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In vivo toxicity study of quatro stimuli nanocontainers in pregnant rats: Gestation, parturition and offspring evaluation

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ABSTRACT

The aim of the present study was to investigate the impact of intravenous administration of newly fabricated nanocontainers (NCs) on the last third of pregnancy in rats. Fifteen pregnant 3-month-old Wistar rats were separated into 3 groups. On the 15th and 17th day of pregnancy all animals received an intravenous administration of 1 ml of 15 mg of NCs (Group A), 1 ml of 5 mg NCs (Group B) while Control group received 1 ml of 0.9% NaCl. On the 14th and 17th of pregnancy ultrasonography was performed and the parameters evaluated were the width of placenta, the length and width of the embryonic sac, the foetus length and the heart rate. On parturition the number of pups per dam was evaluated. Half of the pups were euthanised the day after parturition and their liver and kidney was histologically evaluated and for the rest of the pups the body growth curve was evaluated until the age of 14 week. At the end of the 14th week the remaining pups were euthanised and their liver and kidney was histologically evaluated. At weaning the dams were euthanised and their liver and kidney was histologically evaluated. Ultrasonography: Baseline measurements of the width of placenta, the length and width of embryonic sac, the foetus length and the heart rate on the 14th day of pregnancy, revealed no statistical significant differences between groups. Comparison of the same values on the 17th day of pregnancy after 2 intravenous administrations of NCs showed no statistical significant effect on the respective parameters. The administration of NCs had no impact on the mean number of pups per dam. Additionally, no impact of the NCs on the body weights of the pups was observed on the 1st day after parturition. Moreover, comparisons between groups, for both sexes showed no difference on growth rate. During the histological evaluation no inflammatory, degenerative or neoplastic lesions were observed as far as the newborn, adult offspring and dams were concerned. According to our results no toxic impact of the low and high doses of the NCs was observed on the parameters selected to be evaluated.

1. Introduction

A quest for the ideal pharmaceutical substance, which will be able to treat successfully the pathologic entity is meant for, without exerting any adverse effect on healthy cells or tissues is as old as modern medicine. Many diseases in western societies require long term therapies, especially those of degenerative and neoplastic origin. Long treatments are associated with adverse effects, varying from mild to severe, and often reluctance of patients to further discipline on their schedule (Maningat et al., 2013; Sabaté; 2003). This is mainly true for cancer regimens, where adverse effects are closely related to their mechanism of action, which is the insult of rapidly multiplying cells. Although this is an intrinsic characteristic of neoplastic cells, they are not the only ones in living organisms possessing it (Chan et al., 2012). Bone marrow, digestive and integumentary system, are all structured by fast multiplying cells and are severely affected by the systemically administered antineoplastic therapies. Developing carriers which will enable therapeutic regimens to be delivered to target cells or tissues and released after specific internal or external stimuli is a new approach of quantitatively and selectively delivery of drugs.

In a previous study we have investigated synthesis, characterization, evaluation of their colloidal properties in simulated body fluids (SBF), as well as their *in vivo* biological properties (biodistribution and pharmacokinetics) of multi stimuli responsive NCS@P(MMA-*co*-DMAEMA-*co*-DVB-*co*-PEG360-*co*-AA-*co*-DS) (NCs) in adult animals (Efthimiadou et al., 2017; Tapeinos et al., 2016). Concerns arose about their impact during pregnancy. Under the term nanomaterials a wide variety of diverse small sized object (< 100 nm) is described. Except from efficacy,

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safety is a prerequisite property of materials expected to serve as drug carriers. According to literature there are only few examples about how nanostructured materials may cause toxic phenomena during pregnancy, to the dams and pups. A detailed understanding of the effects of nanoparticles on pregnant animals remains elusive. For example silica and titanium dioxide nanoparticles with diameters of 70 nm and 35 nm respectively, have been already studied, and found to cause complications when injected intravenously into pregnant mice (Yamashita et al., 2011), while other studies fail to detect any adverse effect of nanoparticles on pregnancy. The aim of the present study was to investigate the potential impact of NCs *per se* on pregnancy, embryo development and the development of offspring till adulthood on the rat animal model.

2. Materials and methods

2.1. Ethical statement

The study was performed in the animal facility of the Centre for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens and was evaluated and authorized by the Veterinary Service of the Prefecture of Athens, as mandated by Greek legal requirements for animal experimentation. The facility is registered as a "breeding" and "experimental" facility according to Greek Presidential Decree 160/91, which harmonises national legislation with European Community Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes.

