Local antitumor effects of intratumoral delivery of rIL-2 by sustained-release PLGA/PLA microspheres

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ABSTRACT SUMMARY

In this study, we formulated a rIL-2 loaded sustained-release PLGA/PLA microsphere, mimicking the paracrine mechanisms of cytokine action, to investigate its local antitumor efficacy. The presented microspheres were formed in two steps: rIL-2 was firstly loaded into dextran particles to keep its bioactivity by a unique method of stabilizing aqueous-aqueous “emulsion” (Dextran aqueous-PEG aqueous phase) (1); subsequently, the particles were encapsulated into PLGA/PLA. A stable sustained release behavior in vitro was achieved for a period of about 25 days. In the subcutaneous colon carcinoma BALB/c mice models, a single dose of microspheres was introtumorally administrated and compared with multiple doses of rIL-2 solution to investigate the long acting effect of microspheres on tumor. The animal experiments showed the local efficacy at tumor site mediated by rIL-2 from a single dose of microspheres was better than that of multiple rIL-2 solution injections. Based on the experimental results, we conclude that rIL-2 loaded sustained-release PLGA/PLA micro-spheres represent a promising approach for local cancer immunotherapy.

INTRODUCTION

Recent years, studies of tumor immunotherapy have entered the mainstream in cancer research. This recipe rejects and destroys tumors by inducing, enhancing the immune response of body. In fact, it has been demonstrated that the growth of tumor is the result of the immunological escape of tumor cells by down-regulating the paracrine mechanisms of cytokine action. In addition, tumor growth is also under the control of an immunotherapy that relies on the contribution of the host immune system. In light of these observations, exploitation of body immunity to control tumor is warranted. Recombinant interleukin-2 (rIL-2) is a multifunctional cytokine that enhances proliferation and activation of T cells, NK, LAK and TIL cells and has been exhibited to be one of the most effective inducers of potent antitumor immunity. During the past decades, rIL-2 has been used as an immunomodulator for tumor-bearing hosts. However, systemic administration of rIL-2 in vivo has not been effective in inducing antitumor responses because of ignoring the paracrine nature of its action (2). A lower local cytokine concentration and short duration failed to enhance potent enough antitumor response. Furthermore, previous studies have showed that the inflammatory responses induced by the high local secretion of cytokines at tumor sites often resulted in the ultimate destruction of surrounding tumor cells.

In this study, we formulated an rIL-2 sustained-release PLGA/PLA microspheres based on the unique method of stabilizing aqueous-aqueous “emulsion”, mentioned above invented by Jin laboratory. The morphology, size distribution and release kinetics in vitro were investigated. Furthermore, to evaluate the long-acting antitumor efficacy, we chose multiple doses of rIL-2 solution as control and experiments were carried out in BALB/c mice bearing colon carcinoma.

EXPERIMENTAL METHODS

rIL-2 solution was added into a self-standing aqueous-aqueous emulsion with dextran forming the dispersed phase and PEG being the continuous phase. After freeze-drying the emulsion and washing the PEG continuous phase using organic solvents, rIL-2-loaded dextran particles, 1-2 µm in diameter, were harvested. These rIL-2-loaded dextran particles were suspended in solution of PLGA/PLA and formulated composite PLGA/PLA microspheres. These rIL-2 microspheres were subjected to a number of assays as in vitro release, SEC-HPLC analysis, in vivo PK test and efficacy.

RESULTS AND DISCUSSION

Fig. 1 presents the in vitro release profile of rIL-2 loaded PLGA/PLA microsphere in PBS (pH=7.4). Fig. 3 presents the in vitro release profile of rIL-2 loaded sustained-release dextran/PLGA-PVA core/shell microspheres in PBS (pH=7.4). ELIAS assay showed that rIL-2 was released from microsphere in a steady and gradual way which lasted less than one month. During this period the cumulative release amount of rIL-2 reached 90±4.7% of total loadings. The burst release within the first day was 13±2.2%.

The rIL-2 encapsulation efficiency was 89.65±3.23%. Considering the physical loss of
microspheres in the process, maybe the factual encapsulation efficiency is higher.

Fig. 2. The in vivo efficacy of rIL-2 loaded sustained-release dextran/PLGA-PLA core/shell microspheres in BALB/c mice bearing colon carcinoma. All mice were euthanized on day 22, and tumors were stripped, weighed and photographed. (A) Representative photographs of tumors; (B) Representative photographs of BALB/c mice bearing tumors; (C) Tumor volumes in the different groups (Blank microspheres, rIL-2 solution, and rIL-2 loaded microsphere) as a function of day after treatment. Arrow represents the day that each formulation was administrated for the first time. (D) Tumor weights of day 22 after mice were euthanized, and the data were expressed as Mean±SD (n=4).

As shown in Fig. 2C, the suppression growth of tumor volume in rIL-2 microsphere group exhibited excellent results (P<0.05) compared to blank microsphere group and the tumor growth was significantly suppressed at 22 days while tumors in blank microsphere group showed rapid growth. Meantime, mice of rIL-2 solution group presented similar results to that of rIL-2 microsphere group and there was no significant difference (P=0.131) between these two groups. Furthermore, weight of tumors on day 22 in each group (Fig. 2D) also demonstrated that rIL-2 microsphere group (1.25±0.33 g) was more effective in growth inhibition of tumors than blank microsphere group (4.63±0.82 g, P<0.01) and inhibition ration is 27%, or rIL-2 solution group (2.18±0.34 g, P<0.01) and inhibition ration is 47% compared with blank group.

Fig.3. Hematoxylin-eosin staining of tumors in BALB/c mice treated with (A) Blank microspheres, (B) rIL-2 solution, and (C) rIL-2 loaded sustained-release dextran/PLGA-PVA core/shell microspheres.

To further investigate the antitumor effect of rIL-2 loaded sustained-release dextran/PLGA-PLA core/shell microspheres in vivo, tumor specimens from mice sacrificed on day 14 were fixed, embedded, sectioned and analyzed by Hematoxylin-eosin staining. As shown in Fig 3, tumors that received rIL-2 microspheres (Fig. 3C) presented significant necrotic region with weak staining and poorly defined borders, while tumors treated with blank microspheres (Fig. 3A) exhibited entirely whole area of proliferating tumor cells with strong staining and regular array. At the same time, a smaller but apparent necrosis was also observed in rIL-2 solution group (Fig.3B).

**CONCLUSION**

In summary, rIL-2 loaded PLGA/PLA microspheres demonstrated a stable sustained release behavior in vitro. Further investigations also evidenced that rIL-2 loaded PLGA/PLA microspheres were capable of acting as artificial paracrine depot of cytokine action in vivo. The local efficacy at tumor site mediated by rIL-2 from a single dose of microspheres was better than that of multiple rIL-2 solution injections. Based on the experimental results, we conclude that the rIL-2 loaded sustained-release PLGA/PLA microspheres may enjoy a potential use in cancer therapy. Meantime, detailed and intensive studies about its further applications should be carried on.

**REFERENCES**


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