Supplementary Information

Comparative evaluation by scanning confocal Raman spectroscopy and transmission electron microscopy of therapeutic effects of noble metal nanoparticles in experimental acute inflammation

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Methods

Section S1: In vivo toxicity evaluation

112 female Wistar rats (10 weeks old, 140-160 g body weight) were used for acute systemic toxicity test. The animals were randomly divided into 3 treatment groups of 16 animals each. The results were compared to one group (n=16) treated with vehicle (PBS). For acute systemic toxicity test, one hundred µl amounts of silver and gold nanoparticles suspensions were administered intravenously (via a tail vein) to the rats, at different doses (low, middle and high doses): 0.3, 0.8 and 1.5 mg/kg body weight. At 30 minutes, 24 hours and 7 days after administration, the animals were euthanized and blood samples

were taken for biochemical and hematological investigations (Sysmex XE 2100 Analyzer (Sysmex America, Inc.). Triglyceride, cholesterol, glucose (ChemiTech, France), glutamic oxaloacetic transaminase (GOT, Diagnosticum Rt (Hungary), glutamic pyruvic transaminase (Diagnosticum Rt (Hungary, GPT), red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Ht), white blood cell (WBC) and platelets (PLT) levels were evaluated. At each time-point, the animals were anesthetized with ketamine xylazine cocktail (90 mg kg^{-1} ketamine, 10 mg kg^{-1} xylazine) and weighed. At 7 days, liver and spleen were collected for routine histopathological examination. The liver samples were collected according to goRENI standard (http://reni.item.fraunhofer.de/reni/trimming/index.php). To detect possible toxic effects, the histological activity index of Knodell was quantified in liver and was evaluated in spleen the red and white pulp. All the microscopic analyses were done using an Olympus microscope (BX41; Olympus). Pictures were acquired using a DP25 camera (Olympus) under a 400X magnification and processed using the Cell B software (Olympus). The animals were also observed for toxic symptoms signs (weight loss, water and food consumption, behavior, macroscopic changes of skin and fur) and were noted the number of survivors.

Results

Section S2: Physiological stability

The successful use of colloidal gold and silver nanoparticles as therapeutic agents would require proves for their robustness in simulated physiological conditions. Herein, we investigated as-synthesized nanoparticles for their stability under physiological conditions by incubating 500 μL of colloidal solution with 100 μL of a solution of phosphate buffer saline (PBS)/PBS containing 10 % fetal bovine serum. Fig. S1 compares the UV-vis absorption spectra of the prepared colloidal nanoparticles in the absence and presence of PBS/PBS containing 10 % fetal bovine serum. We noticed that the initial absorption spectra of silver/gold nanoparticles were fully recovered which rule out any possible aggregation of the colloidal solutions in simulated physiological conditions. However, a red shift was observed after the addition of PBS/PBS containing

10% fetal bovine serum, which is most probably due to environmental changes which lead to an increase in the local refractive index.