2.2. Housing (food, water and bedding)

All cages were kept in the same animal room with a HEPA-filtered air supply, 15 ACH, at a room temperature of 24 ± 2 °C, relative humidity of 55 ± 10%, 12 h:12 h light/dark cycle (0700/1900), light intensity of 300 lx, as measured 1 m above the floor in the middle of the room, and positive air pressure of 0.6 Pa within the room. All animals were free of a wide range of pathogens including Kilham rat virus, rat parvovirus, Toolan's H-1virus, Sendai virus, pneumonia virus of mice, reovirus type III, murine encephalomyelitis virus, sialodacryoadenitis virus, rat min virus, Hantaan virus, lymphocytic choriomeningitis virus, CAR bacillus, MAD 1 and 2, rat rotavirus, rat coronavirus, Mycoplasma pulmonis, Clostridium piliforme, Bordetella bronchiseptica, Pasteurella spp, fur mites and pinworms. All Wistar rats had ad libitum access to filtered tap water in drinking bottles and pelleted chow, which contained 18.5% protein, 5.5% fat, 4.5% fiber, 6% ash (irradiated vacuum packed, 2918, Harlan, Italy). Each cage contained ≈140 g of corncob bedding (RehofixMK 2000, J. Rettenmaier & Soehne, Rosenberg, Germany). Cages were cleaned twice weekly.

2.3. Materials

Acrylic Acid (AA) and Dimethyl Amino Ethyl Methacrylate (DMAEMA) were purchased from Sigma Aldrich and distilled before their use. Divinyl Benzene (DVB) and Poly(ethylene glycol) methacrylate (average Mn = 360) (PEG-360) were also purchased from Aldrich and used as received. Methyl Methacrylate (MMA) which was purchased from Merck was freshly distilled before its use and Potassium persulfate (KPS) was purchased from Panreac and used as received. Ethylene Glycol (EG) provided by Merck, Iron (II) Chloride tetrahydrate ($FeCl_2$.$4H_2O$) provided by Riedel-de Haën, Potassium Nitrate (KNO_3) provided by Acros and Hexamethylenetetramine (HETM) provided by Alfa Aesar were used as received. 95° commercial ethanol was used as received. The synthesis of N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(2-methylacrylamide) (Disulfide) was previously described in detail.

2.4. Equipment

Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM) images were obtained on an FEI Inspect microscope operating at 25kV and a FEI CM20 microscope operating at 200kV, equipped with a Gatan GIF200 Energy Filter utilized for EF-TEM elemental mapping respectively. An ultrasonic bath was used for sonication (Elma Sonic, S 30H).

2.5. Synthesis of NCs

The synthesis of NCs consisted of two steps; in the first step the polymethacrylic methacrylate (PMMA) core was fabricated while during the second step the shell was created on the seed surface *via* emulsion polymerisation. The detailed synthetic procedure of hollow NCs has been previously presented (Efthimiadou et al., 2017; Tapeinos, 2014). Briefly, the PMMA core synthesis carried out by emulsion polymerisation, MMA (1.18 gr, 1 ml) was added into a 25 ml round flask filled with water (24 ml) and agitated under nitrogen atmosphere. Then a condenser was placed in the flask and temperature was raised at 90 °C. When temperature stabilised at 90 °C, the initiator (KPS) was added (20 mg, 0.5 ml aqua solution) in order for the polymerization process to be initiated. After the KPS addition the solution became cloudy which progressively turned into white. After 3 h, the polymerisation was completed, the white solid was collected by centrifugation (3 × 10000 rpm for 10 min each). Afterwards, the PMMA core (0.4 g) was suspended in a mixture of water/ethanol (90/10). The mixture was sonicated in an ultrasonic bath for 30 min at 55 °C and was agitated in a magnetic stirrer for 3.5 h at 55 °C, under nitrogen atmosphere. In order to fabricate the multi-stimuli shell, MMA (0.38 ml, 0.46 g), DMAEMA (0.104 ml, 0.0972 g), DVB (0.136 ml, 0.124 g), and PEG360 (0.041ml, 0.045 g), AA (0.043 ml, 0.0455g) were added dropwise and the mixture was agitated for 1 h. When temperature raised at 80 °C, 2 ml aqueous solution of the initiator KPS (32.5 mg) was added. After 10 min of agitation, 1 ml ethanolic solution of Disulfide (0.136 g) was added dropwise. The reaction was completed 18 h later and the product was isolated and purified by centrifugation (3 × 7000 rpm for 5 min each). During purification process NCs were washed and dispersed in distilled water. The dispersant was mixed with ethylene glucose and agitated for 40 min under nitrogen atmosphere. One ml of aqua solution $FeCl_2 \cdot 4H_2O$ (0.018 g), 1 ml of aqua solution of HETM (0.188 g) and 1ml KNO_3 (0.027 g) were added. The pH of the solution was adjusted at 9. The reaction completed and the final product was isolated by centrifugation (1 × 6000 rpm for 5 min and 2 × 5000 rpm for 5 min) (Fig. 1).

