Are experimental models useful for analysis of pathogenesis of changes in central nervous system in human diabetes?

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Incidence of diabetes and complications associated with the course of the disease make diabetes an important clinical problem. Clinical studies have not provided sufficient data on pathophysiological and therapeutical possibilities of treating the disease and diabetic complications. Published work shows that certain animal models and human diabetes have very similar pathophysiology and clinics. Many similar features have been described in streptozotocin-induced diabetes, which is the most frequently used model. Reported similarities include, e.g., presence of oxidative stress with depletion of endogenous antioxidants, increased lipid peroxidation, changes in metabolism of different cells, development of nephropathy and cataract, decrease of cerebral blood flow, and cognitive function impairment. However, the influence of ageing, long duration of the disease and development of vascular complications (including changes in the walls and endothelium of blood vessels, specific for human diabetes) are difficult to reproduce under laboratory conditions. In the case of central nervous system (CNS), relatively scarce data indicate that observations in animal CNS are different from those in humans. Inter-species differences among interspecies and the possibility of different pathomechanisms of human diabetes should be taken into consideration when interpreting study results.

**key words:** brain, nervous system, streptozotocin, hyperglycaemia

**INTRODUCTION**

Diabetes is one of the most common chronic diseases. In the USA and European countries, according to WHO, the incidence of diabetes is about 3–7% in people aged > 30 years old. In Poland the incidence of diabetes is about 3.5%. Therefore, it is one of the most important challenges for investigators and clinicians. Because of heterogeneity of etiopathogenesis of diabetes, it is difficult to improve the diagnostic methods and to develop better methods for its treatment. Similarly, as in the case of other pathologic states, an important question arises — not what to investigate but how the investigation could be or should be performed. In case of diabetes, clinical materials are easily available; tissue or cellular level studies can be done in vivo as well as post-mortem. However, it is still much more difficult to explore intracellular processes and reactions on the level of particles, ions, receptors, etc. These studies can only be performed on laboratory models — animals or tissue/cell cultures. For laboratory studies, it is necessary to artificially induce diabetes, use inbred diabetes models or, in some cases, maintain hyperglycaemic conditions. The question has arisen: to which degree the changes observed in models of diabetes/hyperglycaemia reflect features known to accompany the human diabetes?
This article reviews models of diabetes, with focus on the most extensively used streptozotocin (STZ)-diabetes, and analyses their usefulness in investigating the pathogenesis of human diabetes. Particular attention has been paid to the central nervous system (CNS) in diabetes.

**EXPERIMENTAL MODELS OF DIABETES**

A substantial number of models of experimental diabetes have been described, so far.

**Surgically evoked diabetes**

Total pancreatectomy was used as a model of experimental diabetes in dogs [53]. However, interpretation of changes observed in such a model is complicated because total pancreatectomy impairs many other excretional functions of the pancreas, leading to metabolic disturbances of the whole organism.

**Genetically-based experimental diabetes and diabetes of autoimmuneological type**

Genetically based human diabetes has been extensively studied and is generally acknowledged. Several models of idiopathic diabetes in animals have been described.

Insulitis is a key event in type I diabetes in humans. For this reason, attempts to induce autoimmuneological damage of beta cells may serve as a good model of insulin-dependent diabetes. In many genetically based models of diabetes, genetic changes lead to autoimmuneological response, which damages the pancreatic islets or beta-cells.

In the BB/W or (BB-biobreeding) model, insulin-dependent diabetes evokes spontaneously secondary to an immune-mediated destruction of the beta-cells [15]. Another model is the NOD mice (non-obese diabetes) — genetic changes of the beta-cells and modifications in MHC (major histocompatibility complex) lead to autoimmuneological reactions against pancreatic islets. In NOD-mice model, diabetes is evoked in 3–6-month old mice (mostly female) and insulin substitution is required [38].

Some genetically based models without autoimmuneological damage to the beta-cells have been used as well. One of them is transgenic mouse with overexpression of MHC genes on beta-cells. This overexpression probably leads to a competition between production of MHC and insulin particles, as they have common initial steps of synthesis of their peptic chain. As a consequence, insulin production is impaired [5]. Another interesting model is Zucker diabetic fatty rat, which is known as an experimental type 2 diabetes [56]. Goto-Kakizaki rats are used as an experimental model of non-insulin dependent diabetes [21]. Recently, Japanese investigators have established two spontaneous models of non-insulin dependent diabetes mellitus: “TSNO” model — diabetes without obesity and glucosuria and, resembling human type 2 diabetes, “TSOD” model — diabetes with obesity, glucosuria, stable hyperglycaemia, hyperinsulinemia and the hypertrophy of pancreatic islets without insulitis or fibrosis [55].

