *S. aureus* living bacteria percentage, besides growth inhibition [38]. When compared to silver phosphate nanoparticles (SPNPs), an equivalent antibacterial activity was reported for 18-21 nm sized SPNPs and 50-100 nm sized SeNPs. It was proved the inverse dependency between the SeNPs antibacterial activity effectiveness and their particles size, while SPNPs exhibited antimicrobial effect even when particles are hundreds nanometers [39].

Food can also be a vector for pathogenic microbial agents, and severe infections appear after contaminated aliments ingestion. Certain foodborne strains were studied for the impact of SeNPs on each of them and their biofilms. The antimicrobial efficacy of antibiotics or sanitizers is strongly diminished for biofilm-associated pathogens. The SeNPs active levels for each of the above-mentioned assays were not connected to any significant toxicological impact on *Artemia* larvae [42] Nevertheless, biofilm removing capacity is controversial, as figured in Table 1 [43].

3.4. Antiviral

SeNPs were less investigated for antiviral effect. However, antiviral activity was tested on vero cloned cell lines of type-1 Dengue virus. The enhanced inhibitory effect on the viral growth along with increasing Se concentration, and the maximum was recorded for 700 ppm SeNPs [26].

3.5. Antifungal

In 2015, SeNPs have tested for ***antifungal*** properties against *Candida albicans* and *A. niger*. Biosynthesised SeNPs spherical particles of 50-200 nm size revealed a good antifungal activity, especially on *C. albicans* [44]. *C. albicans* along with another *Aspergillus* strain, *fumigatus*, have undergone treatment with biogenic 80-220 nm sized SeNPs and antifungal activity was proved and this treatment was proposed as an alternative or complement for nystatin drug therapy [45].

Another example of premises towards systemic and also topical therapy are those issued by Kheradmand et al. [46] Biogenic SeNPs

synthetized from selenium dioxide were added to *Lactobacillus plantarum* and *L. johnsonii* cultures, and for the resulted enriched bacteria, antifungal activity of *Candida albicans* was tested. *C. albicans* cells were mixed with *Lactobacillus* strains, Se-enriched and Se-free. After 30 min, it was observed that about more than one hundred times *C. albicans* cells died in case of Se-enriched *Lactobacillus*, proving the synergistic antifungal effect of enriched SeNPs with the Lactobacillus strains [46]. The comparison of chemically produced SeNPs with the biogenic ones in what concerns their activity against *C. albicans* revealed that biogenic nanoparticles are more efficient inhibitors of *C. albicans* biofilm synthesis and are also able to disaggregate the mature exopolysaccharide matrix produced by this fungus. Yet, great minimal inhibitory concentration (MIC) values concluded that SeNPs have no growth inhibitory effect on *C. albicans* and *C. parapsilosis* strains. This increased antimicrobial potential of biogenic SeNPs was correlated with the presence of a bacterial protein layer that coated them [47].

Post antibiotic effect (PAE) test was performed for *C. albicans* and *A. niger* treated with SeNPs at sub-inhibitory concentrations, and no inhibitory effect was observed. Moreover, Se nanospheres considerably stimulated the growth of *A. niger*. This calls attention for taking precaution to limited exposure to sub-inhibitory concentration of SeNPs, in case of infections caused by these pathogens [48].

3.6. Chemoprevention and chemotherapy

Studies have suggested that SeNPs possess great selectivity between cancer and normal cells, regarding cytotoxicity and cell apoptosis. Thus, SeNPs display potential application in cancer chemoprevention and chemotherapy [49]. The mechanism mostly claimed for the effect of SeNPs against cancer cells is related to SeNPs antioxidant activity and will be discussed later. Also, Chen and co-workers [50]. proved that SeNPs induced a dose-dependent increase in depletion of electric mitochondrial membrane potential, inducing apoptosis in human melanoma cells through mitochondrial dysfunction.

