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ОГЛЯДИ

STREPTOZOTOCIN-INDUCED TYPE 1 DIABETES MELLITUS: FORMATION MECHANISMS AND PRACTICAL ASPECTS OF EXPERIMENTAL MODELING (literature review)*

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Experimental modeling of type 1 diabetes mellitus (DM) by using streptozotocin (STZ) is an indispensable tool for researching both the mechanism of the pathology development (e. g. for determination of immune response associated with insulin-dependent DM (IDDM)), its complications (in particular, for defining the role of mitochondrial glucose toxicity) and the methods of their correction (firstly for preclinical testing of new insulin analogues, pancreas/Langerhans islets transplantation methods) in humans [1–3]. The development of fundamental diabetology in this area during past years has determined the formation of different approaches to investigate the principles of type 1 DM initiation by STZ and working out a large number of methods for representation of absolute insulin deficiency in an experiment based on using the particular substance. In this regard the aim of the current review was to summarize existing views about the mechanisms of STZ-induced IDDM development in laboratory animals and generalize some methodological recommendations on experimental modeling the pathology with this pharmacological method.

Streptozotocin Structure and Diabetogenic Properties

STZ (streptozocin, 2-deoxy-2-(3-methyl-3-nitrosourea)-D-glucopyranose, $C_8H_{15}N_3O_7$) is a naturally occurring antibiotic with a broad spectrum of action and it is produced by the specific strain of soil gram-positive bacteria Streptomyces achromogenes [4]. STZ molecule is formed by methylnitrosourea moiety — N-methyl-N-nitrosourea, and glucosamine derivative — 2-deoxyglucose (Fig. 1) [5].

Just N-methyl-N-nitrosourea as a part of STZ molecule determines initiation of some biochemical reactions by the ß-cytotoxin (e. g. DNA and proteins alkylation, nitric oxide (NO)

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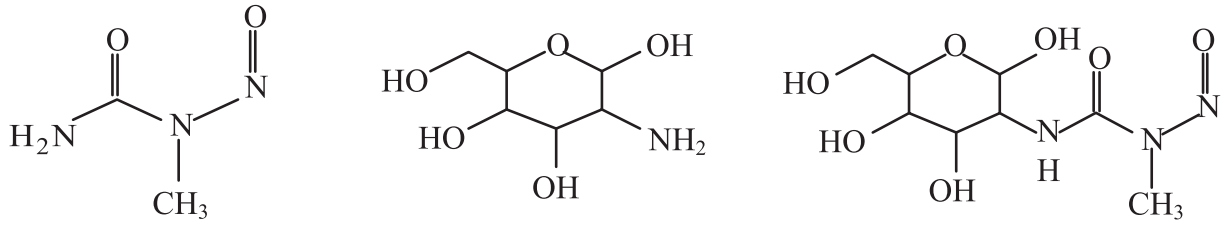

N-methyl-*N*-nitrosourea 2-deoxyglucose streptozotocin

Fig. 1. Structure formula of N-methyl-N-nitrosourea, 2-deoxyglucose and streptozotocin

donation etc.) in the organism of laboratory animals, which in turn predetermine the diabetogenic effect of particular substance, namely, the ability to suppress insulin-producing function and apoptosis/necrosis of ß-cells in islets of Langerhans (see section «Realization Mechanisms of Streptozotocin Diabetogenic Effect») [6–8].

Selectivity of Streptozotocin Transport into Internal Organs

Selective STZ entrance in the ß-cells of the islets of Langerhans underlies the manifestation of the substance diabetogenic influence in laboratory animals.

The first and fundamental attempt to explain the selective transport of STZ into pancreatic insulin-producing apparatus was hypothesis proposed by P. S. Schein and S. Loftus in 1968 [9]. It was about the significance of glucosamine as a part of the mentioned substance for input of the last one into ß-cells (see Fig. 1). The validity of this scientific statement was confirmed in the follow-up works of W. Schnedl and co-authors, M. Elsner and colleagues, where the evidences of STZ entrance in the ß-cells of the Langerhans islets similarly to realization of glucose transport in their intracellular compartment were received [10, 11]. Later it was revealed that the difference between the powers of ß-cellular expression of GLUT 2 in laboratory animals determines the presence of interspecies differences for value of diabetogenic STZ dose [12]. The features of localization of GLUT 2 expression in mammals cause STZ entrance not only into pancreatic ß-cells, but in hepatocytes, renal tubules cells and penetration of its insignificant amount into skeletal muscles, small intestine mucosa [13]. In this regard, animal models of type 1 DM based on using STZ, as a rule, provide minimization/elimination of STZ side effects by means of applying the minimum required dose (taking into account the dose-dependent nature of STZ hepato- and nephrotoxicity) or/and using preventive measures regarding the concomitant damage of animals' internal organs (e. g. hydration with saline infusion) [14]. In addition, there is an assumption that the development of adverse pathological abnormalities in laboratory animals after the excretion of STZ from their body is a result of diabetic hyperglycemia and it may be considered as a way of reproducing the complications of type 1 DM in an experiment [3].

Realization Mechanisms of Streptozotocin Diabetogenic Effect

The mechanism of STZ diabetogenic action in laboratory animals remains not accurately determined until now. It is assumed that there are four ways by which the nitrosourea derivative disturbs structure and function of the ß-cells in the islets of Langerhans, which leads to IDDM formation in laboratory animals [15].

