O5-3

Improving Safety of Venom Immunotherapy using Microspheres of PLGA for Antigen Delivery

R. A. Trindade[1#], V.C. Rescia, O.A.B. Sant'Anna, M.V.A. Lopes-Ferreira, F.M. Bruni, P.S. Araújo, R. Nicolete, D.F. Santos, and M.H. Bueno da Costa[2*]
[1] Federal University of Piauí – Parnaíba - Brazil[2] Institute Butantan - São Paulo, Brazil.
* Supervisor # Contact email : rtrindade@ufpi.edu.br

INTRODUCTION AND OBJECTIVES

Currently, Venom Immunotherapy (VIT) is the only definitive treatment for venom allergic disease reaching up to 90% of efficacy. It consists in repeated and gradually increased subcutaneous allergen injections in order to promote a shift in immune response from Th_2 (production of IgE) to Th_1 (production of IgG) also called desensitization (ACAAI 2003).

However, it presents some inconvenient, such as, high risk of anaphylactic reactions during venom injections owing to direct contact between antigen and organism. In addition, VIT is a long term treatment, in some cases up to 5 years, and high number of injections required (30-70) which discourages allergic patient adhesion to therapy, so that less than 5% choose undergo this treatment (ACAAI, 2003).

The use of microspheres (MS) prepared with PLGA (poly D,L-lactide-co-glycolide) for delivery system presents numerous advantages, such as: allows antigen release over weeks or months following continuous or pulsatile kinetics from a unique administration; protects direct contact between antigen and organism (Langer 1990, Saéz 2007). They are readily recognized and ingested by macrophages and dendritic cells (APC – Antigen Presenting Cells) stimulating the immune system, among other important properties (Johansen 2000).

So, the combination of efficiency of VIT with the feasibility and advantages of PLGA microspheres could form an ideal formulation to overcome the troubles related to traditional treatment with free antigen (BVP), which is our main goal in this work.

MATERIALS AND METHODS

Microspheres of PLGA were prepared with polymer of 34 kDa and free carboxi-end region (COO-H) and Lactic / glycolic acid ratio 50:50. The preparation method used was double emulsion ($W_1/O/W_2$).

The dynamic of alterations in the microcirculatory environment was determined using intravital microscopy by transillumination of mice cremaster muscle after topical application of different formulations (BVP-PBS; unloaded MS, and BVP-MS). After the stabilization of the microcirculation (baseline count), the number of rolling cells in the postcapillary venules was counted in 10, 20 and 30 min after treatment application. Five mice were used for analysis of each formulation.

J774 macrophage cell line was used for phagocytosis assays. These cells (5×10^6/24-well) were incubated for 4 h with 50 µg/ml of empty MS or BVP-MS. Control: cells incubated only with RPMI medium. After incubation period, the medium was aspirated and non-ingested MS were washed. Cell suspensions were collected, cytocentrifuged and identified by panoptic staining. MS uptake was assessed microscopically by counting the percentage of cells that had ingested at least one MS. The phagocytic index (PI) was also calculated: PI = number of engulfed MS×number of cells containing at least one MS/total number of cells. Triplicate of experiment was performed.

For evaluation of immunological response, the mice groups were previously twice sensitized with 5 µg of BVP adsorbed in $Al(OH)_3$ and desensitized with six doses of 0,3 µg BVP-PBS or BVP-MS-PLGA. They were challenged with 15 µg of BVP-PBS. Sera were bled through the ophthalmic plexus after: 1st and 2nd sensitization; immunization; and challenge, totalizing 4 bleeds. The sera obtained were analyzed by ELISA for specific IgG_1, IgG_{2a}, and IgE. Serum titration included a serum from naïve mice used as control.

RESULTS AND DISCUSSION

Microspheres prepared with PLGA 34 kDa-COOH were capable to protect the organism against inflammatory reaction caused by BVP exposition, expressed by ability to increase leukocyte migration in a microcirculatory environment of a mouse cremaster muscle (Figure 1). The free BVP triggered two folds more leukocyte migration than BVP within MS, whilst empty microspheres did not trigger this phenomena.

