The effects of dexpanthenol in streptozotocin-induced diabetic rats: Histological, histochemical and immunological evidences

K. Gulle, N.G. Ceri, M. Akpolat, M. Arasli and B. Demirci

Introduction

Type-1 diabetes mellitus (T1DM) is a metabolic endocrine disease with autoimmune background, caused by the massive destruction of insulin-producing pancreatic β-cells localized in islets of Langerhans. This condition leads to insufficient insulin production, which in turn lead to an excessive increment in blood glucose levels with multiple local and systemic pathological effects (Eizirik and Mandrup-Poulsen, 2001).

Various cellular interactions including pro-inflammatory cytokines, autoreactive T cells and macrophages have been implied in pathophysiological process of T1DM. When T cells are activated, they trigger the necrotic process in target cells by releasing some enzymes such as perforin and granzymes, by producing several pro-inflammatory cytokines including interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) or by activating the Fas receptors on target cell surface. All these mentioned mechanisms have also been described as responsible factors for pancreatic islet β-cell death process (Kagi et al., 1997; Petrovsky et al., 2002). In particular, it has been suggested that some cytokines secreted by macrophages and T cells such as interleukin-1β (IL-1β), TNF-α and IFN-γ have a more
significant role in T1DM development than formerly considered. Iwahashi “et al.” have reported that these cytokines are the main inducers of β-cell stress responsible for the remarkable levels of β-cell death in experimental animal T1DM models (Rabinovitch et al., 1994; Iwahashi et al., 1996).

Increased production of reactive oxygen species (ROS) along with the decreased synthesis antioxidant enzymes can trigger the formation of oxidative stress, which has deleterious effects on different cell and tissue types (Sies, 1997). Cytokines can induce multiple stress pathways in β-cells. IL-1β, TNF-α and IFN-γ induce the ROS production and nitric oxide (NO) via inducible NO synthase (iNOS) expression (Rabinovitch and Suarez-Pinzon, 1998). Generated free oxygen and nitrogen radicals can react to form peroxynitrite, which is a very strong oxidant. Oxygen and nitrogen free radicals, as well as the radical peroxynitrite can interact with and damage a range of cellular proteins, which in turn can block metabolic functions and induce β-cell death (Azevedo-Martins et al., 2003). It has been indicated that β-cells are especially susceptible to such oxidative stress, attributed to their low levels of antioxidant enzyme production (Lenzen et al., 1996; Tiedge et al., 1998).

Furthermore, oxidative stress may also cause several adverse effects commonly seen in diabetes mellitus (Hamden et al. 2009). Antioxidants have been shown to prevent the β-cell damage via inhibiting the peroxidation chain reaction, and thus may be helpful in diabetes management (Jain, 2006; Liang et al., 2013).

Dexpanthenol (Dxp) is an anti-inflammatory and antioxidant agent with cellular restoration effect, which is converted to pantothenic acid (PA) in tissues (Slyshenkov et al., 1999; Ceylan et al., 2011; Altintas et al., 2012) in addition to its cellular repair capability (Hayes and McLellan, 1999). There are numerous studies conducted to investigate the protective effect of PA and its derivatives against ROS activity in tissues (Slyshenkov et al. 1998; Wojtczak and Slyshenkov 2003; Slyshenkov et al., 2004). It has been reported that Dxp exhibited an anti-inflammatory effect on experimental ultraviolet-induced erythema (Tauschel and Rudolph 1982; Stozkowska and Piekos, 2004). Beneficial effects of Dxp have been observed in patients who have undergone skin transplantation / scar treatment, or therapy for burn injuries and different dermatoses (Ebner et al., 2002).

This study was designed to investigate whether Dxp has protective effect against hepatic and pancreatic damage induced by streptozotocine (STZ)-induced diabetes in an experimental in-vivo rat model. We have evaluated the histopathological and immunological data established from this study in order to reveal and interpret the possible effects of Dxp.

Material and methods

Animals

Wistar albino male rats were provided from the Adnan Menderes University Animal Care and Research Unit (Aydin, Turkey). All animals were daily fed tap water and pellet foods (Gebze Food Factory, Kocaeli, Turkey), including 21% pure protein under optimum laboratory conditions (temperature, 22±2°C; humidity, 50-55%; light/dark period: 12 h/12 h). All the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US Public Health Service. The study was approved by the Institutional Animal Ethical Committee of Adnan Menderes University (Aydin, Turkey).

Experimental design

Twenty-four Wistar albino male rats were randomly allotted to one of four experimental groups: control, Dxp, STZ-induced diabetic (STZ) and diabetic treatment with Dxp (STZ-Dxp) groups; each contained six animals.