The first way deals with the alkylating properties of STZ, primarily with its ability to methylate ß-cellular DNA [15, 6, 8]. The interaction of methyl radical of STZ with DNA binding sites (oxygen in the sixth position of guanine, circular nitrogen and exocyclic oxygen atoms of pyrimidine bases) leads to dehydration of the last one and the loss of its hydrogen bonds, which lead to development of conformational and functional disorders of this

molecule-carrier of hereditary information [16–18]. In these conditions the activators of cellular reparation mechanisms related with poly-(ADP)-ribosylation of nuclear proteins through poly (ADP-ribose) -polymerase (PARP) (7-methylguanine, O6-methylgyanine, 3-methyladenine and 7-methyladenine) are formed [17, 18]. STZ-induced excessive PARP activation causes the depletion of ß-cellular reserves of NAD^+ and ATP, which in turn leads to declining proinsulin and insulin synthesis and apoptosis/necrosis of insulin-producing cells as well [19, 20]. Despite the ability of STZ to methylate proteins, it is considered that DNA structure modifications, induced by STZ, is of first importance in this particular mechanism of the ß-cells destruction in the islets of Langerhans [6, 8].

The second way is based on the property of STZ to induce the generation of NO in pancreatic ß-cells [15, 6, 8]. Accumulation of NO in the insulin-producing apparatus can occur due to both metabolism of STZ and indirect stimulation of free radical synthesis by the diabetogen. In the first case, STZ is a donator of NO due to present in the substance structure the nitroso group [21–23]. In the second one, the multiply administration of sub-diabetogenic STZ dose causes the development of insulitis in experimental animals. The latest is accompanied by infiltration of the Langerhans islets with macrophages producing proinflammatory cytokines (IL-1ß, TNF-α and INF-γ) that, in turn, are able to activate expression of inducible NO-synthase and, respectively, generation of NO by the pancreatic islets ß-cells [2, 24–27]. Despite the fact that production of NO directly with macrophages can not be excluded, it is assumed that the main role in the development of type 1 DM, has been induced in laboratory animals by multiple low-dose STZ, plays the property of the cytokines to produce generation of the free radical in ß-cells [26]. In addition, it has been proven that IL-1 directly stimulates NO production, and TNF-α, INF-γ potentiate this process by means of increasing the first cytokine synthesis [24, 28, 29]. This is additionally confirmed by the ability of TNF-α and INF-γ to stimulate in the islets of Langerhans the expression of chemokines CXCL9, CXCL10 and CCL2 which are able to intensify the further migration of macrophages to these cellular formations and, therefore, to increase insulin-producing apparatus destruction [30]. The pathological influence of NO on pancreatic ß-cells is related with its effect on mitochondrial enzymes that are involved in glucose metabolism (e. g. aconitase) resulting to reduce the enzymes activity, disruption of glucose metabolism, suppression of insulin-producing function and ß-cells death [31]. It is assumed that increased cGMP formation under these conditions is only the indicator of NO synthesis, but no evidences have been received regarding the absence of an indirect connection of this substance with NO-induced suppression of insulin production by the ß-cells in the islets of Langerhans till now [31]. The ion channels as well as I and II complexes of the mitochondrial electron transport chain are two other targets for the NO influence, related with suppression of ß-cellular insulin production [32]. Under these conditions the important role in initiating of pancreatic ß-cells apoptosis belongs to NO-induced cleavage of DNA into nucleosomal fragments [29], despite the possible endogenous mechanism of prevention STZ cytotoxicity, associated with the increases of osteopontin synthesis in pancreas [33].

The third way is related to the property of STZ to provoke the generation of free radicals, oxidative stress development and the disruption of mitochondrial electron transport chain function in the ß-cells of the islets of Langerhans [6, 8, 15]. STZ-induced disturbance in ATP synthesis due to DNA alkylation and generation NO in its intracellular compartment (see above) is a trigger of increasing free radical production in pancreatic insulin-producing apparatus [34]. Under these conditions the generation of reactive oxygen forms initiates reduction of Nrf-2 (the main regulator of antioxidant defense) expression level, intensification of synthesis Bax and NOS-2, the cleavage of caspase-3 and PARP in ß-cells that leads to its apoptosis [35, 36]. Also the fragmentation of ß-cellular DNA under the influence of free radicals is not excluded [37]. STZ-induced formation of oxidative stress determines disruption of electron transport chain function (the suppression of activity of its I, II/III and IV complexes) which is also associated with destruction of the ß-cells in the islets of Langerhans [38].

The fourth way is associated with the STZ property, on the one hand, to enhance proteins glycosylation in cytosole of the ß-cells of the Langerhans islets and, on the other one, to inhibit the activity of N-acetyl-ß-D-glucosaminidase (O-GlucNAcase) that cleaves N-acetylglucosamine from the protein structure during the last reaction of this process [39, 40]. As the result O-glycosylated proteins accumulation in pancreatic insulin-producing cells, which leads to intensification of its apoptosis, is enhanced [39, 40].

Methodological Recommendations in Experimental Modeling of Type 1 Diabetes Mellitus by Using Streptozotocin

Methodological recommendations for reproduction of STZ-induced IDDM in laboratory animals are presented according to eight criteria used in the analysis of appropriate experimental models.