2.6. Study design and experimental procedures

A total of 15 pregnant female Wistar rats (HsdOla:WI) 3 months of age were studied. The animals were obtained from the breeding colony of the animal facility and were being used for the first time for reproduction. The animals were weighed before mating and vaginal smears were obtained in order to define their oestrous cycle. They were caged in pairs in H-Temp Polysulfone type III cages 425 mm long 266 mm wide 185 mm high (Tecniplast, Varese, Italy). On proestrous they were introduced into the cage of a male rat of proven fertility and remained until the day sperm was detected on their vaginal smear. All cytologic examinations were performed by the same person. The day on which sperm was detected, was designated as embryonic day 0 (D0) and they were allocated into 3 groups: group A high dose (n = 5), group B low dose (n = 5) and group C Control (n = 5). At 14th and 17thday post-coitum pregnant animals were evaluated ultrasonographically. The examination was performed with the animals being restrained in a supine position by an experienced person. Anaesthesia was avoided in order to exclude any complicating factor. Abdominal hair was clipped, in order to achieve full contact of the

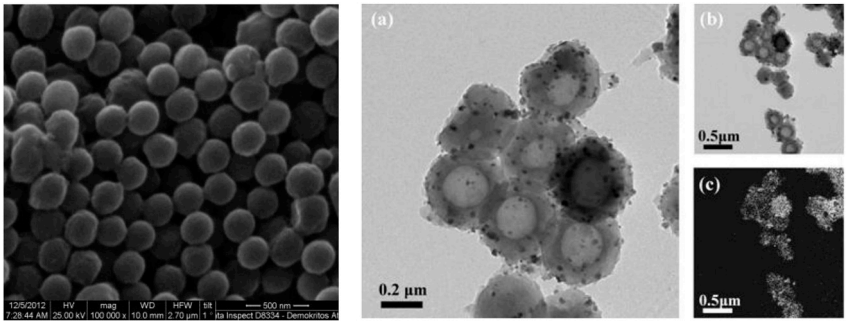

Fig. 1. A. Scan Electron Microscopy image of quarto stimuli NCs, B. a) Transmission Electron Microscopy image of, b) bright field micrograph used for sulfur map and c) EF-TEM sulfur elemental map.

probe with the skin in the region of interest and acoustic gel (Skintact, Leonard Lang, Innsbruck, Austria) was applied. In this way, the phenomenon of "grimy acoustic shadow" was reduced to a minimum and the resolution of ultrasound imaging was greatly improved. The equipment used was a Vivid I ultrasound machine (GE Medical Systems, Tirat Carmel, Israel) with the linear transducer probe 12 L-RS (GE Yokogawa Medical Systems, Tokyo, Japan), which has a variable frequency of 5–13 MHz. To achieve detailed imaging of the reproductive tract and embryos, a frequency of 13 MHz was applied, with one focal zone set at a depth of 0.5–2 cm. The total duration of the examination for each animal was approximately 10 min. Immediately after the end of each study the animals were returned to their cages. The 15thand 17th day of pregnancy an intravenous injection of NCs was administered by the coccygeal vein (after restraining the animals in a plastic restrainer (Rodent Restrainer MLA5022) and vasodilatation of their lateral coccygeal vein was achieved through immersion of their tail in lukewarm water). Group A was injected with 15 mg/ml of NCs (1 ml), Group B with 5 mg/ml (1 ml) while Control group received 1 ml of 0.9% saline. The intravenous administration was performed by the same experienced person.

2.7. Euthanasia, blood and tissue collection

Half of the pups were euthanised a day after their birth by decapitation and the liver and kidneys were harvested. The remaining pups were weighed twice weekly on a digital balance (DSW200D, DI Delmac Instruments, Athens, Greece) in order to evaluate their growth rate. The balance was precalibrated on each measurement by using known weights of 100, 5, and 1 g. Their gender was determined at day 10 and at the 28th day were distributed two in a cage according to their sex. Euthanasia was performed when the pups reached the age of three months. The dams were euthanised at the end of weaning. All the adult rats were euthanised by anaesthesia and exsanguination from the posterior vena cava. An autopsy was performed to examine for any macroscopic pathologic finding and the liver and kidneys were harvested for further histopathological evaluation. The organs were fixed in 10% formalin. Following fixation, the specimens were processed routinely, embedded in paraffin, sectioned at 3μm and stained with Hematoxylin–Eosin (H&E).

2.8. Statistical analysis

Data were analysed by using a statistical software program (SPSS version 16.0 for Windows, SPSS, Chicago, IL). The normality of the distribution was determined by the Kolmogorov–Smirnov and the Shapiro-Wilk tests. Comparisons of absolute values of the variables between the 3 groups were performed by using the one-way analysis of variance model (repeated measures test). When the distribution was normal, pairwise multiple comparisons were performed by using the Bonferroni test, while, if data presented non-normal distribution the Mann-Whitney test. Statistical significance was set at 5%. All data are presented as mean ± standard deviation (SD).

3. Results

3.1. Ultrasonographic evaluation

The parameters selected for ultrasonographic evaluation were the width of placenta, the length and width of embryonic sac, the foetus length and the heart rate. Baseline measurements carried out on the 14th day, revealed no statistical significant differences between groups in all parameters examined (Table 1). Additionally, comparison of the values on the 17th day of pregnancy revealed that the administration of the NCs had no statistical significant effect on the parameters under examination. The data are expressed in centimeters and beats per minute (Fig. 2 and Fig. 3).

