Mechanisms of Toxic Effect of Streptozotocin on β-Cells in the Islets of Langerhans

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Administration of streptozotocin caused selective damage to endocrine β-cells in rat pancreatic islets, which was related to activation of apoptosis. The cytotoxic effect of streptozotocin was associated not only with DMA damage due to dysfunction of the antioxidant defense system, but also with activation of the caspase cascade and TNF-related apoptosis-inducing ligand.

Key Words: *diabetes mellitus; endocrine β-cells; apoptosis; streptozotocin*

Diabetes mellitus (DM) is a global medicosocial problem in various countries all over the world. The disease is associated with early disability and death resulting from the development of late complications [1].

Selective β-cytotoxins, including alloxan, uric acid derivatives, and streptozotocin (STZ), are extensively used to produce DM in animals [11]. These substances are not characterized by individual variability of β-cytotoxicity, because they cause damage to β-cells in all animals of various species. Administration of STZ suppresses DNA synthesis and cell proliferation, inhibits mitosis, and activates apoptosis [12]. However, specific mechanisms for STZ-induced apoptosis are poorly understood.

Here we studied the mechanisms for the toxic effect of STZ on endocrine β-cells in rat pancreatic islets.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 300-340 g. The animals were divided into 2 groups, which consisted of intact (n=10) and control rats (n=10). Severe DM (blood glucose level >15 mmol/liter) in control animals was induced by intravenous injection of STZ (Sigma) in a daily dose of 20 mg/kg for 5 days [14]. The study was conducted in accordance with the requirements of the Regional Ethics Committee (order No. 43-2006). The rats were euthanized after 3 days. Histological study of the pancreas was performed routinely. Immunohistochemical study was conducted at the Laboratory of Morphology and Immunohistochemistry (Department of General and Experimental Pathology, Volgograd Research Center, Russian Academy of Medical Sciences). Monoclonal antibodies to caspase 3 (clone JHM62), TRAIL (apoptosis-inducing tumor necrosis factor receptor; clone 27B12), MDM2 (clone 1B10, Novocastra), and Bcl-2 (clone sc-7382, Santa Cruz) and polyclonal antibodies to p53 proteins (DakoCytomation) and Bax (BD Pharmingen) were used.

RESULTS

Bax-negative staining and weak positive cytoplasmic staining for the Bcl2 protein were revealed in endocrine cells of the pancreatic islets in intact animals. Slight expression of p53 protein was detected in the nuclei of some cells. Most nuclei in β-cells were positively stained with antibodies to MDM2 protein. Cytoplasmic staining for caspase-3 and TRAIL was moderate. These antigens were identified in individual endocrine cells (Fig. 1). In control animals, the expression of p53 and

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Bcl2 was weak, Bax was not expressed. Nuclear staining for MDM2 protein was revealed in a small number of islet cells. The majority of endocrine cells were positively stained for caspase-3 and TRAIL (Fig. 1).

Selective toxicity of STZ is related to destruction of the antioxidant defense system and DNA fragmentation in β-cells [7]. Previous experiments showed that DNA alkylation is the major cause of STZ-induced death of β-cells [4]. The exposure of cells to STZ is followed by the formation of toxic compounds (superoxide anion, peroxynitrite, and NO) [3]. It should be emphasized that NO plays an ambiguous role in the cytotoxic effect. Intracellular NO in low concentration has an inhibitory effect on

