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In Vivo Study on the Biodistribution of Silica Particles in the Bodies of Rats

Badania *in vivo* dystrybucji kul krzemionkowych w organizmach szczurów

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Abstract

Background. Biodegradable carrier materials with nontoxic degradation products are very valuable for delivering drugs and biologically active molecules. Many organic systems (such as liposomes, micelles and polymeric nanoparticles) and inorganic systems (metal oxides and silica) have been researched for delivering active substances to organs. Silica seems to be one of the most interesting and promising materials.
Objectives. The aim of this study was to investigate the SiO_2 elimination process from rats' organisms and to ascertain the distribution and prospective accumulation sites of the silica particles.
Material and Methods. A suspension of silica particles (∅ 150 nm) in 0.9% NaCl solution was introduced into rats' circulatory system. The degradation of these particles over time and their accumulation in the heart, lungs, kidneys and liver were observed.
Results. It was found that 36% of the introduced silica particles were excreted with urine after four days. The remaining particles were accumulated in the kidneys and lungs, probably in the lung air sacs and kidney glomerulus.
Conclusions. Silica seems to be promising carrier material. Silica particles dissolve in the rat's body and are eliminated in urine (**Adv Clin Exp Med 2012, 21, 1, 13–18**).

Key words: rats, silica spherical particles, sol-gel method, drug carrier.

Streszczenie

Wprowadzenie. Obecnie trwają poszukiwania nośników leków, które po spełnieniu swojej funkcji transportowania substancji aktywnych rozłożą się w organizmie żywym do nietoksycznych produktów. Bada się układy opierające się na związkach organicznych (liposomy, micele, polimery) oraz tlenków metali i niemetali. Interesującym materiałem wydaje się krzemionka.
Cel pracy. Określenie stopnia eliminacji kul krzemionkowych z organizmów szczurów oraz określenie miejsc ich ewentualnej akumulacji w organach zwierzęcia.
Materiał i metody. Zawiesina kul krzemionkowych (∅ 150 nm) w roztworze soli fizjologicznej została wprowadzona do układu krwionośnego szczurów przez iniekcję. Badano degradację kul krzemionkowych w czasie (96 h), a następnie ich akumulację w głównych organach zwierząt: sercach, płucach, nerkach i wątrobach.
Wyniki. 36% wprowadzonych kul krzemionkowych zostało wydalonych z moczem w ciągu 4 dni. Kule, które nie rozpuściły się i nie zostały wydalone znaleziono w nerkach i płucach. Jest to spowodowane agregacją kul i ich zatrzymaniem w małych strukturach, takich jak pęcherzyki płucne i kłębuszki nerkowe.
Wnioski. Kule krzemionkowe są obiecującym materiałem do transportu substancji aktywnych. Materiał ten rozpuszcza się wewnątrz organizmów szczurów i jest wydalany wraz z moczem (**Adv Clin Exp Med 2012, 21, 1, 13–18**).

Słowa kluczowe: szczury, kule krzemionkowe, metoda zol–żel, nośnik leków.

Conventional forms of drug administration like tablets or bolus injections are not always optimal for effective treatment. Similarly, conventional cancer treatments such as surgery, radiation and chemotherapy are limited by the accessibility of the tumor. Chemotherapy as a cancer treatment is restricted by a lack of selectivity toward tumor cells and often causes severe side effects in healthy tissues. Various new drug delivery systems have been proposed recently. Among the materials researched as carriers are liposomes, micelles, polymeric nanoparticles, metal oxides and spherical silica particles. The chemical and mechanical stability, hydrophilicity and biocompatibility of silicon dioxide particles are properties that make these particles very promising as a new universal drug delivery system [1, 2]. Another quality that makes silica especially interesting is the possibility to tailor the particles' surface reactivity and electrical surface potential (zeta potential) through surface modification [3]. Separated silica particles have a negative charge of nearly –40.5 mV at neutral pH [4]. The surface charge of the carrier plays an important role during endocytosis [5]. Negatively charged particles are repelled by the negatively charged cell membrane, so these particles are gradually detected and rejected, and this helps to avoid the particles being eliminated from the body. It is possible to modify the silica surface by functional groups (amines, thiols, etc.) that can react with corresponding functional groups of enzymes, proteins, DNA and pharmaceutical substances [6, 7]. Amorphous silica, in contrast to crystalline silica, is a nontoxic and highly biocompatible material that causes no adverse tissue reactions. Silica is bioresorbed via hydrolysis of siloxane bonds into $Si(OH)_4$, which diffuses into the blood and lymph system and is excreted through the kidneys [8].