1. Species, breed, line of laboratory animals. STZ-induced type 1 DM may be reproduced in different species of laboratory animals: mice, rats, Chinese hamsters, guinea pigs, rabbits, dogs, primates and pigs [1, 15]. However, the existence of species difference in sensitivity to ß-cytotoxic effect of STZ has been proven: rats are most sensitized to the diabetogen and guinea pigs, rabbits and pigs are least sensitized [12, 41]. Taking it into account and existing features in the work with above mentioned laboratory animals species, mice, rats and primates are often used in the experimental modeling of type 1 DM. Moreover, using the latest animal is associated with conduction certain scientific researches concerning type 1 DM. For example, the research of autoimmune component of mechanism type 1 DM development and the action of new insulin analogs are usually carried out by using mice or rats models of STZ-induced IDDM, but the studding transplantation of pancreas/islets of Langerhans and subsequent immunosuppressive therapy are done by using primates.

Mice. Experimental modeling of IDDM by using STZ is carried out in mice of the following lines: C57BL/6; C57BL/KsJ; BALB/c; CD-1; DBA/2; C. B17-SCID; BALB/c Nude; CD-1 Nude; MRL/Mp and 129/SvEv. Among them there are rodent lines with high sensitivity to diabetogenic effect of SZT (C57BL/6, ICR and DBA/2c) and with reduced metabolic response to the influence of this substance (MRL/Mp, BALB/c and 129SvEv) [42]. Furthermore, the tendency towards the variation in sensitivity to the ß-cytotoxic influence of STZ in the subgroups of the same mice line has been proven [43, 44]. Hence, under the conditions of using animals of a certain line, age and gender, but with some genetic differences, one and the same dose of the particular diabetogen may cause severe hyperglycemia in most animals, but be ineffective for others or lead to their death [42]. In this regard, if the value of laboratory animal' death after SZT influence exceeds 10–20 % of individuals in the experimental group, the estimation of the diabetogen safety in mice is recommended to carry out [43]. In the experimental modeling of type 1 DM using the mice IRC with genetically determined PARP ($Parp^{-/-}$) deficient has been excluded due to their absolute resistance to the diabetogenic effect of particular nitrosourea derivative [45].

Rats. Wistar and Sprague-Dawley rats are used in modeling IDDM by using STZ. The possibility of using SHR rats that are genetical model of arterial hypertension corresponding to essential hypertension in human has also been proven [46, 47].

Rabbits. It is considered that rabbits are resistant to STZ diabetogenic effect [41]. Successful initiation of IDDM under the condition of using STZ has been described in New Zealand rabbits which are prone to this pathology development [48].

Monkeys. In scientific literature the initiation of type 1 DM induced by STZ has been described in different species of monkeys: rhesus macaque (Macaca mulatta) [49, 50], cynomolgus macaque (Monkey fascicularis) [51, 52], vervet monkeys (Chlorocebus aethiops) [53] and baboons (Papio hamadryas) [54]. In the majority of cases the absolute insulin deficit is reproduced by this way in macaque and vervet monkeys. This feature is related with the fact that macaque and vervet monkeys compared with baboons have a less body size and weight, a more pronounced physiological similarity with a human, in this case it provides a relatively easy use of these monkey breeds in an

experiment, their less expensive maintenance and free extrapolation results in humans [55]. Moreover, unlike other primate breeds, vervet monkeys with STZ-induced IDDM are not characterized by spontaneous restoration of functioning endogenous ß-cells [1]. Along with it one must remember that this monkey breed is naturally infected with a virus B (Cercopithecine herpesvirus 1; it belongs to biological agents of biosafety level 4) and keeping the primates in vivarium does not reduce the frequency of their infection by this virus [56]. In this regard, the attention should be given to the need for observing appropriate preventive measures under the condition of using macaque in experimental researches [1].

2. Gender of laboratory animals. With a view to reproduce STZ-induced IDDM in laboratory animals it is recommended to use males because females are less sensitive to hyperglycemic effect of particular diabetogen (Table 1) [57].

Table 1

Characteristics of some animal models of STZ-induced type 1 DM

Animal species	Animal breed, line, gender	Animal age, body weight	STS dose, solvent, route of administration	Additional conditions	Target level of fasting glycemia in animals	Source
Single STZ injection						
Mice	C57BL/6, males	6 weeks, —	200 mg/kg b.w., citrate solution (0.1 M citric acid and 0.2 sodium phosphate; H = 4.5), i/p	Starvation of animals during 12 hours before STZ injection	> 400 mg/dl 7 days after STZ injection	Yang B.Y., Li M., Shi Z.-G. et al. *Anadolu Kardiyol Derg* 2012; 12(8): 621–627
	BALB/c, males, females	—, ~ 20 g	200 mg/kg b.w., 0.09 M citrate buffer (pH = 4.8), i/p; 160 mg/kg b.w., 0.09 M citrate buffer (pH = 4.8), i/v; 180 mg/kg b.w., 0.09 M citrate buffer (pH = 4.8), i/v	—	≥ 200 mg/dl 3 days after STZ injection in all doses; ≥ 300 mg/dl 7 days after STZ injection in all doses	Paik S.G., Fleischer N., Shin S.I. *Proc Natl Acad Sci USA* 1980; 77(10): 6129–6133. Sakata N., Yoshimatsu G., Tsuchiya H. et al. *Exp Diab Res* 2012; 2012: e256707
	ICR, males	8 weeks, —	95 mg/kg b.w., citrate buffer (pH = 4.5), i/p	—	> 300 mg/dl 24 hours after STZ injection	Kim H.K. *Evid. Based Complement Alternat Med* 2012; 2012: e439294
Rats	Wistar, males	—, 200–220 g	60 mg/kg b.w., 0.1 M citrate buffer (pH = 4.8), i/p	—	Plasma glucose level ≥ 250 mg/dl 48 hours after STZ injection	Sameni H.R., Ramhormozi P., Bandegi A.R. et al. *J Diabetes Investig* 2016; 7(4): 506–513