Diabetes was induced in STZ and STZ-Dxp groups by a single intraperitoneal (i.p) injection of STZ (50 mg/kg) (Kanter et al., 2009). Three days after STZ treatment, development of diabetes in two experimental groups was confirmed by measuring (IME-DC® Glucosticks) blood glucose levels in a tail vein blood sample. Rats with blood glucose levels of 200 mg/dL or higher were considered to be diabetic. Serum glucose levels in control animals remained normal for the duration of the study. Control rats were injected with the same volume of physiologic saline as the diabetic animals that received STZ. The rats in Dxp treated groups were given Dxp (300 mg/kg) once a day i.p. for 6

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</table>
| 1st day | Randomization<br>
Administration of physiologic saline to the rats in groups control and STZ by i.p. injection.  
Administration of STZ to the rats in group STZ by i.p. injection.  
Administration of Dxp to the rats in groups Dxp and STZ-Dxp by i.p. injection. |
| 3rd day | Measurement of blood glucose levels in STZ administered rats.       |
| 6 week  | Killing of the rats in groups control, Dxp, STZ, and STZ-Dxp.       |
weeks. Control and diabetic untreated rats were also given the same volume of physiologic saline as the diabetic treated animals that received Dxp (Table 1).

**Histopathological and histochemical examination**

The rats were anesthetised with an intraperitoneal injection of ketamine 90 mg/kg (Ketalar, Eczacibasi, Istanbul, Turkey) and xylazine 10 mg/kg (Rompun, Bayer, Istanbul, Turkey). Biopsies from the liver and pancreatic tissues of the rats were harvested, and tissue fragments were fixed in 10% neutral buffered formalin solution, embedded in paraffin. In order to clarify the severity of glycogen depletion in the liver, special histochemical staining of periodic acid shift (PAS) was conducted. The evaluation criteria were as follows: zero for no detectable, 1 for mild, 2 for moderate and 3 for severe glycogen depletion (Table 2). Additionally, the hematoxyline-eosin (H-E) and Masson's trichrome staining was performed on the liver samples to evaluate any diabetes-induced histological damages. For each animal in the tests and control groups, at least three slides from the liver samples were prepared and scored. The histopathological examinations were performed by a histologist who was completely unaware of the study purposes. The preparations were evaluated by means of a bright-field microscope and photographed (Carl Zeiss Axio Lab A1, Germany).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zero (0)</th>
<th>Mild (1)</th>
<th>Moderate (2)</th>
<th>Severe (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dxp</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STZ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>STZ+Dxp</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Changes in hepatic glycogen depletion score (HGD) in various experimental groups. (n=6).

Fig. 1. Changes in histology of liver tissues in the control (A), Dxp (B), STZ (C) and STZ-Dxp (D) groups. Hematoxylin-eosin. Scale bars: 50 μm.
**Immunohistochemistry**

The pancreatic tissues collected were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 4-6µm thickness. Paraffin sections of the pancreas were deparaffinised in xylene and rehydrated in graded alcohol. Antigen retrieval was performed by boiling the sections in 0.1M sodium citrate followed by treatment with 3% H₂O₂ in deionised water for ten minutes to quench endogenous peroxidase activity. After blocking with 10% normal serum for one hour at room temperature, sections were incubated with anti-insulin antibody (18-0066; Zymed, San Francisco, CA, USA) for one hour, and then at room temperature the sections were washed in PBS (3x5 minutes). The specimens were then incubated with biotinylated secondary antibodies for 30 minutes at 37°C followed by incubation with ABC reagent (VECTASTAIN Elite ABC kit; Vector Laboratories, Burlingame, CA) for 30 minutes at 37°C. The sections were developed using a DAB substrate kit (Vector Laboratories) for three minutes and counterstained hematoxylin.

**Analysis of cytokine and chemokine levels**

Blood samples were obtained by cardiac puncture of the anesthetised rat. After the blood clotted, the samples were centrifuged and serum was immediately stored at -80°C until assayed for analysis. Proinflammatory cytokine (IL-1α) and chemokine (MCP-1) concentrations were determined in each serum sample of all the rat groups. IL-1α and MCP-1 were determined with the commercially available fluorescent bead FlowCytomix Multiplex Rat Cytokines 6 plex immunoassay (eBioscience, Vienna, Austria), according to the manufacturer’s instructions. The samples were analysed by the Beckman Coulter Cytomix FC500 Flow Cytometer (Miami, Fl, USA). The resulting data were

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**Fig. 2** Changes in connective tissue of portal area in the control (A), Dxp (B), STZ (C) and STZ-Dxp (D) groups. Masson’s trichrome. Scale bars: 20 µm.
analysed using FlowCytomix Pro 2.3 software (eBioscience, Vienna, Austria).