Multiple types of cancer were targeted by combining chemical therapy using SeNPs capped with polyethylene glycol with X-radiotherapy. The synergistic activity is due to the responsive property of the nanosystem towards X-rays. DNA fragmentation and activation of caspase-3 were induced by the SeNPs-X-rays co-therapy, resulting in cell growth inhibition and cancer cell apoptosis. Cancer cells apoptosis was also the result of ROS overproduction, induced by SeNPs, causing mitochondria fragmentation [51]. One may notice this completely different behaviour of SeNPs on cancer cells and normal cells, in terms of ROS production. In cancer cells SeNPs induce a ROS overproduction, while in normal cells the ROS production is lowered.

X-ray therapy considerably suppresses immunity. When bone marrow (BM) injury occurs, white blood cells, red blood cells and platelets would decrease numerically. Orally administered SeNPs for 30 days revealed satisfactory properties of bone marrow restoration, as well as significant increase of lymphocytes and neutrophils. For more intense X-radiation, where 100 μg/day could not keep up with the damage rate, a different route of administration or dose were considered as further research proposals [52].

Prostate cancer already benefits of a SeNPs-based treatment. SeNPs prepared using crude peptone solutions induce prostate cancer cell apoptosis more efficient than the most potent Se organic form, methyseleninic acid. This novel drug is indicated to be used also in chemoprevention, not only in chemotherapy [53].

SeNPs are conjugated with folate (FA) to target folate receptor-overexpressing cancer cells. The FA-SeNPs increase the sensitivity of the cells and fight against multidrug resistance of R-HepG2 drug-resistance of hepatocellular carcinoma (HCC) cells. Therefore, ABC protein family expression is inhibited. These nanosystems get inside the cancer cell and lead to its apoptosis by initiating ROS overproduction. The low *in vivo* toxicity for healthy cells was once again demonstrated, which strengthens the identity of multidrug resistant cancer nanodrug of FA-SeNPs [54].

HepG2 cancer cell line was also the most sensitive to the cytotoxic capacity of SeNPs, when comparing to other cancer cell lines such as MCF-7, A549, Hela, etc. At 500 μg/mL SeNPs, only 27.7% of HepG2 cells survived, whilst normal cells were poorly affected [55]. Another *in vivo* assay on HCC in male albino rats revealed complex beneficial properties of SeNPs, such as significant ameliorative effect on liver enzymes AST and ALT. Remarkable decrease could be noticed in tumor markers α-fucosidase, α-fetoprotein and carcinoembryonic antigen serum levels. The effect of administering SeNPs, pre- or post induction of HCC, was correlated with the down-regulation of β-catenin, survivin and Ki-67 proteins. All these results underline the potent role that SeNPs could play in the retraction of HCC [56].

Hepatitis-B virus-infected liver cancer was retracted by a baicalin-SeNPs-FA nanosystem that induced apoptosis by targeting lysosomes, down-regulating ROS generation and inhibiting HBxAg expression. These particles were faster internalized by the infected cell line than by the healthy one, and also had the ability to inhibit cancer cell migration and invasion [57].

Great efficiency over human melanoma cell line was found when combining SeNPs with 5-fluorouracil (5-FU-SeNPs), Caspase-9 activation, breakdown of mitochondrial membrane potential

and ROS enhanced production were associated with SeNPs presence in the cancer cell. 5-FU-SeNPs acted very selectively on melanoma cells, without affecting normal cells [58].

Amino acid-functionalized SeNPs proved to be effective in inducing dose-dependent apoptosis, for example in breast adenocarcinoma cells. It seems that alkaline amino acids (such as lysine) conjugated SeNPs are more efficient as cancer cytotoxic agents. The proposed mechanism inside cancer cell is the same: ROS over-production - caspase activation - mitochondrial dysfunction [59] An immunomodulatory potential was outlined for biogenic SeNPs also for breast cancer in an *in vivo* study performed on mice. According to the study, when associated to 4T1 crude antigens, better results were seen compared to the antigen-alone at mice group. The improvement consisted of weigh gaining, tumor tissue necrosis and anti-metastatic effect, the decrease of TGF-β, but also an increase of IL2 [60].