The use of genetic models is limited due to relatively low availability of genetically impaired animals and related high costs. Nevertheless, they are both interesting and important, and may imitate some conditions occurring in the course of the disease in human.

A model not based on genetic changes (or role of genes is unknown in this model), but with inflammatory response, leading to diabetes is also known. Multiple injections of very small dose of STZ lead to this type of diabetes, probably via autoimmuneological reaction, with insulitis and overactivation of lymphocytes [50].

**Experimental hyperglycaemia**

Apart from diabetes, hyperglycaemia alone, particularly of acute type, is studied. These models are well accepted and useful for studies on influence of hyperglycaemia on acute disorders, such as stroke or infarcts. Acute hyperglycaemia in rats can be, for example, induced by infusion of 25% glucose solution through the tail vein [34].

**Chemically-induced diabetes**

**Alloxan-induced diabetes**

Alloxan has been the longest known and the most potent cytotoxic agent damaging the pancreatic beta-cells. Injection of alloxan in susceptible animals induces permanent hyperglycaemia within 48–72 hours. Alloxan acts via damaging the mitochondria and DNA within the beta-cells, and irreversible oxidation of -SH groups of glucokinase [36]. However, alloxan injection in rats caused high mortality (37%) within few days [16]. It should be taken into consideration that alloxan express general toxicity and damages not only the beta-cells, but also the liver, kidneys, lungs, gonads, etc. [36].

The most extensively used model is STZ-induced diabetes [11]. It seems that it is relatively the easiest and cheapest model of diabetes. Besides, it is a well-described model and the toxicity of STZ is relatively low.

**Streptozotocin-induced diabetes**

The STZ-induced diabetes is regarded as a meaningful model of type 1 diabetes [47]. However, it has been
demonstrated on mice that low doses of STZ may produce a NIDDM (non-insulin dependent diabetes mellitus) and the elevation of blood glucose levels may be due to the increase in insulin resistance rather than to its impaired secretion, whereas high doses of STZ lead to IDDM (insulin dependent diabetes mellitus) [27].

Chemically, STZ is a broad-spectrum antibiotic extracted from Streptomyces acromogenes, which exerts antileukaemic and carcinogenic activity [26]. Rakieten et al. [49] were the first to report that STZ also exerts diabetogenic activity. After injection, STZ destroys pancreatic beta-cells relatively selectively [41], leading to insulin deficiency and hyperglycaemia. As early as 1 h after injecting STZ, degranulation of beta-cells occurs, and after 7 h, necrosis of beta-cells is seen [31]. The half-life of STZ is 5-35 minutes and it cannot be detected in the plasma beyond 3 hours after administration. Metabolites of STZ may be detected in plasma up to 24 hours after intravenous (i.v.) administration [1]. There is almost no transport of STZ into cerebrospinal fluid but metabolites of STZ readily enter the spinal fluid, and 2 h after administration the metabolites reach almost the same levels as that in the plasma [1]. No STZ concentrates in the brain [9].

In animal studies on experimental diabetes, mainly rats (different strains) have been used.

Table 1 presents doses of streptozotocin used to induce diabetes in different species of animals [23, with modification].

Hyperglycaemia is observed within days after STZ administration in either way (i.v., i.p. or s.c.) and is a permanent state. Glycaemia increases to very high values even up to 5-fold in comparison with control animals unless it is treated, e.g., with insulin, [42]. Two weeks after i.p. STZ, in Wistar rat assumed as diabetic, the content of glycaemia of over 180 mg/dl was noted in the tail vein blood. At the end of the tenth week after STZ administration, the content of glycaemia increased to 230–460 mg/dl [45, 46].

Animals treated with STZ gained little or no weight during the experiment, whereas non-diabetic control animals almost doubled in weight; antioxidant treatment had no effect on the body weight of the diabetic rats [42]. At four months after i.v. administration of STZ, diabetic rats were emaciated but did not appear severely ill, mortality during that interval was 8%. STZ-diabetic rats develop cataract, however it is probably a dose-dependent complication [6].