Continuation of table 1

Animal species	Animal breed, line, gender	Animal age, body weight	STS dose, solvent, route of administration	Additional conditions	Target level of fasting glycemia in animals	Source
Rats	Sprague-Dawley, males	3 months, 250–300 g	60 mg/kg b.w., 0.1 M citrate buffer (pH = 4.5), i/p	—	> 15 mmol/l 3 days after STZ injection	Radovits T., BömickeT., Kökeny G. et al. *Br J Pharm* 2009; 156: 909–919
Rabbits	New Zealand, males	—, 2.5 kg	65 mg/kg b.w., citrate buffer (pH = 4.0), i/v	Animals starvation during 24 hours before STZ injection	≥ 150 mg% 24 hours after STZ injection. Postprandial glycemia ≥ 300 mg% 3 weeks after STZ injection	Kedar P., Chakrabarti C.H. *Indian J Physiol Pharmacol* 1983; 27: 135–140
Monkeys	Vervets (*Chlorocebus aethiops*), males	6–12 years old, 5.9–10.0 kg	45 and 55 mg/kg b.w., 0.9 % NaCl, i/v	Animals starvation during 12 hours, sedatation with ketamine hydrochloride 15 mg/kg b.w., i/m before STZ injection	≥ 200 mg/dl 2 days after STZ injection in both doses	Kavanagh K., Flynn D.M., Nelson C. et al. *J Pharmacol Toxicol Methods* 2011; 63(3): 296–303
	Cynomolgus monkeys (*Macaca fascilularis*), males	3–6 years old, 3.1–9 kg	55 and 100 mg/kg b.w., 0.9 % NaCl, i/v	Animals starvation during 12 hours, sedatation with ketamine 10–15 mg/kg b.w. i/m, hydration with 50–60 cc 0.9 % NaCl before STZ injection	> 400 mg/dl 24 hours after STZ injection in both doses	Koulmanda M., Qipo A., Chebrolu S. et al. *Am J Transpl* 2003; 3: 267–272
Multiple STZ injection						
Mice	C57BL, males	6 weeks, 16–18 g	60 mg/kg b.w./day × 6 days, citrate buffer (pH = 4.5), i/p	—	≥ 16 mmol/l 6 days after the last STZ injection	Yuan X., Xiao Y.C., Zhang G.P. et al. *Drug Des Devel Ther* 2016; 10: 2729–2737
	NOD, males, females	4.5–9 weeks, —	40 mg/kg b.w./day × 5 days, citrate buffer (pH = 4.0), i/p	—	≥ 20 mmol/l 8 days after the last STZ injection	Elliott J.I., Dewchand H., Altmann D.M. *Clin Exp Immunol* 1997; 109(1): 116–120

Continuation of table 1

Animal species	Animal breed, line, gender	Animal age, body weight	STS dose, solvent, route of administration	Additional conditions	Target level of fasting glycemia in animals	Source
Mice	CD-1, males	—, 35–40 g	40 mg/kg b.w./day × 5 days, citrate buffer (pH = 4.5), i/p	—	Plasma glucose > 200 and > 400 mg / 100 ml 1 and 6 days after the last STZ injection	Rossini A.A., Like A.A., Chick W.L. et al. *Proc Natl Acad Sci USA* 1977; 74(6): 2485–2489
	Swiss Wistar, males	—, 20–30 g	Three-day course: 1 day – 85 mg/kg b.w. 2 day – 70 mg/kg b.w. 3 day– 55 mg/kg b.w., citrate buffer (pH = 4.8), i/p	Injection of STZ to fasting animals	≥16 mmol/l 7 days after the last STZ injection	Kennedy J.M., Zochodne D.W. *Brain* 2000; 123(10): 2118–2129
Rats	Albino Wistar, males	2.5 months, 220–250 g	40 mg/kg b.w./day × 5 days, 0.1 Na citrate buffer (pH = 4.5), i/p	—	> 20 mmol/l 24 hours after the last STZ injection	Arambašić J., Mihailović M., Uskoković A. et al. *Eur J Nutr* 2013; 52(5): 1461–1473
	Sprague-Dawley, males	—, 190–200 g	15 mg/kg b.w./day × 5 days, citrate buffer (pH = 4.5), i/v	—	Plasma glucose ≥ 250 and ≥ 350 mg / 100 ml 1 and 5 days after the last STZ injection	Rossini A.A., Like A.A., Chick W.L. et al. *Proc Natl Acad Sci USA* 1977; 74(6): 2485–2489

On the one hand, this feature is explained by the effect of sex hormones on autoimmunity and, respectively, additional influence on the level of proinflammatory reaction in islets of Langerhans due to multiply STZ injection. On the other one, it is explained by the property of estradiol to protect ß-cells from apoptosis of induced oxidative stress (see section «Realization Mechanisms of Streptozotocin Diabetogenic Effect») [58].