The anticancer effect of SeNPs was also tested on head and neck squamous cell carcinoma line (HNSCC). It was compared to that on human dermal fibroblast (HDF) as regularly affected human cells during cancer therapy. The effect on the cancer cells was four times higher than that on HDF, the best benefit/risk ratio being obtained for

SeNPs in media in the range of 20 - 55 μg/mL; a slight toxicity was noticed against HNSCC and almost none for HDF [61]. Hence, the resulted PEG-coated SeNPs are a promising alternative for treating this type of carcinoma.

4. Antioxidant molecular mechanisms proposed for SeNPs

Basically, almost all the effects assigned to SeNPs were largely attributed to their antioxidant properties. In normal mammalian cells, they act like ROS scavengers and thus reduce the cellular oxidative stress, along with the levels of harmful

Table 2

Effects of selenium nanoparticles in oxidative stress studies / *Efectul nanoparticulelor de seleniu în stresul oxidativ*

Biologic material	Assayed parameters and observations	SeNPs concentration or dosage	Reference
In vitro			
HUVEC in normo- or hyperglicemic media	ROS level (fluorescecent probe); no fluorescence increases of the probe due to ROS in SeNPs treated cells, in both media	N/A	65
BSA, glucose	protein glycation ↓; glyoxal formation ↓; ROS scavenging ↑; α-dicarbonyl formation ↓	0.375 – 6 mg/mL	2
Aqueous media, Fe^{2+} + H_2O_2	Hidroxyl radical level ↓	55.3 μg/L	14
BABLC 3T3 Caco-2	ROS ↓; superoxide anion ↓; antioxidant activity (DPPH, ABTS tests)	50 - 500 μmol/L	62
Blood cells	superoxide anion ↓; antioxidant activity better than ascorbic acid and trolox (DPPH test)	0.5 to 5.0 μM (antiox. activity) 1-16 μM (superoxide anion)	49
In vivo			
Rats (Adult male Wistar, diabetic)	MDA ↓; NO ↓	0.1 mg of SeNPs/kgbw, p.o.	66
	GSH – lower rate of reduction		
	SOD, CAT, GPx, GR activity – stimulated by SeNPs, leading to recovery of their activity		
Rats (male adult albino)	MDA ↓; NO ↓; BCHE ↓; γ-GT ↓; DNA fragmentation ↓; GSH - lower rate of reduction	0.5 g/kgbw, i.p.	67
Rats (Wistar)	CAT ↓; PC ↓; GPx↓; SOD ↑	0.1 mg/kgbw, p.o.	68
Rats (Wistar)	CAT; GPx – restoration with consequent level increase; TBARS↑	250 μg/kgbw, p.o.	29
Rats (Wistar, male albino)	NOx ↓; TNF-α ↓; PGE_2 ↓; GSH ↓	2.55 mg/kgbw, p.o.	30
Mice (male, C57BL/6 J)	GPx ↑; MDA ↑; IL-6 ↓; iNOS ↓; TNF-α generation ↓; NF-kB expression ↓;	N/A	31
Rats (adult male, albino)	SOD ↑; CAT ↑; GPx ↑; MDA ↑; GPx↓	6.7 μg/kg bw	63
Mice (Kunming, male)	TrxR ↑; GST ↑	35 and 70 μg Se/kg bw, p.o.	69

Abbreviations: ↑ - increase; ↓ - decrease; kgbw - kg body weight, HUVEC - human umbilical vein endothelial cells, MDA = malondialdehyde, NO - nitric oxide, GSH - glutathione, SOD - superoxide dismutase, CAT - catalase, GPx - glutathione peroxidase, GR - glutathione reductase, BCHE - butyryl cholinesterase, γ- GT - gamma-glutaryltransferase, PC - protein carbonyls, NOx = total nitrite/nitrate, TNF-α: tumor necrosis factor-α, PGE_2 - prostaglandin E2,iNOS: inducible nitric oxide synthase, NF-κB: nuclear factor κ-B, GSE - grape seed extract, TrxR - thioredoxin reductase, GST- glutathione S-transferase, TBARS - thiobarbituric acid reactive species.

