11th International Conference on Advanced Materials
ICAM2009
Rio de Janeiro Brazil
September 20 - 25

Insulin-loaded polymeric nanoparticles: Cell viability and cytotoxicity studies

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Abstract – Insulin polymeric nanoparticles were prepared by double-emulsion technique resulting in particles size distribution of 796 nm (P.D.I=0.49) and encapsulation efficiency of 90%. The particles were additionally characterized by SEM. The studies of cell viability were assayed in rat pancreatic MIN6 cells. Cell viability and proliferation were not affected by using the nanoparticles expect to the treatment in 72 h of 550 $\mu g\ ml^{-1}$. These results showed that this system is promissory to improve the delivery of insulin by subcutaneous injection due to a possibility of a reduction in the number of dosages.

Modern drug nanocarrier systems play an important role to improve therapeutic action, such as increase of efficacy (lower number dosages) and a sustained release. More than 30 million people in the world suffer from insulin-dependant diabetes mellitus and require daily parenteral injections of insulin. This treatment is probable because of regulatory glucose homeostasis. [1] The aim of this study was to prepare and characterize polycaprolactone (PCL) nanoparticles loading insulin by double-emulsion method to be administrated "*in vivo*" by subcutaneous injection, and to investigate a possible cytotoxicity in MIN6 insulin-producing cells. Human insulin loaded nanocapsules were prepared as follows: insulin water solution was added to a solution containing polycaprolactone and Span 60 in dichloromethane. It was stirred under 10,000 rpm for 1 min. to form the W1/O primary emulsion. This first emulsion was added in another solution containing Pluronic F68, and it was stirred under the same condition to produce a W1/O/W2 emulsion. The organic solvent was removed under reduced pressure. The aqueous suspension of particles was freeze-dried. Particles size was determined using a Zetasizer Nano ZS equipment. Determination of insulin encapsulation was determined by HPLC, using the difference between the total amount of insulin used and the free drug collected in the supernatant after centrifugation. The morphology of the particles was investigated by scanning electronic micrograph (SEM). [2] MIN6 insulin-producing cells was cultured in RPMI 1640 medium, supplemented with 10 mmol/l glucose, penicillin, and streptomycin in a humidified atmosphere at 37°C and 5 % CO_2. MIN6 cells were exposed to 112, 225 and 550 $\mu g\ ml^{-1}$ of nanoparticles, for 6, 24 and 72 h. Cell proliferation rate of viable cells was measured by a colorimetric method of reduction of tetrazolium salt into soluble formazan (MTS) and protein expression of clivated caspase-3 was performed by Western-blotting [3]. The encapsulation efficiency was 90.6% (±1.6) (n=3); the size of particles was 796 nm (P.D.I. = 0.49). Figure 1 shows a representative image of the particles that presents spherical morphology. There was no significant increase in clivated caspase-3 expression and no difference in the cell proliferation rate and viability, with exception to 550 $\mu g\ ml^{-1}$ in nanoparticle-exposed cells for 72 h compared to the controls, as stated by MTS test. An explanation to such results is that "in vitro" conducted assays may not reflect the nanoparticle effects that would be observed in animal tests. In this way, more studies are necessary to confirm nanoparticles effects in animals. These results indicate that 112 and 225 $\mu g\ ml^{-1}$ of nanoparticle exposures do not impair cell growth and probably do not alter cell survival. In conclusions, these results show that this system is promissory to improve the delivery of insulin by a reduction in the number of doses. Therapeutic use in rats is being conducted in our laboratories.

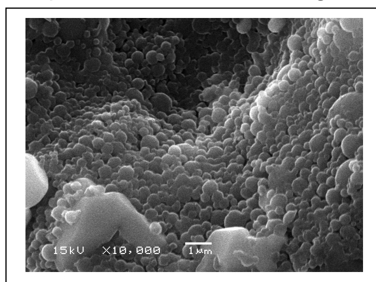

Figure 1: scanning electronic micrograph.

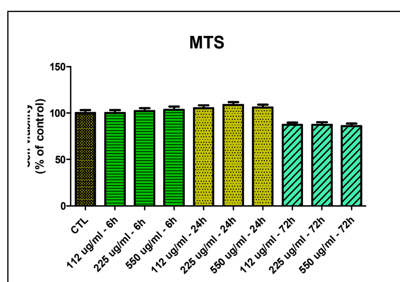

Figure 2: MTS assay (the graphics in blue showed significant differences, *P*<0.05).

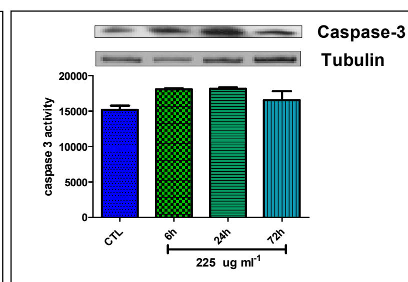

Figure 3: Caspase 3 clivage. (*P*<0.05).

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