NANOPARTICLES WITH GENTAMICIN TO TREAT INTRACELLULAR *BRUCELLA* INFECTIONS

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Introduction: Brucellosis is a worldwide zoonosis caused by different species of the genus *Brucella*. The ability of this pathogen to survive and multiply within macrophages makes difficult the antibiotic access to its niche [1] and relapses remain as a problem [2]. As alternative to current treatment, polymeric drug delivery systems containing gentamicin have been developed. These particulate carriers target the drug into mononuclear-phagocytic system, where the pathogen resides and allow the antibiotic reach therapeutic concentrations intracellularly after particle degradation. Besides, particle uptake may induce macrophage activation, increasing the production of reactive oxygen intermediates, involved in host defense against the pathogen [3]. The aim of the present work was then to study the suitability of polymeric nanoparticles (NP) for gentamicin (GM) entrapment in view to treat brucellosis. Furthermore, *in vitro* macrophage activation upon NP phagocytosis and *in vivo* distribution of the nanocarriers in the target organs for *Brucella* (liver and spleen) were also studied.

Material and methods: Poly(lactide-co-glycolide) (PLGA) NP containing gentamicin (GM) were prepared by a water-oil-water solvent evaporation technique [4]. Particle size was determined by photon correlation spectroscopy. Drug content in particles was quantified by first dissolving the polymer in diclorometane followed by extraction with phosphate buffer pH 6. GM in the supernatant was analyzed by o-phthalaldehyde derivatization and measured by fluorimetry [5]. J774 murine monocyte activation after 1 h incubation with particles was stablished by the Bursttest® kit (Orpegen, Germany) and by flow cytometry, to determine the percentage of phagocytic cells which produce reactive oxidants. PMA (phorbol 12-myristate 13-acetate) was used as positive stimulant, and culture medium as negative control.

In vivo nanoparticle distribution study was carried out by the method described by Peyre *et al* [6], with some modifications. Briefly, 2 mg of particles loaded with rhodamine and gentamicin were administered intravenously, in 1% lecithin in saline. At different times post-administration (4 h, 1 week and 2 weeks) animals were sacrificed, liver and spleen were embedded in a tissue proceeding medium (O.C.T.®, Sakura, Netherlands), immersed in melting isopentane (Fluka, Buch, Switzerland) and stored at -20 ºC . Tissue samples were cut into 5 µm sections in a cryostat (2800 Frigocut E, Reichert-Jung, Germany). The distribution of NP was studied in liver and spleen by using fluorescence microscopy, and the fluorescence quantified by an image processor program (Micro Image® 4.0; Olympus Optical Co., USA).

Results and discussion: All the NP formulations presented homogenous size with a mean diameter of 320 nm and with an encapsulation efficiency of 6.23 µg of GM/mg of nanoparticles. No differences on surface charge were observed between loaded and empty nanoparticles, suggesting that the drug was entrapped inside the nanocarriers.

As showed in Figure 1, nanoparticles activated murine monocytes more significantly than negative control and PMA. Oxygen reactive production upon phagocytosis plays a significant role in intracellular destruction of the pathogen.

Direct fluorescence studies demonstrated that PLGA nanoparticles loaded with rhodamine and gentamicin were actively taken up by spleen and liver of mice after intravenous administration. As shown in figure 2, higher fluorescence intensity was observed in liver compared to spleen. Moreover, the intensity of fluoresccence observed was quite high 4 h after nanoparticle administration however little fluorescence was observed 1 and 2 weeks post-administration.

In conclusion, the results obtained in the study indicated that the designed nanoparticle formulations may be suitable gentamicin delivery system to control brucellosis.

References:

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Figures:

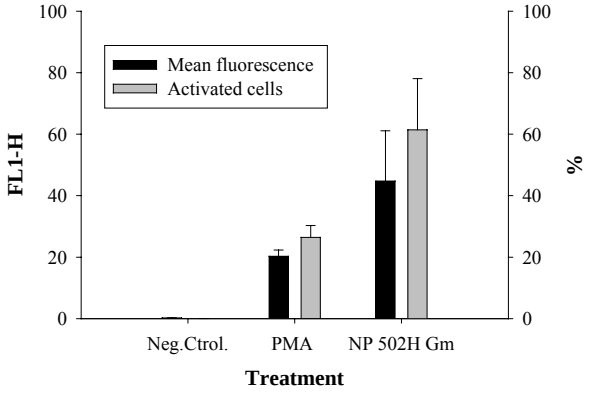

Figure 1. Oxidative burst of J774 macrophages after gentamicin-loaded NP uptake. Results expressed as percentage of activated cells and mean fluorescence.

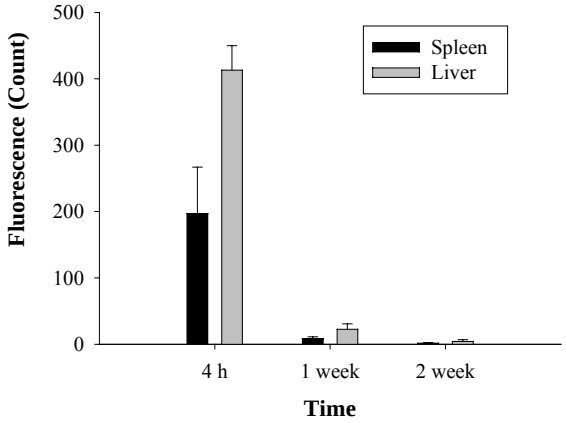

Figure 2. Fluorescence intensity observed in spleen and liver of mice at different times post-administration of 502H NP.

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