oxidation by-products [62, 63]. The opposite situation is in case of bacteria and cancer cells, bur the mechanism is still unclear, ROS production being hyper-stimulated by SeNPs. Consequently, mitochondria are damaged, and the cell dies.[59] Normal cells differ from cancerous cells because of the basal ROS level, much higher in the latter. So neoplastic cells are more vulnerable to subsequent oxidative stress as the capacity of their own antioxidant systems is easily overwhelmed. This vulnerability is a promising direction for designing novel anticancer agents [64].

Table 2 presents examples of studies considering the oxidative stress parameters and *in vitro* and *in vivo* effects of SeNPs.

Selenium becomes part of proteins mostly by selenocystein. Selenoproteins are important biomolecules due to their scavenging physiological oxidants [70]. Selenocysteine is required for the activity of antioxidant enzymes, such as GPx and TrxR. Another antioxidant role is played by metal ions coordination in the active sites of dehydrogenases and hydrogenases. GPx is the first selenoprotein identified and it interferes in the catalysis of hydrogen peroxide reduction [71]. GPx1 conserves cells against apoptotic/oxidant by scavenging the physiological H_2O_2 pool, and therefore loses its regulatory role on the transcription of its target genes including Bax (B-cell lymphoma 2 associated X Protein) [66]. Selenium inhibits apoptosis via its ability to modulate Bax/Bcl-2 (B-cell lymphoma 2) expressions, decreasing the ratio of Bax/Bcl-2 [66].

As for nucleic acid antioxidant properties of selenoproteins, 25 μM of selenocystine completely inhibit oxidative DNA damage caused by copper ions and hydrogen peroxide, while the same result is obtained with 1000 μM of selenomethionine [72]. The proposed mechanism, i.e. the ability of certain Se compounds to coordinate different metal ions causing DNA damage, is a quite novel, being called *metal binding*. Apart from copper, iron may also be stopped from altering the DNA structure and function in this way. Not all Se containing chemical structures have this capacity. For example, methylselenocystein, selenocysteine or the inorganic compound SeO_2 inhibit iron-mediated DNA damage, but selenide and selenate anions have no effect. A special case is the selenite, which exhibits antioxidant or pro-oxidant effect, depending on the hydrogen peroxide level in the metal ions-H_2O_2 mixture, so its mechanism of action is not related to metal binding [73].

Selenium compounds also could influence the immune cells. It was noticed that N-acetylcysteine can compensate Se-protein deficiency in T cells and reverse T cell receptor-induced proliferation, so the effect is due to again the antioxidant capacity. By supplementing the medication with 200 μg Se for immunocompromised patients with viral infections, a delay in viral replication was observed, as well as an enhancement of the host defence [70].

SeNPs have an opposite behaviour on cancerous cells than on normal cells, which is partly owed to their different basal oxidative stress level and metabolism [64]. Not all specific mechanisms for the anticancer effect of SeNPs have been elucidated yet, but observations from certain studies are available. For example, the inhibition of prostate cancer cells growth is managed by kinases and caspases modulation.

Other Se compounds act by caspase regulation as well, such as the promotion of caspase-3 in tongue cancer Tca8113. The level of ROS can be controlled in order to achieve anticancer effect. It is supposed that selenium dioxide and sodium selenite induced apoptosis in HSC-3 (human oral squamous carcinoma) cells by influencing the glutathione system. Thioredoxin-1 is another important anticancer drug target, as it is involved in carcinogenesis and cancer evolution, and methyseleninic acid was associated with low thioredoxin-1 levels. Also, p53 protein is involved in the cancer cell apoptosis induced by Se compounds. Thus, seleniumcysteine caused MCF-7 human breast carcinoma cells though multiple pathways, including p53 phosphorylation and ROS generation [64].

4. Toxicity

In order to consider SeNPs as potential antibiotic, anti-inflammatory or other type of drug, their safety had to be investigated. Unfortunately, limited toxicity tests were performed on *in vitro* and *in vivo* models, not always performed according to the OECD guidelines.

