A PEGylated liposome formulation of Tofacitinib targets a site of inflammation and enhances therapeutic efficacy in a mouse calvarial osteolysis model

Xin Wei1, Jianbo Wu1, Ke Ren1, Hongjiang Yuan1, Yijia Zhang1, P. Edward Purdue2, Steven R. Goldring2 and Dong Wang1

1Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198; 2Hospital for Special Surgery, New York, NY 10021, USA. x.wei@unmc.edu

ABSTRACT SUMMARY

A PEGylated liposome formulation of Tofacitinib (Tofa) a selective JAK-3 inhibitor, was prepared to prolong its circulation half-life after system administration and to facilitate its passive targeting to a site of inflammation. When tested in a mouse calvarial osteolysis model, the liposome targeted the site of inflammation and was retained at the calvarial lesion. End point analysis by high resolution X-ray and μ-CT indicated that the liposome formulation was more effective in reducing bone osteolysis compared to a comparable dose of systemically administered free drug.

INTRODUCTION

Orthopedic implant wear particle-induced aseptic loosening is a major cause of clinical failure after total joint replacement surgery. The age of patients receiving this surgery is decreasing, mainly due to high obesity rates and increasing demand from the younger patients. Epidemiologic studies indicate that 10-20% of the implants need to be revised within 15-20 years after arthroplasty due to wear particle-induced osteolysis and implant loosening.

Recently, Tofacitinib, a selective Janus kinase-3 (JAK-3) inhibitor, has been approved by the US Food and Drug Administration (FDA) for the treatment of adults with moderate to severe rheumatoid arthritis (RA) who have not responded adequately to, or are intolerant of, methotrexate. Tofa treatment has been shown to reduce disease activity and to suppress osteoclast-mediated bone erosions and structural damage to arthritic joints.[1] As an immune suppressor, however, Tofa shows dose-dependent toxicities, including risk of severe infections, cancer, liver enzyme elevation and hematologic abnormalities, which may be attributed to its ubiquitous biodistribution and its twice daily dosing frequency that is necessitated by its relatively short half-life. To address these limitations, we prepared a PEGylated liposome formulation of Tofa to increase the drug’s half-life, enhance its therapeutic efficacy and potentially reduce the associated toxicities by preferentially targeting the site of inflammation. When tested in a mouse model of orthopedic wear particle-induced osteolysis, the formulation was found to target the calvarial lesion and to inhibit osteoclast-mediated osteolysis.

EXPERIMENTAL METHODS

1. Tofa-PEGylated liposome preparation.

Tofa hydrochloride (equivalent Tofa 75 mg/mL in water, 15mL) was added to a solution of methanol/ether=1/6, 70 mL, egg PC (800 mg), cholesterol (200 mg) and DSPE (200 mg) under constant stirring. The emulsion was sonicated (conditions: 40 W, amplifier 9) for 5 min with a 5 s on, 5 s off pattern. The organic solvent was then removed by the rotary evaporator to produce the Tofa liposome formulation. After the ultra-centrifugation (48000 rpm under 4 °C for 1.5 hr), the free drug was removed and the liposome pellet was reconstituted to provide the final liposome formulation for in vivo study. The Tofa content was analyzed by UV spectrophotometer.

2. Mouse calvarial osteolysis model treatment protocol

Poly(methyl methacrylate) (PMMA) particles (particle diameter at 1–10 μm, Bangs Laboratories, Fishers, IN) were soaked in 70 % ethanol overnight, then washed and suspended in sterile phosphate-buffered saline (PBS) prior to implantation. Male Swiss Webster mice (6 weeks, Charles River Laboratories Inc.,
Wilmington, MA) were anesthetized with isoflurane through inhalation. PBS (100 μL, for sham control) or PMMA (30 mg suspended in 100 μL PBS) was deposited onto the calvarial surface through a 25G needle, which was inserted locally to the center of the calvaria, after gentle removal of the periosteum on day 0 with the needle tip.

Sixteen male Swiss Webster mice were randomly assigned into the following groups: sham control without particle deposition; saline injection as a negative control (100 μL, i.v. injections for 6 successive days); Tofa hydrochloride as free drug (single dose = 50 mg/kg, i.v. injections for 6 successive days); PEGylated Tofa liposome (single dose=75 mg/kg, i.v. injections on Days 3, 6). On Day 8, all of the mice were euthanized and the upper skulls of the animals were isolated. Calvaria were then fixed in formalin over 48 h and stored in 70 % ethanol for high-resolution X-ray analysis (Faxitron MX20, Faxitron X-Ray, Lincolnshire, IL, USA).

3. Passive targeting of the Tofa-containing liposomes to the calvarial lesion

Mice with or without PMMA deposition were given IRDye labeled PEGylated Tofa liposomes through i.v. injection. Optical images were obtained using a Pearl® Impulse small animal imaging system (LI-COR, Lincoln, NE) at different time intervals for 36 hrs.

RESULTS AND DISCUSSION

The diameter of the Tofa-containing liposome was determined by DLS nanosizer (Malvern Instruments Limited, Worcestershire, UK). (Figure 1) as 167.8 nm with PDI=0.083.

Calvaria X-ray images taken after the different treatments (Figure 1) demonstrate that systemic treatment with the Tofa-containing PEGylated liposomes reduced the size of the calvarial bone erosions compared to the free Tofa treatment.

Mice were imaged at intervals of up to 36h after systemic administration of the IRDye labeled PEGylated Tofa liposomes. Figure 2 demonstrates localization and retention of the Tofa-liposomes at the site of calvarial inflammation.

CONCLUSION

These results demonstrate that a single systemic administration of Tofa-containing liposomes can preferentially localize to a site of inflammation and produce sustained inhibitory activity in the osteolysis model. Tofa-containing liposomes represent a novel approach for targeted delivery of a kinase pathway inhibitor to a site of inflammation, and thereby reducing potential off-target toxicities and sustaining local drug concentration with prolonged therapeutic efficacy.

REFERENCES


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