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Short Reports

ELEMENTAL STUDY OF GENE THERAPY WITH THROMBOPOIETIN

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Thrombopoietin (TPO) is also referred to as the c-mpl ligand or megakaryocyte growth and development factor, inducing thrombopoiesis by proliferation of megakaryocyte progenitors and promoting endoreplication of mature megakaryocytes.[1] The identification of TPO, and the demonstration that administration to normal experimental animals with recombinant TPO results in a marked increase in platelets count,[2] has opened the possibility of using TPO to rescue patients from life-threatening thrombocytopenia with high dose chemotherapy and radiotherapy. But recombinant TPO is a foreign protein and has adverse effects on patients to different extent. This study is to establish a new TPO administration by gene therapeutic methods, i.e. that after transferred into animals, the TPO gene expresses functional TPO *in vivo* and maintains high platelet level.

MATERIALS AND METHODS

Construction of Recombinant Plasmid

Our laboratory obtained human TPO cDNA from total RNA of human fetal liver by RT-PCR. Then TPO cDNA was cloned in pcDNA3 (eukaryotic expression vector), leading to the recombinant plasmid pcDNA3TPO. Before used for injection into animals, pcDNA3TPO was added to 1/10 volume Lipofectin. The function of Lipofectin is to promote transferring of recombinant plasmid into animal cells.

Main Reagents

Enzymes of molecular biology were obtained from Promega Corp., Lipofectin was obtained from Gibco-BRL Corp., pcDNA3 was obtained from Invitrogen Corp.

Animals

Balb/c mice of 6 to 8 weeks age and 18 to 20 g body weight were obtained from the Experimental Center of Academy of Military Medical Sciences. Each experiment included 5 animals for each data point. A single dose of pcDNA3TPO (10 μg, 20 μg, 40 μg, 80 μg, 160 μg) or as a control, null pcDNA3 or vehicle (PBS) were injected in mice hind leg muscle of each experiment group respectively.

Quantification of Hematology Parameters

From the beginning of injection of pcDNA3TPO or null pcDNA3, PBS, blood samples for platelet count were drawn from the tail vein with a capillary pipette once every two days, and the platelet number and white blood cell (WBC) count were determined so

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that the change of the platelet and WBC number was observed.

RESULTS

The hind leg muscle administration of pcDNA3TPO to Balb/c mice induced a significant rise in the platelet count. In general, the magnitude of increase of platelet counts correlated with the dose of gene administered. The number of platelets increased from the 3rd day of injection, reached a peak level at day 5, and decreased to pretreatment level at day 9. The high platelet level maintained about 6 days. No increase in platelet levels was observed with administration of pcDNA3 null vector nor with administration of vehicle. The WBC in the gene injection groups just like the controls, had no change (data not shown) in 10 days (Figure 1).

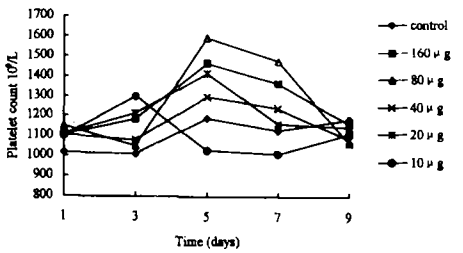

Fig. 1. Platelet count of Balb/c mice with TPO gene therapy. The platelet count of the mice receiving single administration with 10 μg, 20 μg, 40 μg, 80 μg, 160 μg of pcDNA3/hTPO plasmid, respectively, with null pcDNA3 as a control.

DISCUSSION

One report has shown that animals with TPO cDNA delivery by a retrovirus vector containing the TPO cDNA produced TPO and kept high platelet level for over 3 months, resulting in the adverse effects (myelofibrosis and osteosclerosis).[3] So the TPO *in vivo* expression time can not be too long. In this study we made use of eukaryotic expression vector pcDNA3 to transfer TPO cDNA in animals, and the high platelet level of animals administered maintained only 6 days. The number of platelets increased day 3, and decreased to normal level day 9, consistent with the previous results.[4] The transient *in vivo* expression of TPO and the short-term maintenance of high platelet level abrogated the adverse effects because of the long-term expression of TPO and provided an excellent administration for the patients receiving chemotherapy and radiotherapy to overcome the thrombocytopenia. In addition, eukaryotic expression vector is safer than the retrovirus vector. This study also showed that administration of TPO gene had no effect on WBC in mice. We will take a further investigation on TPO gene injection to support the patients receiving chemotherapy and radiotherapy.

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