Toxicology and Applied Pharmacology 282 (2015) 207–214

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Dimethyl sulfoxide inhibits spontaneous diabetes and autoimmune recurrence in non-obese diabetic mice by inducing differentiation of regulatory T cells

Gu-Jiun Lin [a], Huey-Kang Sytwu [b], Jyh-Cherng Yu [c], Yuan-Wu Chen [d,e], Yu-Liang Kuo [f,g], Chiao-Chi Yu [a,c], Hao-Ming Chang [c], De-Chuan Chan [c], Shing-Hwa Huang [a,c,*]

[a] Department of Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan, ROC
[b] Department of Microbiology and Immunology, National Defense Medical Center, Taipei, Taiwan, ROC
[c] Department of General Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC
[d] School of Dentistry, National Defense Medical Center, Taipei, Taiwan, ROC
[e] Department of Oral and Maxillofacial Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC
[f] Department of Medical Imaging, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC
[g] School of Medical Imaging and Radiological Sciences, Chung Shan Medical University, Taichung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 1 September 2014
Revised 19 November 2014
Accepted 24 November 2014
Available online 4 December 2014

Keywords:
Type 1 diabetes
Islet transplantation
NOD mice
Autoimmune recurrence
Dimethyl sulfoxide
Regulatory T cell

ABSTRACT

Type 1 diabetes mellitus (T1D) is caused by the destruction of insulin-producing β cells in pancreatic islets by autoimmune T cells. Islet transplantation has been established as an effective therapeutic strategy for T1D. However, the survival of islet grafts can be disrupted by recurrent autoimmunity. Dimethyl sulfoxide (DMSO) is a solvent for organic and inorganic substances and an organ-conserving agent used in solid organ transplantations. DMSO also exerts anti-inflammatory, reactive oxygen species scavenger and immunomodulatory effects and therefore exhibits therapeutic potential for the treatment of several human inflammatory diseases. In this study, we investigated the therapeutic potential of DMSO in the inhibition of autoimmunity. We treated an animal model of islet transplantation (NOD mice) with DMSO. The survival of the syngeneic islet grafts was significantly prolonged. The population numbers of CD8, DC and Th1 cells were decreased, and regulatory T (Treg) cell numbers were increased in recipients. The expression levels of IFN-γ and proliferation of T cells were also reduced following DMSO treatment. Furthermore, the differentiation of Treg cells from naive CD4 T cells was significantly increased in the *in vitro* study. Our results demonstrate for the first time that *in vivo* DMSO treatment suppresses spontaneous diabetes and autoimmune recurrence in NOD mice by inhibiting the Th1 immune response and inducing the differentiation of Treg cells.

Introduction

Autoimmune diabetes, formally referred to as type 1 diabetes, results from the destruction of insulin-producing β cells in the islet of the pancreas and has been identified as a T cell-mediated autoimmune disease (Kawasaki et al., 2004). The development of T1D is usually diagnosed in young patients; thus, this disease is also termed juvenile-onset diabetes or childhood-onset diabetes. T1D only exhibits 30–50% concordance in monozygotic twins (Barnett et al., 1981), thereby suggesting that both genetic predisposition and environmental factors contribute to the pathogenesis of diabetes (Kaufman et al., 1992; Abdeen et al., 2007; Nejentsev et al., 2007). The NOD mouse model is frequently used for T1D studies. This mouse model spontaneously develops T cell-dependent β cell destruction resembling human T1D and serves as an animal model for this autoimmune disease (Aoki et al., 2005).

The classical therapeutic strategy for patients with T1D is the administration of insulin injections to maintain normal levels of blood glucose. However, this approach is unable to provide real-time blood glucose modulation and is ineffective in maintaining stable blood glucose levels, which frequently leads to clinical complications, such as retinopathy, nephropathy, neuropathy and macrovascular disease (O'Brien and Corrall, 1988). Maintaining stable glucose levels is important to prevent the development of secondary complications in T1D.