All these properties mean that silica seems to be one of the most interesting and promising materials for delivering active substances to selected body organs. The aim of this study was to investigate the SiO_2 elimination process from the organisms of rats and to find the distribution and prospective accumulation sites of silica particles. The main organs (heart, lungs, kidneys and liver) of the rat's organism were studied. The possible application of silica nanospheres as a new drug delivery system was demonstrated.

Material and Methods

Silica Particles in Rats' Organisms

Silica particles were obtained by the sol-gel process. The sol-gel technique and synthesis of silica particles were described in our previous work [9]. All experiments were approved by the First Local Ethical Committee for Experiments on Animals at the Institute of Immunology and Experimental Therapy in Wrocław, Poland.

Two groups of male Wistar rats were placed in special metabolic cages and were analyzed. Rats from the first (research) group (5 individuals) were injected with 0.5 mg of SiO_2 particles suspended in 1 ml of 0.9% NaCl solution. Rats from the second (control) group (5 individuals) were injected with 1 ml of 0.9% NaCl solution. The suspension of silica particles in NaCl solution was introduced through the vein in the rats' tails. The injections were administered once, at the beginning of the observation period. Rat urine samples were analyzed every 12 hours for four days.

After the observation of the silica-particle elimination process (96 hours), the animals' main organs were collected. The hearts, lungs, kidneys and livers were investigated in order to find silica-particle accumulation sites. The average amounts of SiO_2 in these organs were calculated.

Characterization

The morphology and size of the spherical silica particles were determined by transmission electron microscopy (TEM, TESLA BS 500). The level of Si in the urine and organs was measured using atomic absorption spectrometry (AAS, Perkin-Elmer 3110 AAS spectrometer, flame: NO_2/C_2H_4), and the amount of SiO_2 was then assessed. Two-ml urine samples were digested with 3 ml of HNO_3 (Instra-Analyzed for Trace Element Analysis, J.T. Baker, USA) and 0.5 ml of HF (Suprapur, Merck, Germany) in a microwave unit (UniClever II, Plazmatronika, Poland), in accordance with Plazmatronika's recommendations. The digested urine samples were diluted with 10 ml of 4% H_3BO_3. The Si stock standard solution (1 g/ml as $(NH_4)_2SiF_6$ in water) was obtained from CPI International, USA. The working standards were prepared in water in the concentration range of 1–20 mg Si/l. The recovery of Si from samples subjected to prior digestion in a microwave oven (50 mg Si/2 ml of urine) ranged from 98 to 105%. The AAS measurements were performed in conditions recommended for the 3110 AAS apparatus by the manufacturer.

Results and Discussion

Figure 1 presents the TEM picture of the silica particles used in the study. The particles have a spherical shape and are monodispersed. The diameters of the particles are in the 50–200 nm range,

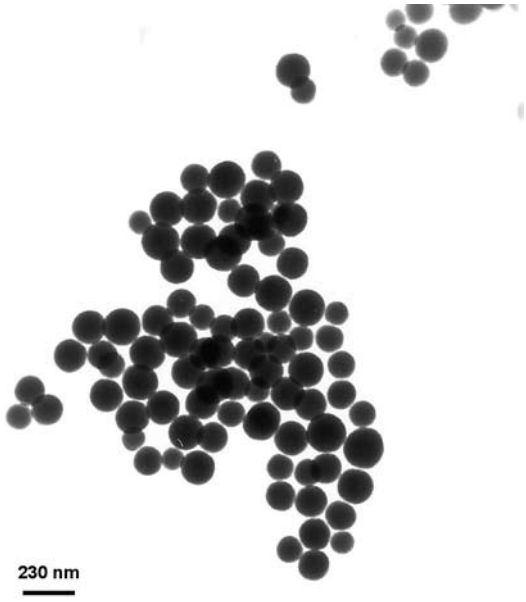

Fig. 1. TEM micrographs of silica particles obtained by the sol-gel method

Ryc. 1. Zdjęcie TEM kul krzemionkowych otrzymanych metodą zol–żel

with a dominant size of 150 nm (Figure 2). Figure 3 presents the levels of Si eliminated from the rats' bodies, determined from rat urine samples during the experiment. After the first 12 hours, approximately 6 μg (1.2%) of the injected silica had been eliminated from the organisms of the research rats. The amount of eliminated silica successively increased to 11, 18 and 31 μg after 24, 36 and 48 hours respectively. The highest amount of silica was eliminated during the third day: 72 μg, which constituted 14.4% of the introduced silica particles. After 96 hours, 181 μg (36%) of the injected silica particles had been eliminated from the rats' bodies in urine. In addition, it was observed that more silica particles were eliminated at night (after 24, 48, 72 and 96 hours) than during the day (after 12, 36, 60 and 84 hours). It can be assumed that the reason for the more intense elimination of SiO_2 at night is the rats' lifestyle: Rats are mainly active at night.