Chemically synthesized (SeNPs C), small and large, were compared to biologically synthesized ones (SeNPs B), by monitoring zebra fish embryo hatching and embryo mortality. The hatching rate for 5 mg/mL decreased after 72h as shown in Figure 3. Embryo mortality was obvious at concentrations above 5 mg/mL and the mortality rate increased in the same order hatching rate decreased [74].

While administering orally for 14 days at male mice, biogenic SeNPs resulted to be less toxic than the chemogenic type, and 26-fold less toxic than selenite [75].

SeNPs were compared with LactoMicroSe (selenium-enriched yoghurt powder, with >95% nanoSe and less than 5% organic Se) and SelPlex (a natural selenium source consisting mostly of biologically produced selenium containing aminoacids). Among these, the nanoparticles were the most toxic regarding subacute toxicity in mice. By the same criteria, SeNPs confirmed their lower toxicity towards inorganic Se (selenite and selenate). Subacute toxicity was assayed through a repeated dose study for 14 days. Three Se

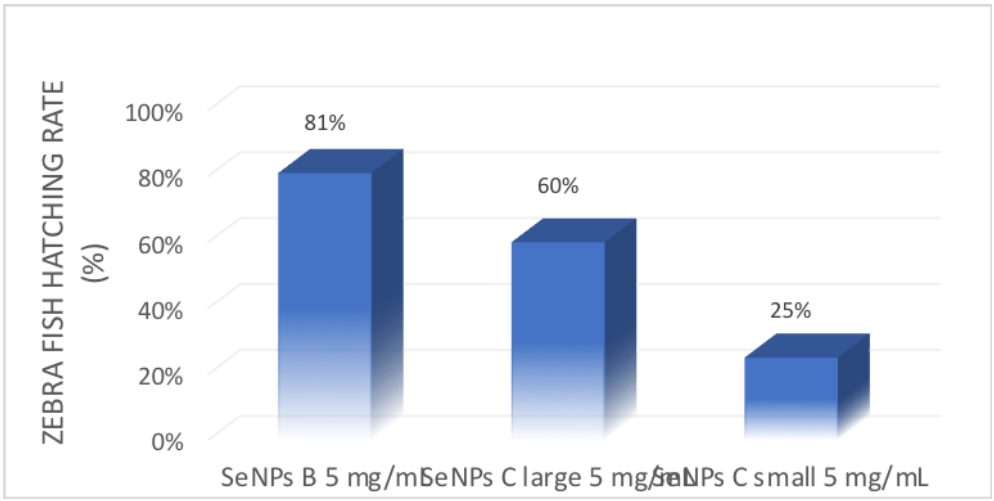

Fig. 3 - Effect of SeNPs B, SeNPs C large and SeNPs C small over zebra fish hatching [74] / *Efectul SeNPs B, SeNPs C mari și SeNPs C mici asupra eclozării la peștele zebra [74]* .