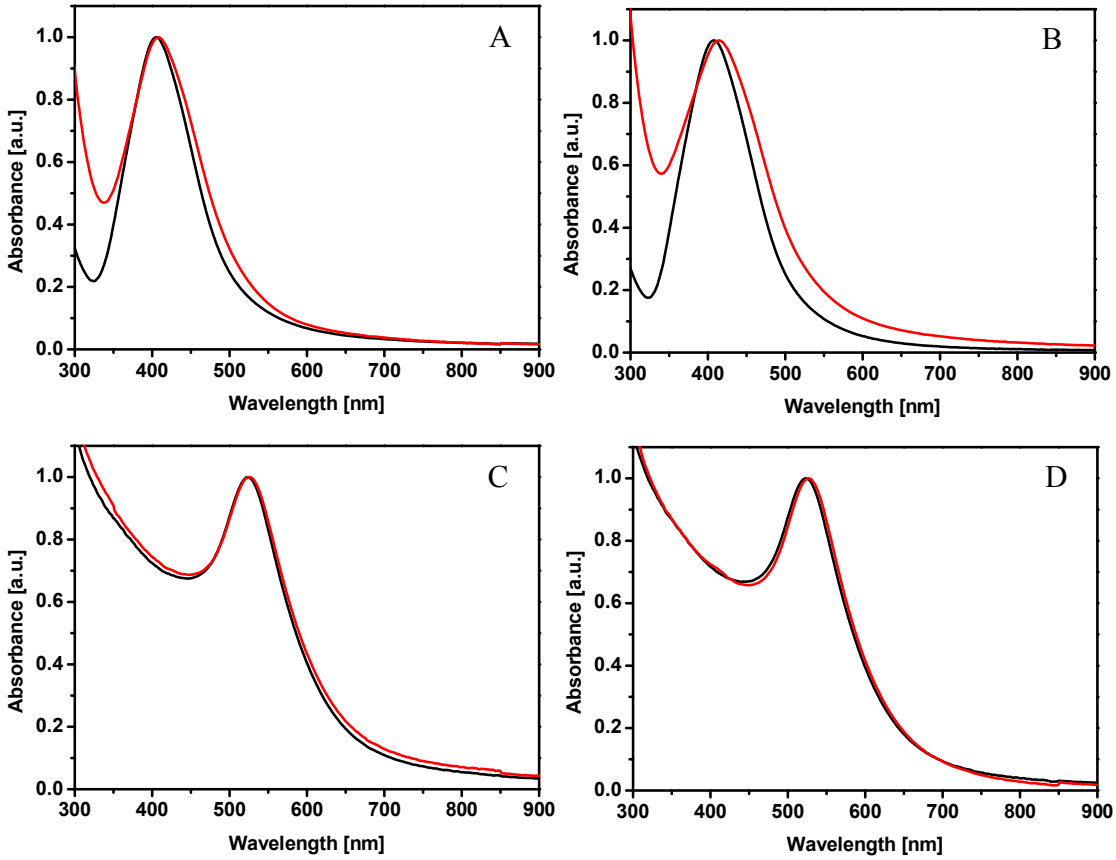

Fig. S1 Normalized UV-vis absorption spectra of colloidal silver nanoparticles: (A) before (black curve) and after the addition of PBS (red curve). (B) Before (black curve) and after de addition of PBS containing 10% fetal bovine serum. Normalized UV-vis absorption spectra of colloidal gold nanoparticles: (C) before (black curve) and after the addition of PBS (red curve). (D) Before (black curve) and after the addition of PBS containing 10 % fetal bovine serum. The spectra were recorded after 4 hours of incubation.

Section S3: General signs, hematology and biochemical parameters

In acute systemic toxicity test no death was recorded during the observation period in treated and control animals. No significant changes in water and food

consumption, behavior and % weight gain of rats were observed (data not shown). There were no significant macroscopic changes of skin and fur of animals and eyes and mucous membranes aspect, respiratory, circulatory, nervous system and somatomotor activity were unchanged.

The administration of high doses of AgNPs-CM and AuNPs-CM induced early and transiently toxicity, especially in liver. Thus, a dose-dependent hepatic cytolysis was noticed at 30 minutes after injection of AuNPs-CM ($p<0.01$). After 24 hours, the liver toxicity decreased to control group and was maintained at low levels at 7 days. High dose of AgNPs-CM had similar effects on liver function at 24 hours ($p<0.01$) as well as high dose of AuNPs-CM, effects that persisted even 7 days. Regarding the glucose level, there was observed a hyperglycemic effect at the highest dose of AgNPs-CM ($p<0.05$) and the lowest dose of AuNPs-CM ($p<0.05$). Gold nanoparticles decreased the cholesterol level independent of dose used ($p<0.05$).

The metallic nanoparticles also induced hematological changes, especially on erythrocyte line and reactive thrombocytosis. Thus, AuNPs-CM decreased early the haematocrit level ($p<0.001$) compared to the control group, independent of dosed used, in parallel with reactive thrombocytosis. The high dose of AgNPs-CM had the same effect on thrombocytes.

