A potential device for oral vaccination of ruminants

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Abstract: This study aimed to develop a device capable of delivering a protein of up to 5.7 KDa for ruminants. Only the treatment which received nanoencapsulated insulin, incorporated into a lipid matrix, was able to promote the intestinal absorption of insulin and preserve its biological activity. These results indicate that this device can be used for oral vaccination in ruminants.

In ruminants, the process of vaccination is mainly performed subcutaneously and with the help of syringes, which are reused for many animals. Such a procedure favors the propagation of blood-borne disease, which puts at risk the bio-safety practices adopted in infectious diseases control programs. An alternative to prevent this problem is to develop alternative forms of vaccination which do not make use of sharp objects. In this context, this study aimed to develop a lipid device, containing chitosan nanoparticles, capable of delivering an intact protein of up to 5.7 KDa through the gastrointestinal mucosa of ruminants. In order to do so, human crystalline insulin (Novonordisk- Montes Claros, Brazil) was encapsulated into nanoparticles through the formation of a spontaneous complex between chitosan (Aldrich – Wisconsin, USA) and sodium tripolyphosphate (Dinâmica - Diadema, Brazil) and posterior mechanical incorporation in a saturated lipid matrix and melted at 28°C. The efficiency of the encapsulation was determined by HPLC-rp (Shimadzu-Kyoto, Japan) with the help of a C18 column and a UV detector. The distribution of nanoparticle sizes and the Zeta potential were determined by Dynamic Light Scattering (Malvern Instruments- Malvern, England), the morphology of the nanoparticles was assessed by a high resolution scanning electron microscope (MEV) (FEG XL30, Philips Electronic Instruments - Mahwah, USA), with 70,000x magnification and a low electron accelerating voltage of 5 kV. The in vivo liberation pattern was carried out in four alloxan-induced diabetic sheep (Sigma- St. Louis, USA), in which blood samples were collected at 0, 1, 2, 4, 6, 10, 12, 14 and 24 hours after the oral administration of 50UI/kg of insulin in the nanoencapsulated form incorporated into the lipid matrix, only nanoencapsulated or in saline solution. In the saline solution samples, the plasma insulin levels were determined by immunoassay of electrochemiluminescence (COBAS Insulin, Roche – Switzerland) and glucose was determined through the enzymatic-colorimetric technique (GLUC-PAP, Randox – Antrim, United Kingdom). The process of encapsulation presented an efficiency of 38.3± 2.8%, producing nanoparticles with Zeta potential of +37± 1.1 mV with diameters ranging from 38 to 396 nm. The predominance of particles with diameters smaller than 100nm was confirmed by the MEV, and they were spherical in shape and had homogenous sizes. During the *in vivo* release pattern (table 1), the three treatments did not present significant alterations in the plasma levels of insulin and glucose in the first fourteen hours of essay. Nevertheless, between the fourteenth and the twenty-fourth hours of essay, only the treatment which received nanoparticles incorporated into the lipid matrix presented changes in the monitored parameters, with an increase in the concentration of plasmatic insulin (42.95±25µUI/ml) and a decrease in glycemia of 20.12± 4.3% depending on the baseline. These results indicate that the device proposed was capable of promoting the oral absorption of a protein with 5.7 KDa, also preserving its biological activity. In conclusion, the device proposed shows potential for use in the oral vaccination of ruminants.

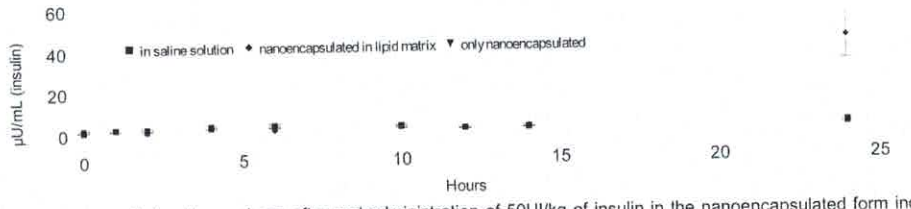

Table1: Profile of plasmatic insulin on sheep after oral administration of 50UI/kg of insulin in the nanoencapsulated form incorporated into the lipid matrix, only nanoencapsulated or in saline solution.

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