Islet transplantation has been reported as an effective strategy to accomplish insulin independence, normoglycemia and long-term hemostasis of blood glucose in T1D patients (Gaglia et al., 2005). Moreover, islet transplantation is relatively simple to administer, as it does not

Abbreviations: T1D, type 1 diabetes mellitus; NOD mice, non-obese diabetic mice; DMSO, dimethyl sulfoxide; Treg, regulatory T cell; DCs, dendritic cells; IFN, interferon; IL, interleukin

* Corresponding author at: Department of General Surgery, Tri-Service General Hospital, Taipei, No. 325, Section 2, Chenggong Road, Neihu, Taipei 114, Taiwan, ROC. Fax: +886 2 87927372.
E-mail address: h610129@gmail.com (S.-H. Huang).

http://dx.doi.org/10.1016/j.taap.2014.11.012

require major surgical procedures. The procedure can perform on an out-patient basis under local anesthesia and can be repeated several times without major discomfort to the patient (Bottino et al., 2002). Islet transplantation achieves nearly perfect blood glucose monitoring and modulation in T1D patients (Gaglia et al., 2005). However, islet grafts have been shown to be destroyed due to allogeneic graft rejection and autoimmune recurrence (Balamurugan et al., 2006). Autoreactive T cells harbor the memory of β cells and are responsible for this autoimmune recurrence. Islet grafts in NOD mice frequently undergo early graft failure because of the immediate destruction of the graft before immunological graft rejection (Gysemans et al., 2000; Okitsu et al., 2001). Young et al. (2004) have also demonstrated that transplanted islet grafts in NOD mice are susceptible to recurrent autoimmunity. Previous studies have reported that human islets from genetically identical twins (Sibley et al., 1985) or cadaver donors (Tyden et al., 1996) were destroyed by recurrent autoimmunity. Therefore, the establishment of a strategy to suppress autoimmune recurrence is critical for islet transplantation in patients with T1D.

Dimethyl sulfoxide (DMSO) is a colorless, hygroscopic liquid chemical that is a powerful solvent for organic or inorganic substances. It is used for a variety of laboratory and clinical purposes. DMSO is frequently used as a solvent in biological studies and a vehicle for drug therapy. Moreover, DMSO has been applied for therapeutic purposes in several clinical diseases. It has been approved by the United States Food and Drug Administration for the treatment of interstitial cystitis by intravesical instillation (Kato et al., 2000; Santos et al., 2003). It also been used to treat localized amyloidosis and improves renal function and proteinuria in renal amyloidosis patients caused by Crohn's disease (Iwakiri et al., 1999; Kato et al., 2000; Amemori et al., 2006). Furthermore, DMSO is a hydrogen-bond disrupter, hydroxyl radical scavenger and cryoprotectant (Amemori et al., 2006). Due to anti-inflammatory properties of DMSO, it has been justified for the treatment of inflammatory diseases (e.g., scleroderma, osteoarthritis, and rheumatoid arthritis) (Kato et al., 2000; Santos et al., 2003), gastrointestinal diseases (Salim, 1991a,b, 1992), some manifestations of amyloidosis (Morassi et al., 1989; Iwasaki et al., 1994; McCammon et al., 1998; Ozkaya-Bayazit et al., 1998), brain edema (Ikeda and Long, 1990) and chronic prostatitis (Shirley et al., 1978).

The immunomodulatory function of DMSO has recently been described. Kloverpris et al. (2010) have reported that proliferative and secretion of inflammatory cytokines in human CD4 and CD8 T cells were reduced following treatment in 2–3% DMSO solution for 7 days. Lin et al. (1995) have demonstrated that a 4-day treatment with DMSO (>2%) induces apoptosis in Burkitt Lymphoma cells. Given these immunomodulation and anti-inflammatory effects of DMSO, we further investigated whether DMSO treatment prevents the onset of spontaneous diabetes and prolongs the survival of islet grafts in a syngeneic islet transplantation model. Our results demonstrate that DMSO treatment significantly prevented the onset of diabetes and prolonged islet graft survival in NOD mice. To elucidate the mechanisms of this protective effect, we investigated the influence of DMSO on immune cells with regard to cytokine profiles and proliferation of T cells. DMSO treatment reduced the proportion of cytotoxic T cells and DCs and increased the proportion of Treg cells in the spleens of NOD recipients. We further demonstrated that DMSO increases the differentiation of Treg cells from the naive CD4 T cells of NOD mice. Overall, DMSO treatment reduced diabetic incidence in NOD mice and prolonged the survival of syngeneic islet grafts. This protective effect is, at least partly, associated with increased differentiation of Treg cells in the modulation of immune cell effector functions.