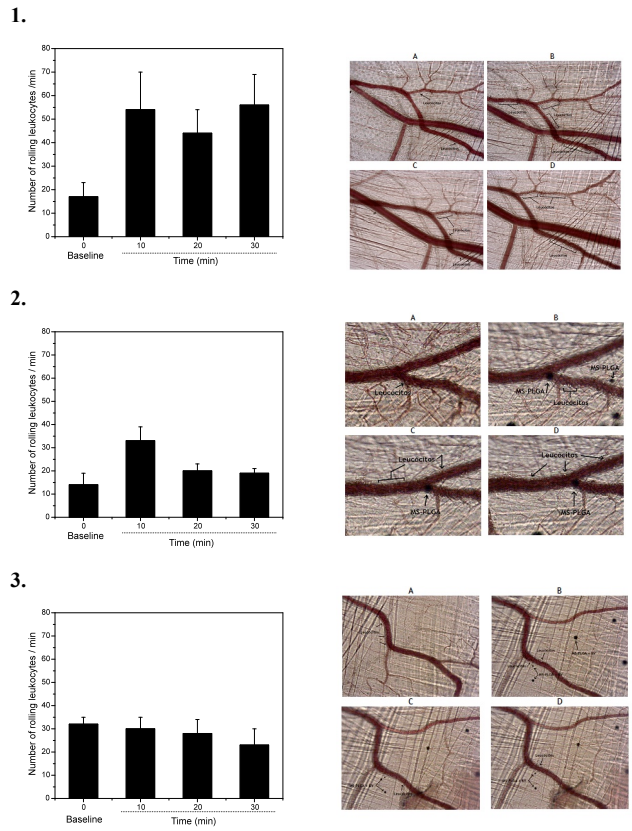

Figure 1. Increase of leukocyte migration in a microcirculatory environment. (1) BVP-PBS; (2) Unload MS-PLGA; (3) BVP-BS-PLGA. Letters in photos represents: (A) baseline (before application); (B) 10; (C) 20, and (D) 30 minutes (after application), respectively.

The ability of macrophages to phagocyte microspheres was improved by presence of BVP within them (Figure 2).

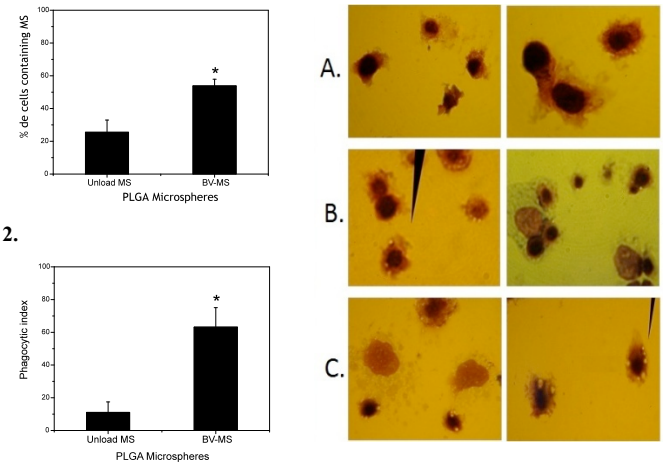

Figure 2. Ability of PLGA-MS to stimulate macrophages to phagocytosis. (1) Phagocytosis induction (%); (2) Phagocytic index. Photos represents cells incubated with: (A) RPMI medium; (B) unload-MS; (C) BVP-MS. Symbol (*) means significant differences (p<0.05).

The formation of IgG_1 in mice which were treated with BVP encapsulated within microspheres was more accentuated than those were treated with free BVP (Figure 3). The more expressive result here was the total absence of IgE along of treatment and even after animal challenge with 15 µg BVP-PBS; furthermore, no animal developed symptoms of allergic reactions. This result showed that IgG_1 and IgG_{2a} were able to neutralize the effects of BVP, and also they were efficient in avoiding the cross-linkage with IgE of mast cells.

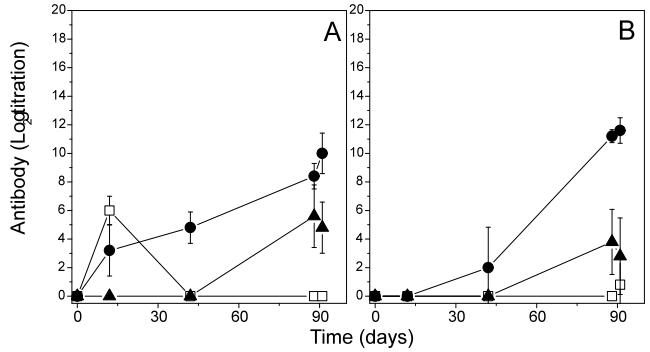

Figure 3. Immunological response expressed by specific antibody produced after BVP injections over time. Mice were immunized with BVP in (A) PBS or (B) MS-34 kDa COOH. Sensitization: 5 µg BVP in $Al(OH)_3$ at 1º and 30° day; VIT treatment 0,3 µg BVP at 45°, 52°, 59°, 66°, 73° e 81° day. Antibody analyzed: IgG_1 (●); IgG_{2a} (▲) and IgE (□).

CONCLUSION

Encapsulating BVP within microspheres of PLGA (34 kDa-COOH) represents a excellent vehicle to administer this allergen in atopic patient because of: (a) they protected tissue from inflammatory damage; (b) they allowed delivery of BVP directly inside APCs; (c) they avoided the formation of IgE and (d) they induced the formation of neutralizing IgG_1 and mature IgG_{2a}, which are efficient in protecting individuals against anaphylactic reactions.

REFERENCES

- ACAAI. (2003) *Allergen immunotherapy: a practice parameter*. Annals of Allergy Asthma and Immunology 90 1-40
- Johansen P. et al. (2000) *Revisiting PLA/PLGA microspheres: an analysis of their potential in parenteral vaccination*. European Journal of Pharmaceutics and Biopharmaceutics 50 129-146
- Langer R. (1990) *New methods of drug delivery*. Science 249 1527-1533
- Saez V. et al. (2007) *Microspheres as delivery systems for the controlled release of peptides and proteins*. Biotecnología Aplicada 24 108-116