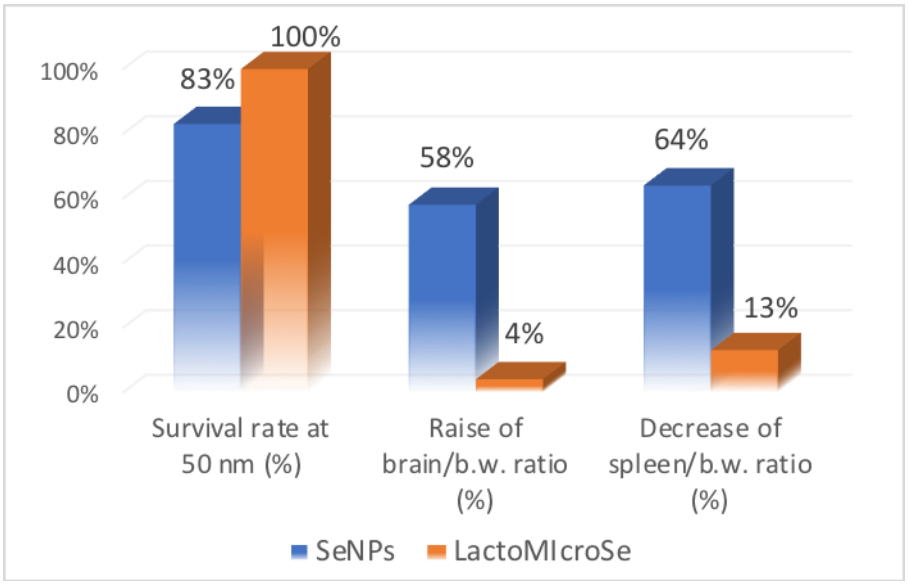

Fig. 4 - Comparison between the toxicity of SeNPs and LacroMicroSe / *Comparație între toxicitatea SeNPs și LactoMicroSe* [76].

concentrations, 0.5, 5 and 50 ppm, of each Se compound, were tested on mice. Survival rate for 50 ppm was lowered for the SeNPs treated mice, while no death occurred for the animals that received SelPlex and LactoMicroSe. As expected, organ toxicity was the highest at 50 ppm Se for all tested compounds. Major distinction could be noticed for the spleen/ b.w. ratio and brain/b.w. ratio (Figure 4). Selenate and selenite provided less spleen damage than SeNPs. There were also different changes in the bone marrow cell number, as SeNPs decreased it by less than half, an average value between the inorganic Se (almost total damage) and LactoMicroSe [76].

Larger SeNPs proved to be more toxic than smaller ones, therefore being more effective as chemopreventive agents, as they accumulate better. But, comparing the nano-sized particles to frequently used Se compounds, the toxicity of SeNPs was lower than that of selenomethionine, and much lower compared to selenite [76].

In vivo studies also illustrated that supra-nutritional levels of nano Se are not toxic and might be appropriate for cancer chemoprevention. Positive influence on the health of male rats was acquired with orally administered 0.2 mg SeNPs/kg b.w., whereas more than 2 mg SeNPs/kg b.w. p.o. induced toxicity. Animals were treated with SeNPs in a repeated-dose during 14 days. The body weight decreased comparing to control only at dosage of 2 mg SeNPs/kg b.w. [77]

As a consequence, there are several aspects that may be taken into account when SeNPs are proposed as a therapeutic solution for different diseases. First, biogenic SeNPs seem to be less toxic than chemically produced ones. Secondly, as far as the literature relates, smaller nanoparticles are more easily accumulated inside

the cell, so particle dimension plays a key role in therapy and toxicity, beside concentration/dosage. Also the synthesis method is considered a source of toxicity. Selenium in the form of nano-sized particles is less toxic comparing to inorganic selenium. The differences vary from *in vitro* to *in vivo* studies, as well as for each of the assays performed for evaluating damage/toxicity. Complex metallic-organic compounds integrating selenium were developed [78] exhibiting a less toxic profile and even enhanced positive effects compared to the particles alone.

In what concerns immunogenicity, SeNPs administration in male Wistar rats enhanced the humoral immune response. A concentration of 150 ppb SeNPs enhanced the animals' immune status better in comparison to 300 ppb SeNPs or 150 and 300 ppb sodium selenite. At this concentration, the mean serum globulin effect was improved, and the serum albumin/globulin ratio was reduced [7]9

Regarding the immunity parameters involved in oxidative stress processes, SeNPs coated with sulphated polysaccharide from *Ganoderma lucidum* managed to inhibit NO production in macrophages. A down-regulation of mRNA gene expression was induced, with a dose-dependent decrease in inflammatory cytokines: inducible NO Synthase, IL-1 and TNF-α. [71]. The immune response against cancer cells was proven in breast tumour mice by increasing serum levels of Th1 cytokines, such as IFN-γ or IL-12. This was correlated with other endpoints, such as rate of survival and the delayed type hypersensitivity (DTH) response of the animals, both increased versus the control [60].

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