3. Age of laboratory animals. Mature animals must be used in experimental modeling type 1 DM by using STZ (Table 1) because immature individuals are more resistant to diabetogenic action of this substance, to overcome it an increase of diabetogen dose is required and that is a factor of forming adverse pathological disorders [52]. It is also known that mature animals have an inverse relationship between the sensitivity to diabetogenic action of STZ and the age that is considered in context of age change levels of sex hormones in the organism [15].

4. Number of STZ administrations. For the purpose of reproducing IDDM in laboratory animals it is allowed either a single or a multiple STZ injection. A single STZ administration assumes the injection of the ß-cytotoxin in a high (diabetogenic) dose, which allows to reproduce IDDM in experimental animals in a short term (in 24–48 hours after injection of particular diabetogen) [59]. However, in this

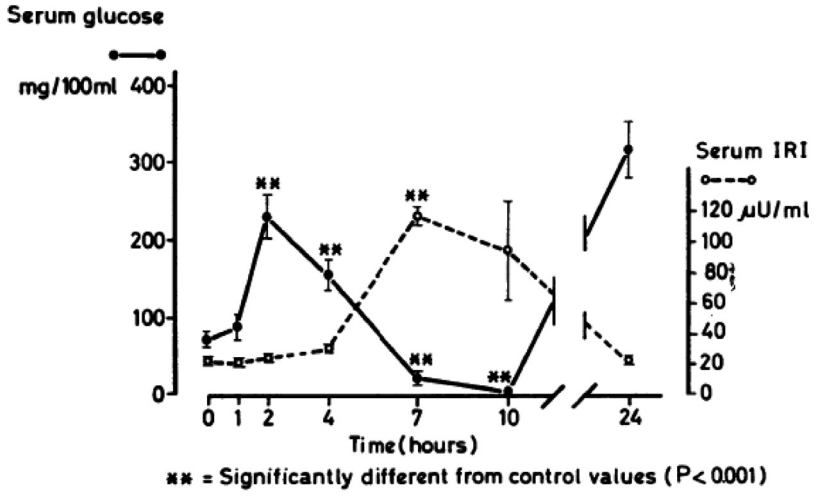

FIGURE 1 Effects of the intravenous (i.v.) injection of 65 mg/kg streptozotocin on serum glucose and immunoreactive insulin (IRI) of rats fasted 16 hr (mean ±SEM).

Fig. 2. Effects of the intravenous injection of 65 mg/kg STZ on serum glucose and immunoreactive insulin of rats fasted 16 hr (mean ± SEM) [59]

case concomitant side pathological abnormalities of a liver and kidneys are often revealed and animals death is registered not uncommon (the animal death rate should not exceed 10–20 % of a total amount of individuals in a group upon the condition of successful initiation of IDDM) [15]. To avoid the latest it is recommended to keep experimental animals over the first 24-hours after STZ administration with considering the phase changes in glycemia and insulinemia in their body (Fig. 2) [59]. It is known that in 2–4 hours after the administration of diabetogenic STZ dose the primary hyperglycemia associated with the absence of compensatory increase of insulinemia can be found in experimental animals. It takes place, apparently, due to the direct suppression of ß-cells insulin-secretory function with STZ (see section «Realization Mechanisms of Streptozotocin Diabetogenic Effect») and/or the elevation of glycogenolysis in a liver.

Hereafter (in 4–10 hours after STZ administration) hypoglycemia as a result of hyperinsulinemia associated with ß-cells necrosis and release of insulin located out of secretory granules of ß-cells, since their damage is not occurred at this time, is developed in the animals [59]. In this regard, delivery of the access of experimental animals to exogenous carbohydrates, e. g. 5 % glucose solution, (especially in the case of the proceeding administration of diabetogen starvation that determines a liver glycogen depletion) permits to avoid their death as a result of severe hypoglycemia at this time [6, 8]. During 12–24 hours after STZ administration the secondary permanent hyperglycemia, hypoinsulinemia, polyuria and glucosuria that persist over 7–28 days are revealed in animals [59]. Taking it into account, for the purpose to be convinced of presence stable symptoms IDDM in animals it is recommended to inject them STZ 5–7 days before the their insertion into the experiment [15].

Multiple STZ injections, as a rule, involve the administration of particular diabetogen in a constant low (sub-diabetogenic) dose each time. In this case STZ administration is not considered as a factor of animals' death, since it provokes gradual increase of blood glucose level which mechanism is related with the formation of insulitis associated with immune reaction of a body that is mediated by autoreactive T-cells and macrophages [26]. Under these conditions the formation of permanent hyperglycemia and hypoinsulinemia is postponed as they can be detected in 6–7 days after the completion of the diabetogen injections course. Multiple low-dose STZ administration makes it possible to reproduce in laboratory animals IDDM that is characterized by autoimmune destructive processes concerning ß-cells in the similar way that it takes place in type 1 DM patients. However, the questions whether the immune reaction in response to insulitis development to be a deter-

minant for ß-cells cytolysis and whether the destruction of insulin-producing cells may not be associated with direct STZ cytotoxic influence, have not been definitely answered yet [57, 60].