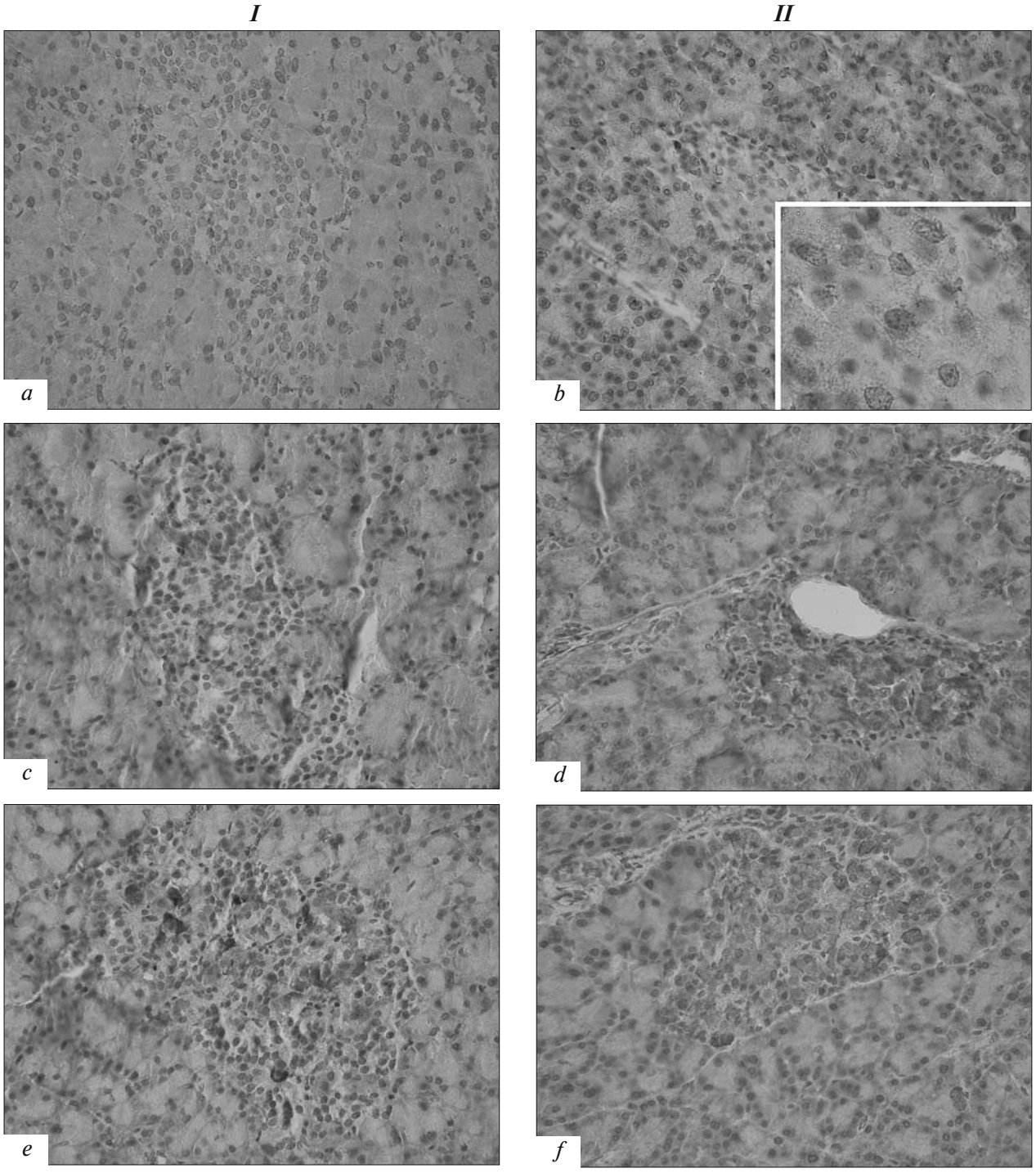

Fig. 1. Pancreatic islets, ×100. Intact group (*I*); control group (*II*). Expression of MDM2 protein (*a*, *b*); expression of caspase-3 (*c*, *d*); TRAIL-positive staining (*e*, *f*).

inducible NO synthase and reduces the degree of DNA fragmentation [6].

DNA damage, dysfunction of intracellular mitochondrial membranes, and cell treatment with strong oxidizers are followed by induction of apoptosis. p53 protein and caspases (family of cytoplasmic proteases) play an important role in the mechanisms of cell death.

p53 protein is a key factor of apoptosis. However, programmed cell death can occur without the involvement of this protein (effect of TNF). MDM2 protein protects the cell from apoptosis, which is related to inhibition of p53 synthesis and acceleration of p53 degradation in the cytosol [2].

Caspases constitute a branched cascade and activate each other [13]. This cascade is initiated by signals from the cytolemma (caspase-8) or mitochondrial factors (caspase-9). Caspase-8 and caspase-9 activate effector caspase-3, and the process of cell death becomes irreversible [8].

However, caspases are not always involved in apoptosis. Oversynthesis of the apoptosis-promoting protein Bax is followed by destabilization of mitochondrial membranes and induction of apoptosis. Mitochondrial membrane permeability is regulated by proteins of the Bcl2/Bax family. Bcl2 protein reduces permeability of membranes and inhibits apoptosis. By contrast, Bax increases membrane permeability. Hence, the mitochondrial mechanism of apoptosis can be induced by Bax protein. Bcl2 protein has a direct or indirect inhibitory effect on the release of mitochondrial cytochrome C, which activates procaspase-9 [9].

TRAIL is a membrane protein. Activation of this protein induces the so-called "rapid apoptosis". Highly differentiated cells gain the sensitivity to TRAIL-induced apoptosis after stimulation with interleukin-2. The induction of apoptosis by TRAIL requires activation of caspases (caspase-8) [10].

Our results indicate that the cytotoxic effect of STZ is associated not only with DMA damage, dysfunction of the antioxidant defense system, and activation of programmed cell death. The progression of apoptosis in endocrine cells after treatment with STZ does not involve p53 protein and destabilization of mitochondrial membranes. This process occurs by the alternative pathway, which is mediated by activation of TRAIL and caspase cascade.

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