3.2. Number of pups per litter and body weight measurements

The intravenous administration of the high and low dose NCs had no adverse effect on the mean number of pups per dam as shown in

Table 1
Parameters evaluated by ultrasonography.

	Placenta width (cm)			Embryonic sac length (cm)			Embryonic sac width (cm)			Fetus length (cm)			Heart rate (beats/min)		
	Mean	SD	p	Mean	SD	p	Mean	SD	p	Mean	SD	p	Mean	SD	p
14th Day															
Group A	0.51	0.05	0.07	1.16	0.11	0.49	0.87	0.03	0.52	0.83	0.07	0.58	228	7.15	0.26
Group B	0.43	0.04		1.05	0.17		0.93	0.12		0.81	0.08		238	9.78	
Control	0.48	0.05		1.06	0.14		0.83	0.10		0.75	0.12		233	6.83	
17th Day															
Group A	0.53	0.04	0.19	1.95	0.29	0.76*	1.18	0.17	0.83	1.50	0.38	0.82	230	11.27	0.57
Group B	0.56	0.03		1.78	0.26		1.16	0.07		1.54	0.23		238	9.06	
Control	0.62	0.04		1.96	0.43		1.09	0.06		1.59	0.34		243	9.82	

*Mann-Whitney test. The lowest p between groups is presented.

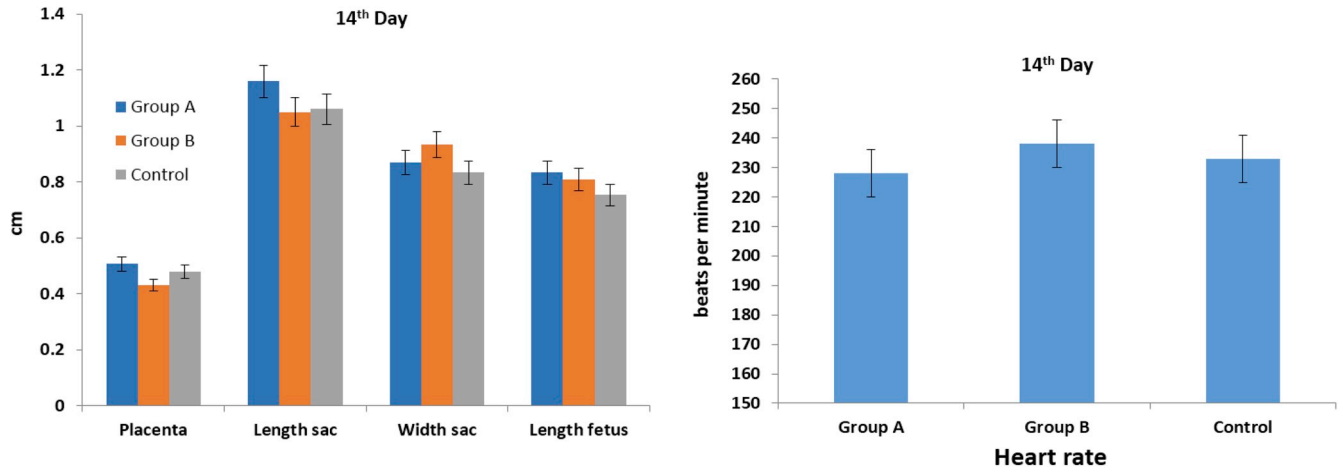

Fig. 2. Evaluation of parameters evaluated by ultrasonography on the 14th day.

Table 2. Furthermore, there was no obvious impact on the body weights of offspring. Body weight did not reveal any statistically significant difference between groups at the day of their birth. Additionally, multiple pairwise comparisons between groups for both sexes showed no statistically significant difference for every time point evaluated, while a noteworthy finding was that the respective growth rate curves were literally identical. Results are shown on Fig. 4.

Table 2
Mean number of pups per litter.

	Mean number of pups per litter		
	Mean	SD	p
Group A	10	1.07	0.069
Group B	8.8	0.79	
Control	10.6	1.58	

3.3. Histopathological evaluation of liver and kidney

Autopsy did not reveal any macroscopic pathological findings. Histological evaluation was based on examination of 3μm-thick, H&E sections, while the Periodic Acid-Schiff reaction (PAS) was performed when required. All histological specimens were thoroughly (i.e. throughout their area) examined, under the 4× and the 10× objectives, while greater magnification (20× or 40×) was used, if needed.

The administration of NCs in increasing dose did not impact on rat pups' liver or kidney microscopic appearance. In detail, liver specimens collected from rat pups of A (Fig. 5c) or B (Fig. 5b) group did not present altered cytoarchitecture when compared to the Control group (Fig. 5a). Hepatic cells appeared to have eosinophilic cytoplasm, euchromatinic nuclei, one or two prominent nucleoli and a nucleus:cytoplasm ratio that varied between 1:2 and 1:3. Their boundaries were hard to distinguish, while sometimes neighboring cells fused into a large syncytium. Furthermore, there were no inflammatory, degenerative or neoplastic lesions observed. Lobular architecture could not be observed, because of small liver size and decreased amount of interlobular connective tissue.

