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Original article

Partial recovery from alloxan-induced diabetes by sodium phthalhydrazide in rats

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ABSTRACT

In the commonly used experimental model of diabetes, a cytotoxic glucose analogue alloxan can selectively destruct pancreatic β-cells, with characteristics similar to the type-1 diabetes (T1D) in humans. Treatment of diabetic rats with sodium phthalhydrazide partially reversed diabetogenic pathology in the alloxan-induced diabetes. The alloxan-treated rats with permanent hyperglycemia, which further received i.p. twenty daily doses 2 mg/kg b.w. phthalhydrazide, showed at 60 days of the experiment a significant amelioration of the diabetes status. Hyperglycemia was decreased by 52%, glycated haemoglobin HbA1c returned to control value, insulin concentration significantly increased from 45,4% (alloxan group) to 59,5% (alloxan + phthalhydrazide) of the control values. Importantly, phthalhydrazide treatment of alloxan-treated diabetic rats markedly decreased the concentrationof interleukin-6 (IL-6) and corticosterone level. Morphometric analysis revealed a marked increase in the number of pancreatic islets/mm^2, and a number of cells/mm^2 in the pancreatic islets. These changes, including 3-fold increase in the number of insulin-producing cells and 2-fold decrease in blood glucose levels, correlated with the increased proliferative activity of pancreatic β-cells in the diabetic phthalhydrazide-treated animals. Interestingly, the number of CD68$^+$ cells/macrophages in the pancreatic islets, which was relatively high in the alloxan group (63,9 + – 16.4/mm^2), markedly decreased after the phthalhydrazide treatment (23,6 + –7,2/mm^2). Taking together with the previous data on the phthalhydrazide-related macrophage silencing, restriction of macrophage quantity in the alloxan-affected pancreatic islets can be possibly one of important events leading to the partial recovery from the β-cell disruption.

1. Introduction

Induction of insulin-dependent diabetes mellitus by chemical treatment of experimental animals is relatively well understood. Several compounds can be used to mimic human diabetes, including alloxan, streptozotocin, vacor, dithizone, goldthioglucose, monosodium glutamate and 8-hydroxyquinolone [1]. Alloxan is an unstable, cytotoxic glucose analogue, rapidly accumulating in pancreatic β-cells through glucose transporter 2 (GLUT2). Reduction of alloxan to dialuric acid with intracellular and redox cycling generate reactive oxygen species (ROS), responsible for the necrotic death of pancreatic β-cells [2]. In addition, due to its central 5-karbonyl group that reacts with thiol groups, alloxan was shown to inhibit pancreatic thiol enzymes such as glucokinase (hexokinase IV) [1,3]. Alternatively, different diabetes models exist, using animals with diabetogenic phenotype, characterized by mutated leptin receptor, spontaneous autoimmune diabetes, glucotoxicity-prone strains, and different obese- and nonobese models [4].

Pancreatic islets are highly vascularized bodies secreting insulin and other hormones in response to glucose and toxic glucose

Abbreviations: ADM, acinar-to-ductal metaplasia; ATP, adenosine triphosphate; DAB, 3.3-diaminobenzidine; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; GLUT2, glucose transporter 2; GSH, glutathione (reduced form); HbA1c, glycated haemoglobin; IGF-1, insulin-like growth factor 1; IL-6, interleukin 6; INF-γ, interferon gamma; LPS, lipopolysaccharide; NOD, non-obese diabetic mice; PDL, pancreatic ductal ligation; ROS, reactive oxygen species; SEM, standard error mediana; T1D, type-1 diabetes; T2D, type-2 diabetes; TGF, transforming growth factor; Th lymphocyte, T helper lymphocyte; TNF-α, tumor necrosis factor alpha.

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analogue, alloxan. Toxicity of alloxan is related to a massive ROS production and inhibition of glucose oxidation, subsequently leading to the reduction of ATP generation, which further suppresses insulin secretion. In microscopic studies of the pancreatic cells alloxan induced necrosis and apoptosis of Langerhans islets. Rapid and selective destruction of β-cells by alloxan is widely used as an experimental T1D model [1–3]. Irreversible β-cell destruction is a key feature of the autoimmune T1D pathology [5]. T1D is a severe progressive disease associated with the increased apoptosis and death of pancreatic islet β-cells, leading to development of insulin deficiency, hyperglycemia and some chronic complications. Monocytes/macrophages and dendritic cells are known to play an essential role in initiating and coordinating autoimmune aggression against insulin-synthesizing cells [6,7]. Infiltrating the tissues in all stages of insulitis, macrophages phagocytize damaged β-cells and present their antigens in local lymph nodes, activating cytotoxic T-lymphocytes [8–10]. It is assumed that the macrophages of the pancreas, possibly of the M1 phenotype, are involved in the secretion of ROS, pro-inflammatory cytokines and cytotoxic factors [11]. However, macrophages of the M2 phenotype prevented the destruction of β-cells, pancreatic islets and the development of diabetic nephropathy in chemically-induced experimental diabetes [12,13]. The change of the macrophage phenotype from M1 to M2 was performed *in vitro* in cell culture and was influenced by an immune modulator, followed by the adoptive transfer of macrophages to NOD mice, leading to normalization of glycaemia and reduction of T1D symptoms [14]. A potential role for macrophage polarization in promoting β-cell proliferation has only recently been appreciated [15,16].