5. Recommended STZ dose. The diabetic STZ dose is a dose of particular ß-cytotoxin that provokes in laboratory animals decline of fasting C-peptide level in circulation to value < 0.5 ng/ml (or to quantity equal 1/3 from the rate that had been determined before STZ administration), the absence of the C-peptide upregulation during a glucose or arginin tolerance test (< 0.3 ng/ml) and persistent hyperglycemia (≥ 200–300 mg/dl) which are a basis for verification their IDDM [14].

Mice. The diabetogenic STZ dose size for mice varies depending on the animals' line and in general it is 90–200 mg/kg b. w. (see Table 1) [15]. Under the condition of multiple STZ administration to mice (usually during 5 days), sub-diabetogenic STZ dose that ranges from 40 to 60 mg/kg b. w. (see Table 1) is used [15].

Rats. For Wistar rats the association between STZ dose in range 25–100 mg/kg b. w. (under the condition of a single administration) and fasting blood glucose and insulin levels is detected [59]. In case of the application of a maximum allowable diabetogenic STZ dose (100 mg/kg b. w.), the formation of absolute insulin lack in experimental animals occurs over the next 24 hours, however, usually these animals die 2–3 days later. Applying STZ at doses 55 and 65 mg/kg b. w. is recognized more appropriate for reproduction of IDDM in rats since it is associated with the formation of steady longstanding (up to 28 days) hyperglycemia and decrease of insulinemia to a basal level just in 24 hours after the diabetogen administration, a low level of STZ hepato- and nephrotoxicity and animals' mortality (see Table 1) [59]. The application of STZ in fewer amounts is not appropriate for initiation of IDDM in rats of this line [59].

Since, in Sprague-Dowley rats similar STZ effect at dose 20–100 mg/kg b. w. had been identified [13], for induction of IDDM in these animals it is recommended to use this diabetogen in an amount of 50–60 mg/kg b. w. [8]. In case of STZ administration to SHR rats at doses 40 and 50 mg/kg b. w., the diabetic hyperglycemia (≥ 300 mg/dL) is established in these animals exactly in 28 and 7 days after that, respectively [46].

Rabbits. The data concerning STZ dose which is effective for initiation of IDDM development in rabbits are contradictory. On the one hand, it is reported that application of STZ at doses 50–150 mg/kg b. w. is not effective for initiation of IDDM in New Zealand rabbits [61]. On the other one, the successful reproduction of this pathology in such animals while applying the given diabetogen at dose 65 mg/kg b. w. has been described [48]. The latest case STZ induces multi-phase changes of glycemia in the rabbits similarly to that had been found in other rodents (see paragraph 4) [62].

Monkeys. There are some differences in representation of the value of diabetogenic STZ dose for primates. A dominating majority of animal models of STZ-induced type 1 DM proposes to express an amount of the diabetogen administered to primates as its fixed mass per unit of animals' body weight (mg/kg b. w.). However, some animal models propose to dose of this ß-cytotoxin as its fixed mass per unit of a body surface area of experimental animals (mg/m^2) [14]. The results of a comparable evaluation of STZ application at doses, represented in accordance with these two ways, have given the evidences of absence advantages in dosing the diabetogen with the latter method. Moreover, in order to avoid the mistakes during the STS dose calculation due to the incorrect estimation of the primates' body surface area, it is recommended to dose STZ in accordance with the body weight of experimental animals. In case of the necessity to compare animal models of type 1 DM with these particular differences, it should be considered that the typically used range of STZ doses 1050–1250 mg/m^2 under these conditions equals to its quantity of 80–108 mg/kg b. w. [14].

The amount of STZ that is used during the reproduction of type 1 DM in primates ranges between 50–150 mg/kg b. w. [1, 15]. The application of high doses of STZ (100–150 mg/kg b. w.) causes the pronounced features of IDDM in primates, however, it leads to the structure and function disruptions in their liver and kidneys [1, 43]. There are contradictory data concerning the application of STZ at doses 30–60 mg/kg b. w. (a single i/v injection) in macaques. It was

established that the administration of STZ at doses 30–40 mg/kg b. w. initiates IDDM in macaca nemestrina and a severe absolute insulin deficiency which requires a replacement therapy to avoid ketosis development in cynomolgus macaques [51, 63]. Conversely, it is reported that the same STZ dose doesn't evoke the development of an absolute insulin deficiency in rhesus macaques [49], but increasing an STZ dose up to 45 or 55 mg/kg b. w. provokes the formation of both type 1 and type 2 DM in these animals [64]. Rood P. P. and colleagues have established that the injection of STZ at dose 60 mg/kg b. w. to cynomolgus macaques doesn't provoke the development of type 1 DM based on the absence of a three-phase changes in glycemia and C-peptide level changes in its blood circulation (> 1.86 ng/ml) in response to ß-cytotoxin action [65] which contradicts the research made by M. Koulmanda and colleagues, according to their results the administration of STZ at dose of 55 mg/kg b. w. is suitable for the reproduction of IDDM in these monkeys and is characterized by a minimal nephro- and hepatotoxic action of the diabetogen [66].