An interesting histological feature was the presence of hematopoietic cells (more prominent in Fig. 5c). This cell population resides inside the lumen of hepatic sinusoids and consists of many different subgroups leading to great variety. After blood islets first appearance in the embryonic yolk sac, hematopoietic stem cells migrate to other fetal organs like liver, so their presence is evaluated as absolutely normal.

Finally, a striking feature of many hepatic specimens (present also in dams and adult offspring rats) was the presence of cytoplasmic vacuoles in all three experimental groups (A, B and Control, best shown in Fig. 5a and c). Cytoplasmic vacuolation differential diagnosis includes a wide variety of pathological conditions like toxicity induced hydropic degeneration (aka cloudy swelling), fatty change, glycogen accumulation etc. However positive Periodic Acid-Schiff (PAS) reaction in our specimens (Fig. 5d) demonstrated that cytoplasmic vacuoles

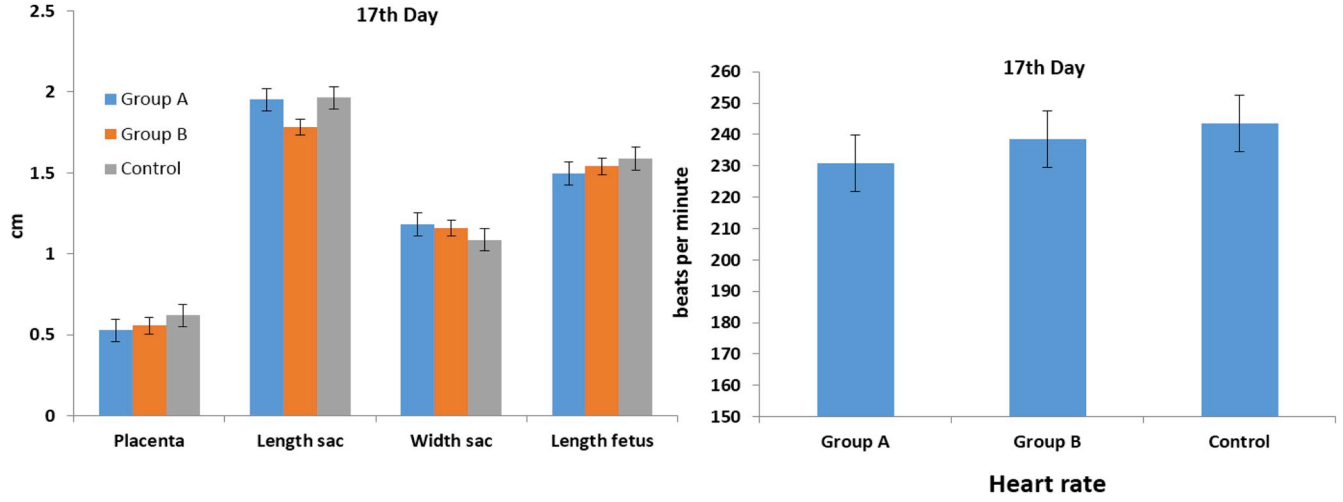

Fig. 3. Evaluation of parameters evaluated by ultrasonography on the 17th day.

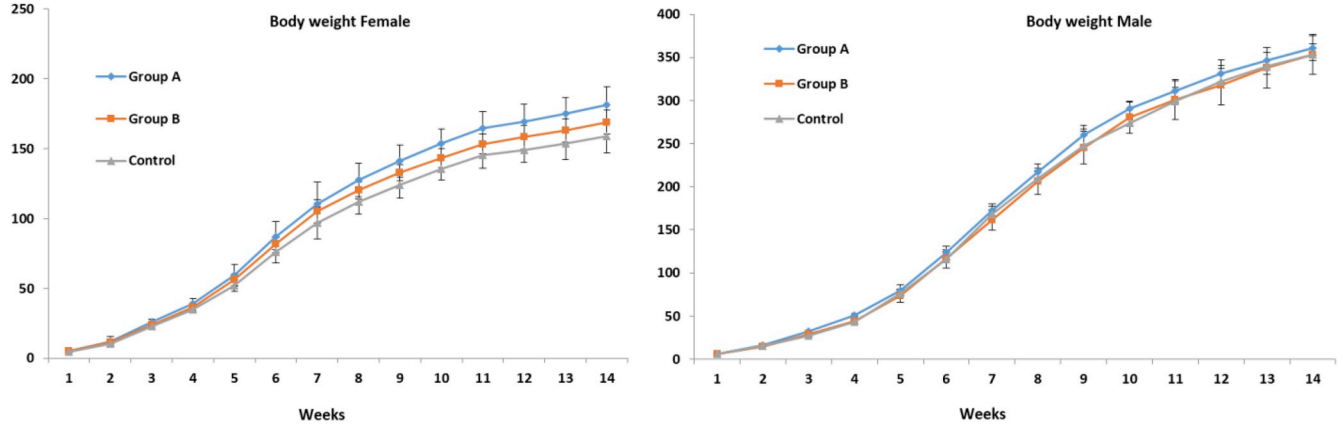

Fig. 4. Body weight curves of the offspring for the 3 different Groups (Group A. NCs 15 mg/ml, Group B. NCs 5 mg/ml, Group C. 0.9% NaCl). Values of body weight are expressed in grams.

represented glycogen aggregates.