**Statistical analysis**

The results were expressed as median (minimum - maximum). The normality distribution of the variables was tested using the one sample Kolmogorov-Smirnov test. Differences in the parameters measured among the groups were analysed by the Kruskal-Wallis test. When a significant difference was found, the Mann-Whitney U-test was used for multiple comparisons. P-values less than 0.05 were considered statistically significant.

**Results**

**Histopathological findings**

The histological examination of the H-E (Fig. 1), Masson's trichrome (Fig. 2) and PAS (Fig.3) stained control liver tissues showed normal architecture of hepatocytes (Figs. 1A, 2A, 3A). Liver section of STZ-induced diabetic group showed vacuolisation of hepatocytes in the liver parenchyma, with significant dilatation of sinusoidal spaces (Fig. 1C) and increasing amounts of connective tissue in the portal area (Fig. 2C). The STZ-Dxp group that received Dxp treatment showed partially normal hepatocyte chords. Neither vacuolation of hepatocytes nor sinusoidal dilatation was seen (Figs. 1D, 2D). Dxp supplementation did not show any remarkable effects in the group treated with Dxp alone compared with the control group (Fig. 1B, 2B).

The histochemical PAS staining showed that STZ-induced diabetes resulted in approximately complete depletion of hepatic glycogen (Fig. 3C), in comparison to the control group, while Dxp was able significantly (p <0.001) to recover the hepatic glycogen level (Fig. 3D).

The numerically estimated effects of diabetes and the

![Fig. 3 Changes in glycogen deposits in liver tissue in the control (A), Dxp (B), STZ (C) and STZ-Dxp (D) groups. Periodic acid-Schiff. Scale bars: 20 μm.](image)
treatment regimens on hepatic glycogen depletion were depicted in Table 3.

The histological examination of the H-E stained control pancreatic section showed normal appearance of pancreatic tissue, with a single islet of Langerhans seen in the centre (Fig. 4A). Pancreatic section of STZ-induced diabetic group showed β-cells with reduction of cytoplasmic mass, reduction of islet size and atrophy of β-cells with vacuolisation. The β-cell cytoplasm was very scanty (Fig. 4C). The severity of degenerative changes was lessened by Dxp supplementation in the STZ-Dxp group, compared with the STZ group, showing the islet cells had a good number of β-cells with abundant basophilic cytoplasm (Fig. 4D).

**Immunohistochemical findings**

Immunohistochemical staining for the pancreatic tissues of control rats elicited strong insulin antigen reactivity in the β-cells of the islets (Fig. 5A). In diabetic untreated rats, the cells were actually negative for insulin-immunoreactivity and only a few β-cells in some islets displayed mild insulin immunopositivity in small granules (Fig. 5C). In diabetic rats with DXP treatment, both the number of insulin-immunoreactive β-cells and the immunopositivity of their granules increased in comparison to that seen in diabetic untreated rats (Fig. 5D). Dxp- treated group insulin antigen positivity was close to control group (Fig. 5B).

**Effects on serum IL-1α, and MCP-1 levels**

To test whether DM induced a persistent and progressive inflammatory process, we assessed the level of serum cytokines and chemokines in all the groups. A significant increase in the levels of serum IL-1α...
(p=0.033) and MCP-1 (p=0.011) was observed in the STZ group, compared with the control group. Dxp-treated diabetic rats IL-1α and MCP-1 levels were similar to control value (Table 3).

**Discussion**

Diabetes mellitus results from selective destruction of the insulin-producing β-cells in the pancreatic islets (Teoh et al., 2010). Hence, search for new agents that protect β-cells from destruction and thereby prevent DM is needed. The present study demonstrated the role of Dxp in protecting against pancreatic β-cell death and hepatic injury caused by STZ-induced diabetes.