6. Preparation of STZ solution. IDDM animal models which involving STZ application determine using citrate or acetate buffer (pH = 4.0–4.5) and more rarely saline solution as a solvent for this diabetogen, since it is considered that the ß-cytotoxin in solution has greather chemical stability at low pH values of the medium than in case of the displacement of its hydrogen index towards the alkali [67]. It is also proven that the temperature regime and the storage duration of STZ solution influence on the diabetogenic properties of this substance [68]. In this regard, the general requirement of all type 1 DM animal models by using STZ is preparation of this nitrosourea derivative solution immediately before (no more than 10–15 min) its administration to the experimental animals. Such requirement is related with a fact that approximately 90 % of the crystalline STZ is contained in the form of α-anomer which has more pronounced ß-cytotoxic properties than its other form — ß-anomer [69, 70]. After STZ dissolution the concentration of α-anomer exceeds the amount of ß-anomer (approximately 10-fold) only for the first 15 min. Hereafter, over 60–90 min after the dissolution of the diabetogen, the mutarotations of glucopyranose ring can be found in its structure, they lead to the establishment of equimolar equilibrium between α- and ß-forms causing an attenuation of STZ ß-cytotoxicity [71]. Nevertheless, an application of anomer-equimolar solution of STZ in experimental modeling of absolute insulin deficient can not be excluded, since it is assumed that achieving in this way a stable diabetogenic activity of this ß-cytotoxin will allow in the future to develop animal models of IDDM which are characterized by a higher degree of reproduction as well as to make the comparison of data obtained by different scientists with higher accuracy [71]. Consequently, the storage of STZ solution is allowed, but it should be taken into account that in case of using 0.1 M sodium acetate buffer (pH = 4.4) the diabetogen concentration daily decreases by 0.03 and 0.4 % at a temperature of 6 and 22 ºC and in case of using 0.1 M sodium citrate buffer (pH = 4.5) the diabetogen concentration daily decreases by 0.1 and 1.0 % at a temperature 4 and 22 ºC, respectively, since 30 % degradation of STZ in solution completely resolves its diabetogenic effect [71]. In the case of using saline solution, it is recommended not to exceed the time interval between the preparation of STZ solution and its injection to experimental animals, which it is defined under the condition of citrate (or acetate) buffer application.

7. Ways of STZ administration to laboratory animals. STZ administration to laboratory animals is realized with intraperitoneal or intravenous injection. It is considered that IDDM animal models which determine the intravenous injection of STZ are more reproducible than those that involve using intraperitoneal way for this ß-cytotoxin administration to experimental animals [42].

Usually the starvation of laboratory animals for 6–24 hours, which allows to increase sensitivity of laboratory animals to STZ and decrease variability of glycemia by the way of the exclusion of its postprandial rise, precedes to STZ administration [72]. However, in this case, a more pronounced hypoglycemia over the first 24 hours after STZ administration (see paragraph 4), the difference in the range of bio-

chemical and hematological parameters as well as the characteristics of gastrointestinal tract function and enzymes' actions involved in metabolism of medication are revealed in these laboratory animals as compared to the fed animals which have got this diabetogen [72]. It is important to take into account during the development of design of experimental study, the analysis and interpretation of obtained data. In case of type 1 DM modeling in primates, as a rule, sedation of the particular species animals by intramuscular injection of ketamine (5–15 mg/kg b. w.) or telazole (3–6 mg/kg b. w.) precedes STZ administration [14]. Since this diabetogen causes nausea and vomiting in primates, it is allowed to use ondansetron (1–4 mg, per os) or promethazine (0.25–1 mg/kg, i/m) 30–60 minutes before STZ administration [14].

8. Correction of STZ-induced type 1 DM by using insulin therapy. During long-term studies which involve animals with STZ-induced type 1 DM there is a demand of application insulin replacement therapy in order to avoid their lethality due to ketoacidosis. Moreover, the application of exogenous insulin in these animals is often determined by the design of the experimental study in connection with the requirement to achieve certain degree of glycemic control by them. The compensation of endogenous insulin deficient in experimental animals is provided by carrying out daily single or double injection of this hormone analogue or implantation of insulin-secreting pallets [14, 73]. Using the insulin implants is considered to be less time-consuming, as well as, providing a better glucose-lowering effect, the absence of pronounced blood glucose level fluctuations and hypoglycemia [73]. Under these conditions it is important to conduct a close control of glycemia in experimental animals due to the possibility of spontaneous recovery of their endogenous pancreatic ß-cells, which can lead to incorrect results of experimental study. The cases of spontaneous regeneration of endogenous ß-cells have been registered in mice, rats and primate with STZ-induced IDDM as a result of the compensation of insulin deficiency or removal/rejection of functioning donor islets of Langerhans, wherein the animals have achieved euglycemia/reduction of hyperglycemia [74–76].