Materials and methods

Animals. Inbred NOD/Sytwu mice (K^d, D^b, L^d, $I\text{-}A^{g7}$) and NOD/scid mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and subsequently bred at the animal center of the National Defense Medical Center in Taipei, Taiwan, under specific pathogen-free conditions. The spontaneous incidence of diabetes in the colony was 80–90% in females and 20–30% in males by 30 weeks of age.

Islet transplantation. Newly diagnosed NOD female mice, with blood glucose levels between 300 and 500 mg/dL for two consecutive days, were used as recipients for islet transplantation as previous described (Lin et al., 2009). The syngeneic islets were isolated from 5- to 8-week-old non-diabetic male NOD mice. The onset of autoimmune diabetes in male NOD mice is later than female; therefore, we can isolate more intact islets form male NOD mice by this age. Islets measuring between 75 and 150 μm in diameters were selected by hand. Islets (700) were transplanted into the kidney subcapsular space of the recipients. Blood glucose levels lower than 200 mg/dL on the first and second day after islet transplantation were considered to represent a successful transplantation.

Blood glucose monitoring. Blood glucose was monitored daily with a blood glucose test strip (MediSense Optium Xceed, Abbott Diabetes Care Inc., Alameda, CA, USA). Graft rejection was defined as blood glucose levels higher than 300 mg/dL for 2 consecutive days.

Histological analysis. Kidneys were harvested from NOD recipients and then embedded in OCT. Sections (4 μm in thickness) were cut and stained with hematoxylin and eosin (H&E) staining and then analyzed via light microscopy.

Insulin secretion test. Islets were isolated from untreated control mice, PBS-treated mice, and DMSO-treated mice. Fifteen islets were incubated in F12K medium (Gibco, Auckland, NZ) containing 16.7 mM or 2.8 mM glucose for 1 h in Transwell plates. At the end of incubation period, islets were removed and the secreted insulin in the medium was measured with a mouse insulin ELISA kit (Mercodia, Uppsala, Sweden). The stimulation index was calculated with the ratio of insulin secreted at 16.7 and 2.8 mM glucose incubated for 1 h.

T cell proliferation. Splenocytes isolated from female NOD mice were treated with Tris-buffered ammonium chloride to eliminate erythrocytes. After washing, cells were resuspended at a concentration of 5×10^6 cells/mL in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. The cells were stimulated with plate-coated anti-CD3 antibody (Clone 145-2C11; BD Biosciences Pharmingen, San Jose, CA, USA), or concanavalin A (Con A; Sigma-Aldrich, Saint Louis, MO, USA). When stimulated with islet antigen, the cells were resuspended in serum-free RPMI-1640 supplemented with 100 U/mL penicillin G, 0.1 mg/mL streptomycin, 0.1 mM MEM non-essential amino acids (NEA, GIBCO Invitrogen, Carlsbad, CA, USA) and 50 μM 2-mercaptothanol (2-ME; Sigma-Aldrich, Saint Louis, MO, USA). After 54 h, the cultured cells were pulsed with 1 μCi of ^{3}H-methyl thymidine (PerkinElmer, Waltham, MA, USA). The plates were then harvested onto a UniFilter-96, GF/C (PerkinElmer) at 72 h. The incorporated ^{3}H-methyl thymidine was measured at 72 h with a TopCount liquid scintillation counter (Packard Instrument Co., Meriden, CT, USA). The level of [^{3}H]-thymidine incorporation for each group was normalized to that of the unstimulated control, thereby producing a ratio referred to here as the stimulation index (S.I.).

Flow cytometry. Lymphocytes were harvested from spleen stained with allophycocyanin-conjugated anti-mouse CD4 (clone GK1.5), phycoerythrin (PE)-conjugated anti-mouse CD8α (clone 53-6.7), fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD19 (clone 1D3), and PE-conjugated anti-mouse CD25 (clone PC61) (BD Pharmingen) for 30 min at 4 °C. For Foxp3 staining, the cells stained with surface molecules were fixed and permeabilized overnight with Fixation/Permeabilization working solution (eBioscience Inc., San Diego, CA, USA).