Citotoxicity and acute oral toxicity tests of nanometer- and submicrometer-sized hollow spheres of chondroitin sulfate as a potential formulation strategy for anti-inflammatory encapsulation

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Abstract - New nano-engineered polymers have created concerns about their toxicity. This study aimed to evaluate the toxicity of nanometer- and submicrometer-sized hollow spheres of chondroitin sulfate (CS). Four variations of this material were tested in mouse fibroblast 3t3 cell assay and their acute oral toxicity (OECD Limit Test) was evaluated by administering 2000 mg/kg of material in 5 mice for each variation. No toxicity signs were observed neither in cells nor in gross necropsy and histopathology of the animals. The tests indicate that the variations are safe for oral acute ingestion.

New nano-engineered polymers have created important concerns about their toxicity. This study aimed to evaluate the toxicity of nanometer- and submicrometer-sized hollow spheres of chondroitin sulfate [1] that have the potential to be used in formulations for anti-inflammatory encapsulations.

Four variations of the material (CS, CS + NIPAAm [N-isopropylacrylamide] 5%, CS + NIPAAm 2.5% and CS + PNIPAAm [Poly-N-isopropylacrylamide] 2.5%) were tested in mouse fibroblast 3t3 cell assay and their acute oral toxicity in mice. No signs of citotoxicity were detected (Fig 1), so the OECD Limit Test [2], which can be done with as few as 5 animals for material, could be performed. All the animals of the study were female, since they are usually more sensitive to toxicity than males. A sighting study starting dose of 2000 mg/kg was administrated by gavage in 4 animals, one animal for each variation of the material. Immediately afterward, they were individually observed during 14 days for external signs of toxicity in their fur, eyes, behavior, etc. The animals were then humanely sacrificed for gross necropsy and histopathology of their organs. Since no signs of toxicity were verified in the sighting study, 16 more mice, 4 for each material variation, received the same dose, following the same methodology. Again, no toxicity signs were detected.

The tests suggest that the 4 variations of the material are safe for oral acute ingestion. Further studies are necessary to verify the effect of subchronic treatments in rodents.

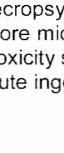

Fig. 1. Citotoxicity of chondroitin sulfate samples. Concentrations were 30 mg/mL, 7.5 mg/mL and 3.75 mg/mL. In the graph, "1:30" means "sample 1 with concentration 30 mg/mL". Each graph column represents the mean (with pattern deviation shown) for 6 wells. It can be seen that, after exposing cells for 24h to nanoparticles, cells viability did not reduce. So, no toxicity was detected in fibroblasts in the range of tested concentrations.
Subtitles - 1 = Micro SC (chondroitin sulphate); **2** = Micro SC + NIPAAM 5%; **3** = Micro SC + NIPAAM 2.5%; **4** = Micro SC + PNIPAAm 2.5%.

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[1] A. V. Reis, M. R. Guilherme, L. H. C. Mattoso, A. F. Rubira, E. B. Tambourgi and E. C. Muniz. Pharm Res., v. 26, no.2 (2009), p.438-444. Epub Nov 13, 2008.
[2] Organisation for economic co-operation and development (OECD). OECD guideline for testing of chemicals, nº 420. Adopted: 17th December 2001.