No toxic effect of STZ injected as diabetogenic agent i.v., i.p. or s.c. on CNS has been described. The only instances of central nervous system toxicity were reported after continuous STZ i.v. infusion over 5 days [52]. That observation was important to rule out direct toxic action of STZ (injected as diabetogenic agent) on the nervous system. Hence, when interpreting the results of studies on STZ-diabetes, changes observed in CNS should not be linked with direct influence of STZ on the nervous tissue, but with STZ-induced diabetes and/or other factors.

**CAN STZ-INDUCED DIABETES BE REGARDED AS A GOOD MODEL OF IMPACT OF DIABETES ON CNS IN HUMAN?**

**Pathophysiology**

Type 1 diabetes (insulin-dependent) is characterised by loss of beta-cells, decreased insulin, and hyperglycaemia. One of the possible mechanisms is that autoimmune activation of macrophages damages beta-

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose of STZ [mg/kg b.w.] i.v.</th>
<th>Dose of STZ [mg/kg b.w.] i.p.</th>
<th>Dose of STZ [mg/kg b.w.] s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey (Rhesus)</td>
<td>40–60</td>
<td></td>
<td></td>
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<tr>
<td>Monkey (juvenile cynomolgus)</td>
<td>150 [57]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>30–50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>Resistant</td>
<td></td>
<td></td>
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<tr>
<td>Rat (Wistar)</td>
<td>25–100 [27]</td>
<td>60 [22]-85 [22, 45]</td>
<td>40 [37]</td>
</tr>
<tr>
<td>Rat (Sprague-Dawley)</td>
<td></td>
<td>75–90 [42, 58]</td>
<td></td>
</tr>
<tr>
<td>Rat (SHRSP)</td>
<td></td>
<td>100 [3]</td>
<td></td>
</tr>
<tr>
<td>Rat (Holtzman)</td>
<td>50–100 [6]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>100–200 [47]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Very high doses required [35]</td>
<td></td>
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</tr>
</tbody>
</table>
-cells through release of NO after iNOS activation, resulting in DNA damage and activation of enzyme PARP (poly (ADP-ribose) polymerase) [47]. DNA damage and PARP activation is a common step in deleterious downstream of type 1 diabetes and STZ action [47]. Therefore, pathophysiology of human type 1 and STZ-diabetes seem similar (Table 2).

**Vascular changes**

Human diabetes is associated with decreased middle cerebral artery blood flow [44] and alterations in the autoregulation of the cerebral blood flow [7]. In a moderate and regionally specific fashion, CBF decreases in animals with poorly controlled, and hyperglycaemic diabetes. This depends on the severity of hyperglycaemia — particularly affected is the hindbrain with minor effect on the forebrain; the reduction has been seen in rats with either acute hyperglycaemia or STZ-induced chronic diabetes [19]. In diabetic patients, modest cerebral atrophy and an increased occurrence of subcortical and brain stem lesions [17] have been reported. Vascular complications were observed in many diabetes clinical observations; they included stroke, foci of malacia, disseminated, small ischaemic foci, transient ischaemic attacks or haemorrhagic episodes [4]. However, it is known that walls of the blood vessels (atherosclerosis, changes related to hypertension) change in the course of diabetes. Therefore, it is very difficult to determine whether metabolic or vascular changes play a more decisive role in the pathogenesis of diabetic complications. It is probable that both factors overlap and cannot be investigated separately. In their study on normotensive and spontaneously hypertensive streptozotocin-induced diabetic rats, Affolter et al. [2] found that vascular and cellular changes in the CNS poorly correlate. This led to the conclusion that microangiopathy plays a relatively minor role in diabetic encephalopathy. In earlier studies on male adult Holtzman rats, it was shown that STZ-diabetes resulted in high incidence of cataracts without retinal pathology and alterations in the kidney tubules (destruction of mitochondria with intact ER, heavy deposition of glycogen) before the development of demonstrable vascular lesions, which may suggest a direct effect of diabetes rather than an effect of vascular origin [6]. However, it remains unclear whether this conclusion is valid