To reverse pancreatic atrophy, several classes of drugs, hormones or growth factors have been successfully tested to stimulate β-cell proliferation in rodents, however, none have proven to be useful for humans [17]. A challenging question is when does physiologic β-cell proliferation occur and what pharmacologic approaches stimulate proliferation of these cells? Common target in many therapeutic efforts to improve patients-relevant outcomes related to chronic inflammation is combating the oxidative stress. High nonphysiological ROS generation can lead to DNA damage, lipid peroxidation, protein modification, and other pathological effects observed in neurodegenerative and autoimmune disorders, chronic viral infections, diabetes and cancer. However, pharmacotherapeutic interventions based on simple chemical scavenging of pro-oxidant molecules can be unsuccessful [18]. Alternative strategies involve silencing of activated macrophages and granulocytes, inhibition of enzymatic elements of oxidative burst reaction, activation of endogenous antioxidant defense systems and possible functional repair of ROS-induced damage. Exploring novel low-molecular, anti-inflammatory drugs, including N-acyl-hydrazone derivatives, phthalhydrazides, and other bioactive chemicals, is remarkable in recent decades [19]. For some targets, the respective pharmacology is advanced to clinical development, for others several drugs are already in clinical use. Monosodium 5-amino-2,3-dihydro- 1,4-phthalazine dione (sodium phthalhydrazide), available for over two decades in Russian Federation under Tamerit®/Galavit® name, has been applied in parallel with different standard therapies for hundreds of patients with viral and bacterial infections, for oncologic patients, and patients with some chronic degenerative diseases [20–24]. In this study we examined the effect of phthalhydrazide on alloxan-induced diabetogenic damage in pancreatic tissue.

2. Material and methods

2.1. Ethics

The use of animals was approved by the Ethical Committee of Institute of Immunology and Physiology of RAS (No-D-TM-2014-26). The study has been conducted on animals that were lawfully acquired. All experimental procedures involving animals were in compliance with the applicable laws and regulations as well as the principles expressed in the National Institutes of Health, USPHS and Guide for the Care and Use of Laboratory Animals.

2.2. Animals

Female Wistar rats (16-weeks old) were obtained from the Institute of Immunology and Physiology, the Ural Brunch of RAS (Yekaterinburg, Russian Federation). The animals were kept under equal conditions (12 h light/12 h dark cycle with lights turned on at 9:00 a.m.; temperature 20 ± 2 °C), were housed 5 animals per cage and were fed according to the customary schedule with free access to water. The animals showed no symptoms of any disease. All animals were randomly divided into following groups:

(i) Control animals (n = 10), b. w. 200 ± 10 g, received i.p. saline injections at day 1 and between 30–60 days, according to the experimental schedule (20 injections in total).
(ii) Sodium phthalhydrazide group (n = 10), The animals (b. w.200 ± 8 g) were treated i.p. with saline at day 1 and with 2-amino-1,2,3,4-tetrahydrophthalazine-1,4-dione sodium salt dihydrate (phthalhydrazide), registered in Russian Federation under the name Tamerit®/Galavit® [19–21], at a dose of 2 mg/kg b.w. between 30–60 days, according to the experimental schedule (20 injections in total). Preparation of sodium phthalhydrazide in a form of dull white powder with high flowability, could be distinguished in a visual comparison and solved by X-Ray single crystal and powder diffraction [25].
(iii) Alloxan group (n = 10). The animals (b.w. 207 ± 10 g) received i. p. single dose 300 mg/kg alloxan monohydrate (Sigma-Aldrich, St Louis, MO, USA) dissolved in 10 mM sodium citrate, pH 4.5, in fasted rats (16 h of food deprivation) and housed in standard conditions until the end of the experiment (60 days).
(iv) Alloxan + sodium phthalhydrazide group (n = 10). The animals (b.w. 210 ± 10 g) received an i.p injection of alloxan, at a dose of 300 mg/kg at day 1 and sodium phthalhydrazide at a dose of 2 mg/kg b.w., between 30–60 days, according to the experimental schedule (20 injections in total).

Alloxan is a toxic glucose analogue that accumulates in the pancreatic cells, resulting in the selective β-cell necrosis and

Table 1
The plasma glucose levels (mmol/l) in controls and alloxan-treated rats.

Group	Days of the experiment			
	3 day	7 day	14 day	30 day
Controls	5.89 ± 0.28	6.02 ± 0.35	5.86 ± 0.23	5.99 ± 0.17
Alloxan-induced diabetes	20.05 ± 3.45*	24.96 ± 4.78*	27.39 ± 4.52*	28.06 ± 3.84*

* $p < 0.05$ as compared to control group.