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Effective long-term immunosuppression in rats by subcutaneously implanted sustained-release tacrolimus pellet: Effect on spinally grafted human neural precursor survival

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ABSTRACT

Achievement of effective, safe and long-term immunosuppression represents one of the challenges in experimental allogeneic and xenogeneic cell and organ transplantation. The goal of the present study was to develop a reliable, long-term immunosuppression protocol in Sprague–Dawley (SD) rats by: 1) comparing the pharmacokinetics of four different subcutaneously delivered/implanted tacrolimus (TAC) formulations, including: i) caster oil/saline solution, ii) unilamellar or multilamellar liposomes, iii) biodegradable microspheres, and iv) biodegradable 3-month lasting pellets; and 2) defining the survival and immune response in animals receiving spinal injections of human neural precursors at 6 weeks to 3 months after cell grafting. In animals implanted with TAC pellets (3.4 mg/kg/day), a stable 3-month lasting plasma concentration of TAC averaging 19.1 ± 4.9 ng/ml was measured. Analysis of grafted cell survival in SOD+ or spinal trauma-injured SD rats immunosuppressed with 3-month lasting TAC pellets (3.4–5.1 mg/kg/day) showed the consistent presence of implanted human neurons with minimal or no local T-cell infiltration. These data demonstrate that the use of TAC pellets can represent an effective, long-lasting immunosuppressive drug delivery system that is safe, simple to implement and is associated with a long-term human neural precursor survival after grafting into the spinal cord of SOD+ or spinal trauma-injured SD rats.

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Introduction

One of the essential requirements for successful translation of experimentally-defined cell-based replacement therapies which utilize the allogeneic or xenogeneic cell grafts into clinical practice is the development of safe and effective immunosuppression protocols that will permit long-term survival and maturation of grafted cells. Current clinical and experimental immunosuppression protocols typically use single or combined immunosuppressive drug regimens with drugs delivered orally, intraperitoneally, intravenously or subcutaneously in a single daily dose or divided into multiple daily doses [see reviews (Barraclough et al., 2011; Halloran, 1996; MacGregor and Bradley, 1995; Wente et al., 2006)]. While in human clinical settings a targeted plasma concentration of a variety of immunosuppressant drugs can effectively be achieved by a drug dose titration, accomplishing comparable consistency in targeted plasma levels in animal studies remains a major challenge.

Besides cyclosporines, mycophenolate mofetil (MMF), rapamycin or prednisolone, TAC (FK-506, Prograf) represents an immunosuppressant of choice and is frequently used as a solo therapy or in combination with other immunosuppressive drugs (i.e., MMF) (Hefferan

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et al., 2011; Reis et al., 1998; Xu et al., 2010) [see reviews (Lama et al., 2003; Su et al., 2011)]. TAC couples with immunophilins, proteins termed FK-506 binding proteins (FKBPs) (Siekierka et al., 1989; Thomson et al., 1995). The formation of a pentameric complex comprised of TAC, FKBPs, calcineurins A and B and calmodulin results in the inhibition of the phosphatase activity of calcineurin (Halloran, 1996; McKeon, 1991). The action of transcription factors requiring dephosphorylation for transport to the cell nucleus is inhibited and leads to the suppression of T-cell proliferation and function (Thomson et al., 1995).

In human clinical allogeneic organ transplantation, the recommended concentration of TAC in blood is in the range of 10–20 ng/ml (Pirsch et al., 1997; Przepiorka et al., 1999; Staatz and Tett, 2004) and is effective in maintaining long-term survival of transplanted solid organs (such as kidney, bone marrow or liver) with tolerable side effects typically presented as nephrotoxicity, neurotoxicity, gastrointestinal toxicity or drug-induced diabetes (Vicari-Christensen et al., 2009). In experimental allograft or xenograft animal studies that use rodents (mice, rats) or minipigs as recipients, TAC is typically administered using a chronically implanted intravenous catheter, intraperitoneally or subcutaneously, with doses ranging from 0.05 to 3 mg/kg/24 h (Gold et al., 1995; Hefferan et al., 2011; Saxena et al., 2007; Tze et al., 1992; Usvald et al., 2010). However, despite the use of such aggressive immunosuppressive protocols, experimental xenograft studies are frequently hampered by inconsistent graft survival particularly seen in long-term survival studies. It is believed that the oscillation in plasma drug concentrations and/or insufficient target plasma levels may in part account for inconsistent graft survival. In addition, the requirements of BID (from latin “bis in die”: two times a day) injections in order to achieve satisfactory TAC levels and to minimize toxicity make this approach i) labor intensive, ii) frequently associated with side effects resulting from repetitive animal injections (such as local inflammatory changes and infection), and iii) associated with systemic side effects such as nephrotoxicity and hepatotoxicity [see reviews (Finn, 1999; Gijsen et al., 2010; Teh et al., 2011)].