Renal specimens examined from pups (Fig. 7a–c) from all three groups (A, B and C) did not present any cytoarchitectural changes. Apart from a mild hyperaemia in few cases in all groups, renal corpuscles were normal, with no evidence of glomerulonephritis. Proximal renal tubules were stained deeply eosinophilic, while their brush-border surface in combination with their large cell size resulted almost in extinguishing their lumen. Finally, inflammatory, degenerative or neoplastic lesions were absent.

Administration of quatro stimuli NCs in increasing dose did not alter liver or kidney histological appearance of dams. Liver specimens (in accordance with liver specimens collected from rat pups), did not present changes between Group A (Fig. 6c), Group B (Fig. 6b) and the Control group (Fig. 6a). Neoplastic, inflammatory or degenerative lesions were absent. Hepatic cells from few animals did have foamy appearance due to glycogen accumulation.

Renal specimens collected from Group A (Fig. 7f) or Group B (Fig. 7e) dams also did not present altered cytoarchitecture when compared to the Control group (Fig. 7d). Apart from mild hyperaemia presented only in few animals of all groups, there were neither toxicity-related nor background lesions observed.

Liver specimens collected from the young adult offspring rats of A (Fig. 6f) and B (Fig. 6e) groups did not reveal any histological differences when compared to the Control group (Fig. 6d). Inflammatory, degenerative, neoplastic or other toxicity-related lesions were not noticed.

On kidney specimens, collected from young adult offspring rats, no altered histological appearance was observed. Comparing specimens of Groups A (Fig. 7i) and B (Fig. 7h), to the Control group (Fig. 7g), there were neither obvious alterations nor toxicity related lesions (e.g. cloudy swelling or fatty change). Finally, there were no inflammatory, degenerative or neoplastic lesions observed.

4. Discussion

Drug selective delivery is an idea conceived a century ago by Paul Ehrlich who envisioned the notion of the perfect agent and described it as ‘magic bullet’. Many of the traditional regimens used against bacteria, are selectively acting, because they target specific and unique features of these cells (Maeda et al., 2009). However, tumors cells are

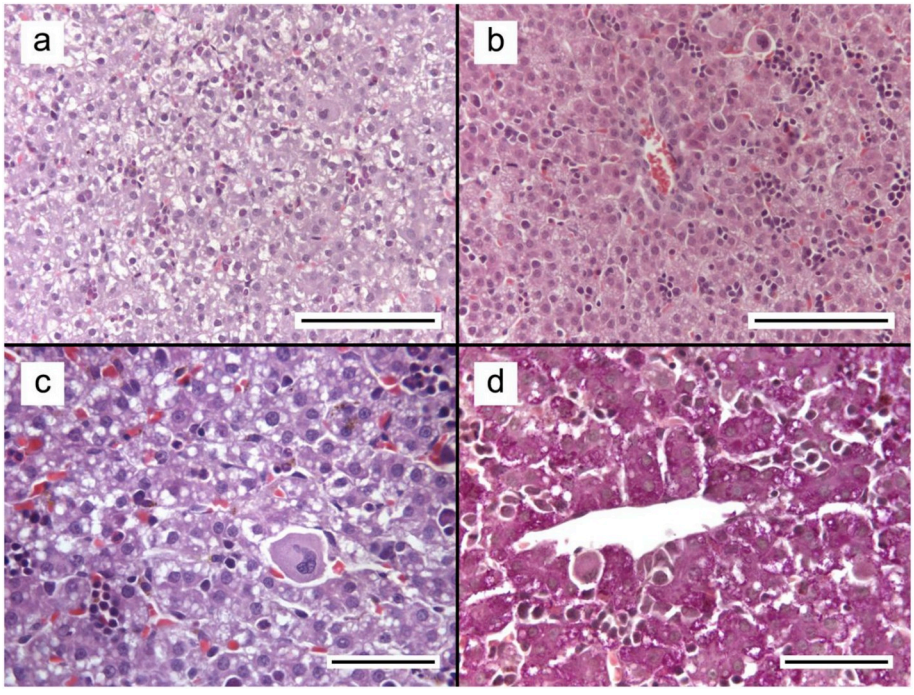

Fig. 5. Liver specimens from rat pups. Vacuoles in hepatocytic cytoplasm are present in animals of all three groups and represent glycogen accumulation: a: Control group, b: group B (low-dose), c: group A (high-dose), d: Periodic Acid-Schiff (PAS) stain revealing glycogen aggregates inside hepatocytes. Hematopoietic stem cells (HSC) can be noticed in all four photographs residing intraluminally in sinusoids. Megakaryocytes can be clearly distinguished from rest HSC. Scale bars in figures a and b represent 0.1 mm and 0.05 mm in figures c and d.