Section S4: Histopathological evaluation

In our study, no significant histopathological changes were observed after 7 days for both types of nanoparticles tested. Only the high dose of AgNPs-CM induced small amounts of focal necrosis in liver (Fig. S2).

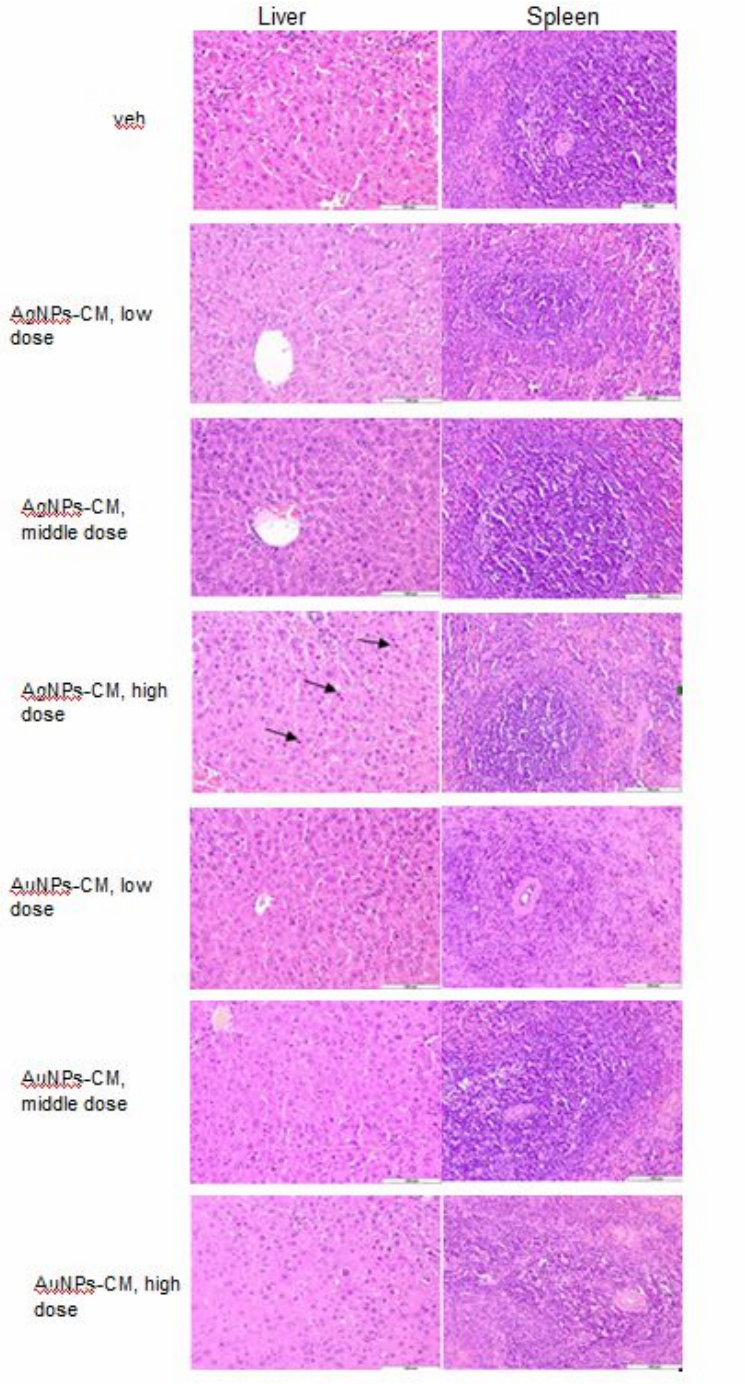

Fig. S2 – Liver and spleen sections stained with hematoxylin and eosin at 7 days following administration of different doses of AgNPs-CM and Au-NPs-CM (arrows indicate necrotic hepatocytes induced by high dose of AgNPs-CM). Scale bar is 100 μm.