Liver and pancreas tissues both act as glucose sensors, and damage to these tissues plays an important role in the onset of diabetes. The pancreatic β-cell possesses the ability to respond to a minor increase in the blood glucose level, thereby maintaining that level

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1α (Min-Max)</th>
<th>MCP-1 (Min-Max)</th>
<th>HGD (Min-Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.17 (17.6-32.2)</td>
<td>230.99 (167.8-269.8)</td>
<td>0.30 (0.1-0.4)</td>
</tr>
<tr>
<td>Dxp</td>
<td>24.26 (15.7-39.2)</td>
<td>246.08 (107.2-307.1)</td>
<td>0.35 (0.3-0.5)</td>
</tr>
<tr>
<td>STZ</td>
<td>46.72 (23.2-60.6)</td>
<td>359.21 (295.1-400.6)</td>
<td>2.60 (2.5-2.7)</td>
</tr>
<tr>
<td>STZ+Dxp</td>
<td>19.54 (11.9-43.0)</td>
<td>269.86 (186.7-369.9)</td>
<td>0.85 (0.7-1.2)</td>
</tr>
</tbody>
</table>

p-value = 0.033 = 0.011 < 0.001

*: compared to control group. #: compared to STZ group

![Fig. 5](image)
The effects of dexpanthenol in diabetic rats

(Hellstrom, 1997). The liver plays a major role in maintaining glucose homeostasis by regulating glucose absorption, accumulation and catabolism through mediation of various metabolic signals (Ferre et al., 1996). Thus, the effect of protective agents on tissues such as the liver and pancreas that regulate glucose metabolism is an interesting area to explore.

In our study, histopathological examination of diabetic pancreas showed the shrinkage of islet cells and the growth of adipose tissue in the pancreas (data not shown). Treatment with Dxp on STZ-induced diabetic rats reduced the changes in the pancreas. Dxp-treated diabetic pancreas showed an increase in the size of the islets and decreased fatty infiltrate around the islets (data not shown). Dxp was able to protect the functional β-cells of the islets by its ability to decrease serum cytokine levels.

Histopathological examination of liver from diabetic rats revealed morphological changes. Diabetic liver showed dilatation of hepatic sinusoids and kupffer cell hyperplasia. Similar histopathological changes were reported in the liver of diabetic rats by Evelson et al. (2005). Dxp treatment showed partially normal hepatic parenchyma and only mild sinusoidal dilatation.

Since glycogen deposition from glucose in the hepatocytes of diabetic animals is defective, glycogen depletion became the main criterion for histopathological evaluation in the current study. Fernandez-Novell et al. (1994) demonstrated that in insulin-deficient animals, the rate of glycogen synthase phosphatase activity was lowered. Therefore, the protective effect of Dxp in normalising the glycogen level might be related to its ability to activate the glycogen synthase phosphatase. In our study, STZ-induced diabetic group exhibited a decreased hepatic glycogen level which was significantly restored by Dxp treatment. Dxp may bring about its anti-hyperglycemic effect by increasing glycogen stores in the liver. This prevents depletion of glycogen there, possibly due to stimulation of insulin release from β-cells. This beneficial effect of Dxp may occur due to its free radical scavenging properties. Dxp has a considerable antioxidant activity: it scavenges reactive oxygen species and may thereby prevent oxidative damage to the important biological macromolecules, such as DNA, proteins and lipids (Zakaria et al., 2011; Altintas et al., 2012). Zakaria et al. (2011) evaluated the effects of Dxp on cerebral ischaemia reperfusion-induced brain injury and suggested that treatment with Dxp effectively inhibits MDA levels (Zakaria et al., 2011). Altintas et al. (2012) examined the biochemical changes associated with ischaemia reperfusion-induced tissue injury in rats responding to Dxp and demonstrated that the systemic administration of Dxp prevents lipid peroxidation (Altintas et al., 2012).

DM is believed to be initiated by physiological β-cell death or islet injury triggering the homing of macrophages and dendritic cells that in turn launch an inflammatory reaction. The infiltrating macrophages secrete pro-inflammatory cytokines, namely IL-1β and TNF-α, as well as various chemokines that attract immune cells such as dendritic cells, macrophages and T lymphocytes. T cells recognising β-cell specific antigens become activated, infiltrate the inflamed islets and attack the β-cells (Baekkeskov et al., 1990; Lieberman et al., 2003; Nakayama et al., 2005). Some of the studies suggest that inflammatory parameters, including inflammatory cytokines TNF-α and IL-1β, are involved in the autoimmune process leading to pancreatic β-cell damage and the induction of DM (Mandrup-Poulsen et al., 1990; Lechleitner et al., 1999). In the present study, IL-1α, and MCP-1 level increased significantly in the serum of the STZ group. Treatment with Dxp significantly ameliorated these parameters.

In conclusion, the present morphological and immunological assessments show that Dxp possesses several beneficial properties including normalisation of cytokine levels, and pancreatic β-cell and hepatocyte protection. Taken together, these findings contribute to its protective effect in STZ-induced diabetic rats. Thus, Dxp may be identified as a preventive agent against diabetes. However, more work is warranted to elucidate its numerous mechanisms of action.

Conflict of interest. The authors declare that they have no conflict of interest.

References


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The effects of dexpanthenol in diabetic rats


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