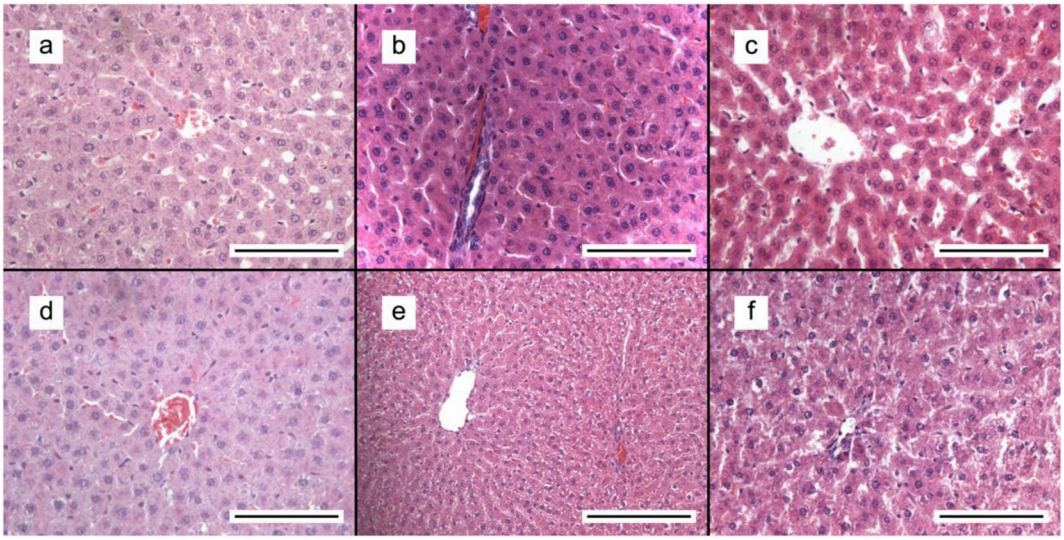

Fig. 6. Liver specimens from dams (a, b, c) and young adult offspring rats (d, e, f). Figures a and d represent Control group, b and e group B (low-dose) and c and f group A (high-dose). In all groups of both dams and young adult offspring no toxicity related lesions were observed. Scale bars in figures a, b, c, d and f represent 0.1 mm and in figure e 0.2 mm.

extremely challenging to be selectively insulted, because they share substantial similarities with the host's cells. Nanomaterials are very promising, since they have been found to be able to exploit successfully these features. Furthermore, they may serve as appropriate media for spatial and temporal targeted drug delivery. However, increasing interest on nanomaterials has raised major concerns about their safety; especially on vulnerable life stages such pregnancy and foetus development. Studies have shown that some nanomaterials may cross the placenta and cause deleterious effects on fetal growth, while others don't. The size and/or surface charge have been incriminated for these differences.

Significant similarities, concerning the placenta, between human and rats has render this animal species widely accepted as model in studies on pregnancy and developmental toxicity. In both species the