Since silicon is an essential trace mineral and particularly high levels of it are found in food derived from plants and in drinking water, it was

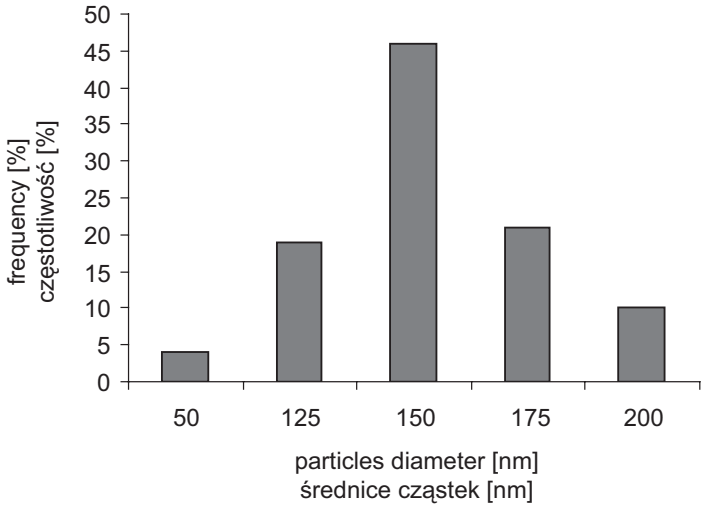

Fig. 2. Size distribution of silica particles

Ryc. 2. Rozkład wielkości kul krzemionkowych

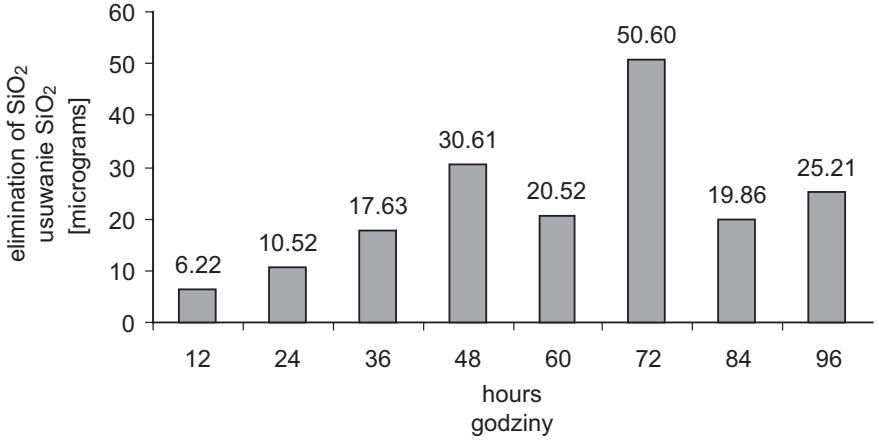

Fig. 3. Elimination of SiO_2 over time

Ryc. 3. Eliminacja kul SiO_2 w czasie

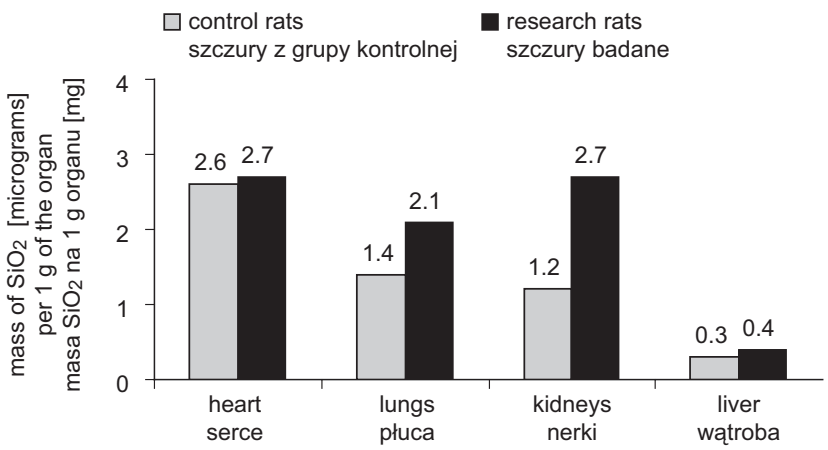

Fig. 4. SiO_2 particle contents in μg per 1 g of organ

Ryc. 4. Zawartość kul SiO_2 (μg) na 1 g organu

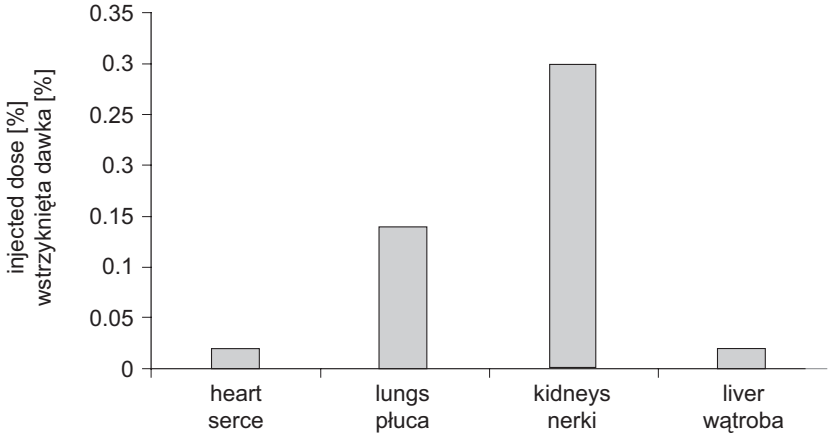

Fig. 5. *In vivo* distribution of SiO_2 in rats 96 h after injection

Ryc. 5. Zawartość SiO_2 w organizmach szczurzych w czasie 96 h po iniekcji

necessary to test the group of control rats. Approximately 50 μg of silica was found in urine samples taken from the control rats after the first 24 hours of the study, and similar results were obtained after the subsequent 24 hours. For this reason 50 μg of silica was taken as a correction value when analyzing the urine samples taken from the research rats.

There are only a few papers describing in vivo experiments performed on animals injected with silica particles. Kortesuo et al. [10] described how the synthesis parameters of sol-gel processed silica microparticles affect the release rate of dexmedetomidine, and also determined the in vitro degradation of the silica gel matrix. They observed that the degradation of the matrix decreased as the water/tetraethyl orthosilicate (TEOS) ratio increased: During a 30-hour dissolution period the degree of matrix degradation changed from 20% to 0.25% when the water /TEOS ratio was 6 and 35 respectively [10]. He et al. [11] investigated a mouse model of biodistribution and urinary excretion of various surface-modified silica