CONCLUSION

Since discovering the diabetogenic features of streptozotocin a great number of research studies has been dedicated to investigate the mechanisms of insulin-dependent diabetes mellitus development induced in laboratory animals by this nitrosourea derivative. A single diabetogenic streptozotocin administration is related with the acute formation of insulin-dependent diabetes mellitus in laboratory animals, its mechanism is associated with the apoptosis of the ß-cells in the islets of Langerhans (primarily due to DNA and proteins alkylation with this diabetogen and NO donating as well). The latter provides only the reproduction of type 1 diabetes mellitus key characteristics (absolute insulin deficiency, hyperglycemia, ketoacidosis and weight loss) in laboratory animals and excludes the possibility of experimental modeling the genesis of the particular pathology. Taking it into account, this type of animal models of type 1 diabetes mellitus can not be defined as pathogenetic, therefore they are significant tool both for research studies related with investigating the correction methods of insulin deficiency and the development mechanism of diabetes-related complications. In the case of a multiple low-dose streptozotocin administration the formation of insulin-dependent diabetes mellitus in experimental animals is prolonged as a result of the initiating the insulitis with streptozotocin which defines necrosis of pancreatic insulin-producing cells (particularly by the way of streptozotocin-induced stimulation of NO production with proinflammatory cytokines). The latest provides the possibility of experimental modeling the determining component of type 1 diabetes mellitus genesis, namely its autoimmune part that is an important tool for researching the features of type 1 diabetes mellitus development in humans.

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STREPTOZOTOCIN-INDUCED TYPE 1 DIABETES MELLITUS: FORMATION MECHANISMS AND PRACTICAL ASPECTS OF EXPERIMENTAL MODELING (literature review)

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In the review the present-day views about mechanisms of streptozotocin-induced type 1 diabetes mellitus development and methodical recommendations for its experimental modeling are presented. The mechanisms of streptozotocin-induced insulin-dependent diabetes mellitus development have been considered according to four ways of the diabetogen influence on ß-cells:

1) DNA alkylating;
2) donating NO/NO expression intensification;
3) oxidative stress development;
4) lowering N-acetyl-ß-D-glucosaminidase activity.

Methodical recommendations for streptozotocin-induced type 1 diabetes mellitus experimental modeling are presented by the criteria:

1) animal species, breed, line;
2) animal gender;
3) animal age;
4) number of streptozotocin administrations;
5) recommended streptozotocin dose;
6) streptozotocin solution preparation;
7) routs of streptozotocin administration;
8) streptozotocin-induced type 1 diabetes mellitus correction.

Key words: streptozotocin, type 1 diabetes mellitus, animal model.

СТРЕПТОЗОТОЦИН-ИНДУЦИРОВАННЫЙ САХАРНЫЙ ДИАБЕТ 1 ТИПА: МЕХАНИЗМЫ ФОРМИРОВАНИЯ И ПРАКТИЧЕСКИЕ АСПЕКТЫ ЭКСПЕРИМЕНТАЛЬНОГО МОДЕЛИРОВАНИЯ (обзор литературы)

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В обзоре освещены современные представления о механизмах развития стрептозотоцин-индуцированного сахарного диабета 1 типа и методические рекомендации по его экспериментальному моделированию. Механизмы развития инсулинозависимого сахарного диабета рассмотрены на основании четырех путей воздействия данного диабетогена на β-клетки:

1) алкилирование ДНК;
2) донирование NO/интенсификация экспрессии NO;
3) развитие оксидативного стресса;
4) снижение активности N-ацетил-β-D-глюкозаминидазы.

Методические рекомендации по экспериментальному моделированию стрептозотоцинового сахарного диабета 1 типа представлены в соответствие с критериями:

1) вид, порода, линия животных;
2) пол животных;
3) возраст животных;
4) количество введений стрептозотоцина;
5) рекомендованная доза стрептозотоцина;
6) приготовление раствора стрептозотоцина;
7) пути введения стрептозотоцина;
8) коррекция стрептозотоцин-индуцированного сахарного диабета 1 типа.

Ключевые слова: стрептозотоцин, сахарный диабет 1 типа, экспериментальная модель.

СТРЕПТОЗОТОЦИН-ІНДУКОВАНИЙ ЦУКРОВИЙ ДІАБЕТ 1 ТИПУ: МЕХАНІЗМИ ФОРМУВАННЯ ТА ПРАКТИЧНІ АСПЕКТИ ЕКСПЕРИМЕНТАЛЬНОГО МОДЕЛЮВАННЯ (огляд літератури)

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В огляді висвітлені сучасні уявлення щодо механізмів розвитку стрептозотоцин-індукованого цукрового діабету 1 типу та методичні рекомендації з його експериментального моделювання. Механізми розвитку інсулінозалежного цукрового діабету розглянуті на основі чотирьох шляхів впливу даного діабетогену на β-клітини:

1) алкілування ДНК;
2) донування NO/інтенсифікація експресії NO;
3) розвиток оксидативного стресу;
4) зниження активності N-ацетил-β-D-глюкозамінідази.

Методичні рекомендації з експериментального моделювання стрептозотоцинового цукрового діабету 1 типу наведені відповідно до критеріїв:

1) вид, порода, лінія тварин;
2) стать тварин;
3) вік тварин;
4) кількість введень стрептозотоцину;
5) рекомендована доза стрептозотоцину;
6) приготування розчину стрептозотоцину;
7) шляхи введення стрептозотоцину;
8) корекція стрептозотоцин-індукованого цукрового діабету 1 типу.

Ключові слова: стрептозотоцин, цукровий діабет 1 типу, експериментальна модель.