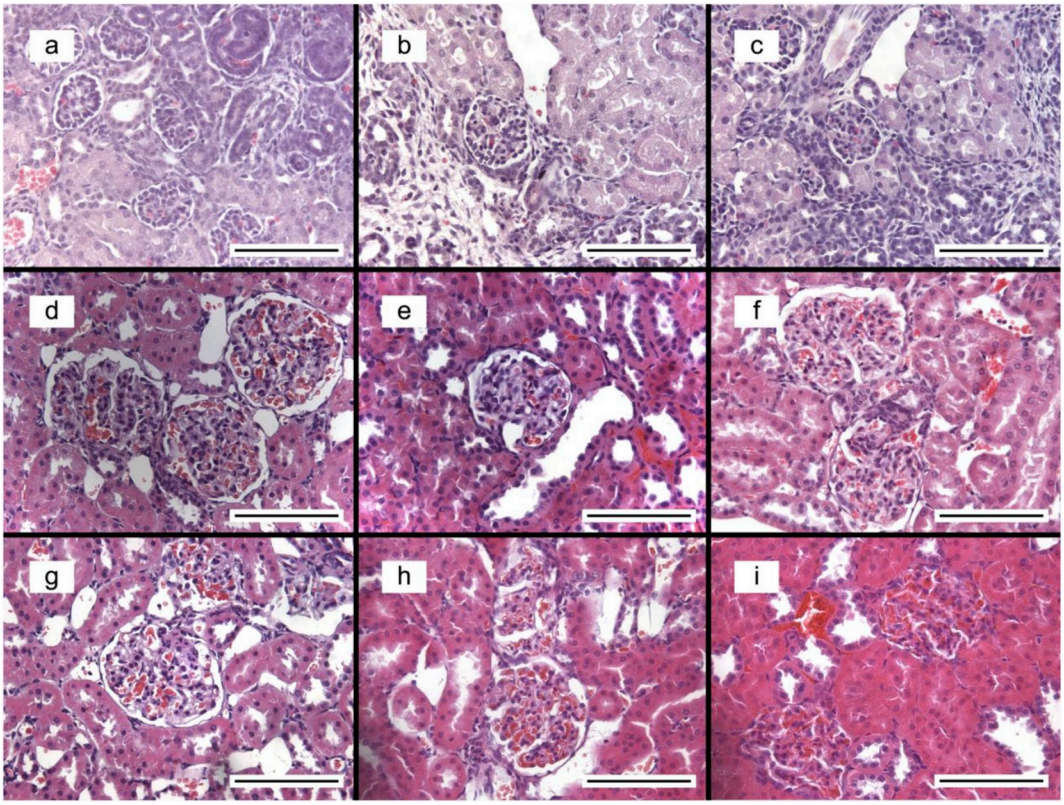

Fig. 7. Renal specimens from rat pups (figures a, b and c), dams (d, e and f) and young adult offspring rats (g, h and i). Left column (figures a, d and g) represents kidney specimens from control animals, middle column (figures b, e and h) depicts renal specimens from group B (low-dose) while right column refers to group A (high-dose) animals. Notice the age-related size differences between infant and adult glomeruli and the nephrogenic zone in upper right area of figure a. All scale bars represent 0.1 mm.

placenta has a discoid shape and belongs to the haemochorial type, while changes, during pregnancy, in blood count and biochemical parameters are closely analogous (Furukawa et al., 2011). To evaluate the normal development of foetuses during pregnancy, ultrasonographic volumetric parameters were recorded. In humans the method is widely used to provide valuable information about the normal progress of gestation while advances in spatial and temporal resolution have expanded its use in laboratory animal settings (Arleo et al., 2014; Stasinopoulou et al., 2014). As expected, baseline measurements on 14th day of pregnancy did not reveal any statistical significant difference between groups as far as the placenta width, embryonic sac length and width and fetus length are concerned (Table 1). On the 17th day of gestation, (after the two intravenous administrations of the NCs) no statistically significant difference on the same parameters was observed in any group (Table 1). Furthermore, the heart rate of the foetuses was obtained. In canine and feline pregnancy heart rate has been considered an excellent indicator to monitor foetal hypoxia and complications related to growth restriction due to placental insufficiency (Vestegen et al., 1993; Zone and Wanke; 2001). In the present study no statistical significant differences between groups in the two time points were evident (Figs. 2 and 3). Additionally, no irregularity or foetal anomaly during ultrasonography was detected. Although, under the present conditions (no anaesthesia, short time for the animals being restrained) the evaluation of the number of pups was difficult, the aforementioned parameters were evaluated on at least four pups for each dam.

In mammals litter size and foetal weight has an inverse relationship (Romero et al., 1992). In toxicological studies, agents may adversely impact either the number or the weight of pups. Consequently, both parameters should be taken into account. In the present study the NCs administration had no adverse effect on the size of the litter (Table 2) or on the mean weight of the pups (Fig. 4). Furthermore, phenotypically no dysmorphogenesis on offspring were observed.

Analyses of body weight growth curves in rats could serve as biological index providing insights about multiple factors which may influence growth and development. In the present study, from the prenatal and postnatal factors that could affect body weight (genotype, age of dams, nutrition of the dams and pups, litter size and environmental conditions) (Eisen, 1976), the only variable parameter was the intravenous administration of NCs. The body weight curves obtained for both male and female offspring indicate that the administration of different doses of NCs had not significantly affect on both sexes. This is evident from the close overlapping of the body weight curves (Fig. 4).

In mammals maternal toxicity plays a predominant role in embryo or foetus development (Khera, 1985). In the present study no adverse effect was evident on dams during pregnancy or lactation. At weaning, when the dams were euthanised, no macroscopic finding was evident in the necroscopic examination. The organs selected for histopathological evaluation were the liver and kidneys. Liver is known as particularly susceptible organ to toxic insults (Acosta et al., 1985) while kidneys may become more vulnerable to toxicity during pregnancy (Baker et al., 1989) the histopathologic evaluation of both organs did not reveal any pathologic lesion. Finally the presence of vacuolation in liver specimens, which may have suggested cell degeneration, was proven to be (after PAS staining) glycogen accumulation.

There is not always a correlation between embryos and foetuses and maternal toxicity (Chahound et al., 1999); this is why the liver and kidneys of newborns and adult offspring were evaluated. Histopathologic findings were analogous to those observed on dams. No evidence of degenerative, inflammatory or neoplastic lesion was noticed.

Conclusively data from the present study were encouraging for the safety of the intravenous administration of the NCs during the last third of the pregnancy. In all the parameters selected to be evaluated, the toxic impact of low and high dose NCs on dams, newborns and adult offspring in the rat model, none was found to differ from the Control group. Although the rat model is one of the most commonly used in reproductive and developmental toxicological studies due to many common characteristics to humans (Fukuwara et al., 2011), one should bear in mind that extrapolation of scientific data from one species to another should always be performed cautiously (Hau; 2003).

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Transparency document

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