nanoparticles. Silica particles with the diameter of 45 nm, surface-modified with hydroxyl groups, carboxyl groups and polyethylene glycol were injected into the tail veins of mince. The authors observed that all the different types of injected silica particles were cleared from systemic blood circulation and partially excreted through the renal excretion route; significant distribution of the particles was observed in the liver, urinary bladder and kidneys [11]. A few papers present in vivo results of silica implants introduced into animals' bodies [12–14]. Silica xerogel implant disks introduced into both sides of mouse backbones showed a 24% weight loss after 14 days and a 55% weight loss after 28 days [12]. Bioactive glass granules (750 mg) loaded directly into proximal tibiae sites in rabbits were totally removed after 24 weeks [13]. Generally, the rate of solubility depends on the surface and morphology of the silica material, the implant location and the species of animal used for the experiments. The authors of the current study found no reports of abnormal inflammation or other adverse effects in any of the available literature on in vivo experiments based on silica implants. These results confirm the biocompatibility of silica materials, but one important aspect has to be taken into consideration. The fundamental difference between *in vivo* experiments performed with silica implants and those performed with silica particles lies in the manner and place where silica materials are loaded into the animal's body. Implants are situated in

a fixed place in the organism, and only compounds formed during its dissolution can get into the blood. Silica particles are directly introduced into a vein and circulate through the blood vessels to all parts of the organism, where they are dissolved.