To extend the half-life of administered drugs in general, several longer-releasing formulations were developed. *First*, the use of TAC-loaded liposomes has been shown to provide moderate prolongation of the TAC half-life in the whole blood of naïve rats in comparison with conventional i.v. injections of TAC diluted in saline (Ko et al., 1994; McAlister, 1998). *Second*, the use of biodegradable microspheres was shown to provide a relatively stable level of TAC in whole blood for up to 10–21 days after single s.c. administration (Miyamoto et al., 2004; Wang et al., 2004). *Third*, the use of implantable biodegradable pellets has been successfully used to deliver a variety of synthetic drugs or hormones in human patients and in animal experimental models and showed up to 3–6 months of stable drug release after a single pellet implantation (Jockenhovel et al., 1996; Packard, 1992; Srinivasan et al., 2002; Studd and Magos, 1987). To our knowledge, no immunosuppressive pellet formulation has been reported to be successfully used in rodent or other animal models of xenogeneic neural precursor transplantation.

Accordingly, the goal of the present study was two-fold. First we characterized the pharmacokinetics of four different subcutaneously delivered/implanted TAC formulations, including: i) caster oil/saline solution, ii) unilamellar or multilamellar liposomes, iii) biodegradable microspheres, and iv) biodegradable 3-month lasting pellets. The optimal TAC formulation, as defined by simplicity of TAC delivery and stable/predictable blood TAC concentration was then selected and used in the second component of our study. The primary goal of the second part of the study was to validate the level of functionally effective immunosuppression in a separate group of $SOD1^{G93A}$ transgenic or spinal trauma-injured SD rats implanted with 3-month lasting TAC pellets and grafted spinally with human fetal spinal stem cells (hSSC) or human ES-derived neural precursors (ES-NPC). The survival of grafted cells was determined at 1–3 months after grafting using human-specific antibodies and confocal microscopy.

In addition, the potency of TAC pellet-induced immunosuppression was validated by the quantitative analysis of the circulating T-cell population (CD45, CD4, CD8) and by the qualitative and quantitative analyses of the infiltrating T-lymphocytes (CD45, CD4, CD8) in cell-grafted spinal cord regions.

Our results indicate that s.c. implanted 3-month lasting biodegradable TAC pellets represent an effective, safe and simple method to achieve long lasting and effective immunosuppression as evidenced by i) consistent xenograft survival and cell maturation, ii) near complete suppression of grafted site T-cell infiltration, and iii) suppression of circulating blood T-cell concentration.

Material and methods

All procedures were approved by the Institutional Animal Care and Use Committees by the University of California, San Diego and by the Czech Academy of Sciences. Adult Sprague–Dawley albino rats (Velaz Praha, Czech Republic and Harlan Industries, Indianapolis) and $SOD1^{G93A}$ ALS rats (SOD+) (UCSD colony, Dr. D. W. Cleveland, San Diego, California; 49–57 days old) were used in experiments. Animals were housed in standard cages with free access to food and water.

Animal experimental groups were divided into 2 principal studies: i) TAC pharmacokinetic study, and ii) spinal grafting of human neural precursors in TAC pellet-immmunosuppressed animals.

TAC pharmacokinetic study

Four different TAC (Prograf®, Astellas Pharma, Deerfield, Illinois, USA) vehicle-delivery systems were used and delivered into the subcutaneous space (see Table 1 for summary).

1) TAC caster oil/saline solution (Group No. 1 and No. 2):
   Because the hydrophobic nature of TAC powder and its poor solubility in water solutions (e.g., saline) (Kino et al., 1987), TAC powder was dissolved in a mixture of 100% ethanol (8% of total volume), caster oil (2% of total volume) and sterile saline for injections (90% of total volume; Fig. 1g). Two dosing designs were studied. In the first dosing design, animals (n = 4; Grp. No. 1) received 3 mg/kg of TAC in 24-h intervals for a total of 5 days. Blood samples for TAC measurement were collected at 2, 9, 24, 72 and 120 h. At 24 and 72 h the blood samples were collected just before subsequent TAC injection. In the second dosing design, animals (n = 4; Grp. No. 2) received 1.5 mg/kg of TAC in 12-h intervals for a total of 5 days. Blood samples for TAC measurements were collected at 2, 12, 14, 24 and 120 h. At 12 and 24 h the blood samples were collected just before subsequent TAC injections.
2) TAC liposomes (Group No. 3 and No. 4):
   Two structurally different liposome designs (unilamellar or multilamellar; Figs. 1I, J) were used (Encapsula NanoSciences LLC, TN). In the first group, TAC-loaded unilamellar liposomes (n = 4; 3 mg/kg; Grp. No. 3) were used. In the second group, TAC-loaded multilamellar liposomes (n = 4; 3 mg/kg; Grp. No. 4) were used. In both groups, TAC liposomes were injected as a single bolus. Blood samples for TAC measurements were collected at 2, 12, 24, 48 and 72 h.
3) TAC microspheres (Group No. 5 and No. 6):
   TAC-containing microspheres were prepared from tacrolimus powder and poly (D,L-lactide-coglycolide) copolymer (Resomer LG 503H, Aldrich) adopting the procedure previously described (Wang et al., 2004). The tacrolimus content in the resulting dry TAC microspheres was 45 mg TAC/g of microspheres as determined by HPLC. Rats were injected with a single bolus of TAC-containing microspheres at a dose of 10 mg/kg (n = 3; Grp. No. 5) or 20 mg/kg (n = 3; Grp. No. 6). Blood samples for TAC measurements were collected at 2, 9, 24 h and at 2, 4, 7, 10, 13, 16, 19, and 22 days.