As mentioned above, in the current experiment after 96 hours only 36% of the introduced silica particles had been eliminated from the rats' organisms; the remaining particles were accumulated in the rats' bodies. In order to find the remaining quantities of the introduced silica particles and accumulation sites, the hearts, lungs, kidneys and livers from the research rats and the control rats were analyzed. The silica particle content observed in these organs are presented in Figure 4. The results clearly show that the amount of SiO_2 particles accumulated in hearts and livers collected from the research group are almost the same as in the heart and livers collected from the control group. Therefore, it is reasonable to conclude that silica particles do not accumulate in livers and hearts. Hence, lungs and kidneys are the organs were the most intensive accumulation of SiO_2 particles take place. The differences between the mass of SiO_2 accumulated in the lungs and kidneys collected from the research and control groups rats were significant: a difference of 0.7 μg SiO_2 per 1 g of lungs and 1.5 μg of SiO_2 per 1 g of kidneys. It can be assumed that SiO_2 particles were trapped in the lungs air sacs and kidney glomerulus. In order to find other possible sites of silica accumulation, samples of the rats' fat were taken. Approximately 5 μg of SiO_2 per gram of fat was ascertained in both the research rats and the control rats.

The findings of the current study indicate that after 96 hours less than 1% of the injected dose of silica had accumulated in the main organs of the rats. The details of its distribution are presented in Figure 5. It is justifiable to assume that the residual amount of SiO_2 must be localized in parts of the animals' bodies that were not researched, such as the spleen, blood, bone, skin and/or muscles. The rats' feces ought to be taken into account as well.

Similar preliminary *in vivo* experiments on silica accumulation within rats' organs were presented by C. Barbé et al. [2], who found that after 48 hours only trace quantities (about 1%) of the injected silica particles were trapped within the lungs, while a significant amount of the particles was trapped in the liver (5% of the injected dose). The amount of silica accumulated in all the investigated organs decreased over time through normal excretion, as the amount of SiO_2 accumulated in urine and feces increased. Kumar et al. reported on "the use of multimodal organically modified silica (ORMOSIL) nanoparticles for in vivo bioimaging, biodistribution, clearance, and toxicity studies" [15]. Those authors observed that 75% of the injected dose of the ORMOSIL nanoparticles (20–25 nm) were accumulated in the liver and spleen, whereas less than 5% of the dose were found in the lung, kidney and heart, and after 15 days hepatobiliary excretion of the particles was observed without any signs of organ toxicity [15]. Huang et al. presented "the effects of particle shape on biodistribution, clearance and biocompatibility *in vivo*" [16], and observed that rod-shaped mesoporous silica nanoparticles (MSNs) intravenously administered to mice are mainly (80%) presented in the liver, spleen and lung. Additionally, they found that "there [are] obvious particle shape effects on in vivo behaviors. Short-rod MSNs [185 nm long] are easily trapped in the liver, while long-rod MSNs [720 nm] distribute in the spleen" and that "short-rod MSNs have a more rapid clearance rate than long-rod MSNs" [16] Thus, Huang et al. clearly showed that the interplay between biological effects and particle shape and size constitutes a very important aspect in strategies for therapeutic applications of silica nanoparticles.

Trying to compare the results of different *in vivo* experiments on potential silica drug-delivery systems, some important features have to be considered. The size of the particles used during the experiments is one of them. The smallest diameter of capillary in the body is 4 μm, and silica particles have to be enough small to be transported through the vascular system. As Barbé et al. noted: "Particles smaller than 50 nm can pass through small intercellular openings in normal blood-vessel walls", while "above 300 nm a significant proportion of the silica particles are trapped in the lungs and liver" [2]. It can be deduced that the silica particles described in the current paper are of an optimal size (150 nm in diameter).

The charge and functional groups on silica particle surfaces are also important features of potential silica drug delivery systems. The hydroxyl groups that pure silica particles have on their surface make the particle surface negatively charged, which causes the particles to repel one another and thus prevents aggregation.

The current authors reported previously that blood vessels are not blocked by aggregated SiO_2 particles with a diameter of 150 nm; similarly, neither inflammatory sites, histological changes nor other side effects in the animal tissue were observed [9]. Thus, the current in vivo data, along with the previously published data, suggest that silica particles have great potential as carriers for the delivery of drugs and other molecules.

The current study shows that spherical silica particles with an average diameter of 150 nm were degradable in the rats' bodies and were gradually

excreted through the kidneys during the experimental timeframe (36% of the introduced amount in 96 hours). Some amount of the introduced silica particles was observed in the lungs and kidneys. It seems probable that during a longer experimental time those particles would also be degraded. Silica seems to be a promising carrier material, but further experiments on animals are needed in order to investigate the pharmacokinetic characteristics of such particles.

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