<table>
<thead>
<tr>
<th>Table 2.</th>
<th>Comparison of STZ-induced diabetes and human diabetes. For references, see the text</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular changes</strong></td>
<td><strong>STZ-induced diabetes (animals)</strong></td>
</tr>
<tr>
<td>Decreased CBF</td>
<td>Yes</td>
</tr>
<tr>
<td>Preischemic hyperglycemia aggravates ischemic insult</td>
<td>Yes</td>
</tr>
<tr>
<td>Development of cataract</td>
<td>Yes</td>
</tr>
<tr>
<td>Renal changes</td>
<td>Yes</td>
</tr>
<tr>
<td>Increase of DAG and PKC in endothelial cells and smooth muscle cells of blood vessels</td>
<td>Yes</td>
</tr>
<tr>
<td>Increased lipid peroxidation</td>
<td>Yes</td>
</tr>
<tr>
<td>Increase of GSH, decrease of GSH reductase</td>
<td>Yes</td>
</tr>
<tr>
<td>Beneficial effect of oral administration of myoinositol</td>
<td>Yes</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>Yes</td>
</tr>
<tr>
<td>Poorer memory in adult rats/elderly patients</td>
<td>Yes</td>
</tr>
<tr>
<td>No effect on or improvement in memory of young rats/young patients</td>
<td>Yes</td>
</tr>
<tr>
<td>Vascular complications including changes in vascular walls</td>
<td>No or mild</td>
</tr>
<tr>
<td>Morphological changes in the brain in treated diabetes</td>
<td>No or mild</td>
</tr>
<tr>
<td>Morphological changes in the brain in untreated diabetes</td>
<td>Yes. Disseminated changes in different structures of CNS</td>
</tr>
<tr>
<td>Influence of ageing</td>
<td>Not studied/probably not significant as most studies carried on young or adult rats</td>
</tr>
</tbody>
</table>
for diabetes in humans. Particularly, when diabetes type II clinically shows, or it can be suspected, that the patient has already some cardiovascular pathology, such as heart disease, atherosclerosis and hypertension, which may be intensified and/or accelerated by diabetes.

**Morphologic changes in CNS in STZ-induced diabetes and human diabetes**

Histopathological studies on CNS in diabetic patients predominantly revealed changes classified as of vascular origin related, as assumed, to hypertension, thrombembolic disease, ischaemic attacks, microangiopathy and/or atherosclerosis. In animal studies, diabetes results in subtle changes predominantly localised in hypothalamus [37] or, if diabetes remains untreated for many weeks, dark neurones and local neuronal loss in many different structures may be noted [46]. Calcium deposits in the walls of the small blood vessels have been documented. Reduced density of cortical capillaries and increased capillary basement membrane thickening has been observed in long-term streptozotocin-induced diabetic animals [30, 40]. Quantitative analyses of cortical structure in rats with diabetes for 1 year showed decreased brain volume and weight and loss of cortical neurones [30]. It can be assumed that damage or changes observed during diabetes within CNS relates to its duration and the treatment applied. Long duration and lack of treatment lead to important damage of CNS structures and cells, whereas short duration of disease/hyperglycaemia and/or anti-diabetic treatment prevent such damages, or they are much less visible [30]. In agreement with findings in human, it has been reported that preischaemic hyperglycaemia worsens the neuronal damage in ischaemia in animals [48].

**Biochemical changes**

It has been shown that hyperglycaemia increases the synthesis of diacylglycerol (DAG) in endothelial cells by enhancing the metabolism of glucose to DAG precursors [33]. The increase of DAG and activation of protein kinase C (PKC) induced by hyperglycaemia have been confirmed in walls of the blood vessels in diabetic animals and patients [32]. DAG seems to predominantly activate certain isoforms of PKC rather than all isoforms [32]. It has been suggested that the activation of PKC is mediated by the effect of hyperglycaemia on gene expression [32]. Increased rates of lipid peroxidation were reported in the plasma of diabetic patients [51] and in tissues from STZ-diabetic rats [43]. In diabetic rats, gradual but sharp increases in GSH (reduced form of glutathione) transferase, and a decreased level of GSH and GSH reductase were found in different tissues (including brain) and blood [43]. The decreased level of GSH in diabetic patients could be caused by the decreased activity of enzymes, such as gamma-glutamylcysteine synthetase, glutathione reductase, or G6PD, possibly due to their glycation by the uncontrolled hyperglycaemia [29]. Additionally, it appears that generation of free radicals by hyperglycaemia [28, 59] causes increased utilisation of GSH and thus lowers its levels of erythrocytes in diabetic patients [29]. It has been documented that the decrease in the GSH content of erythrocytes significantly correlates with the degree of hyperglycaemia in diabetic patients and suggested that lower levels of GSH might have a role in the cellular damage in uncontrolled diabetic patients [29]. After two weeks of STZ-induced diabetes in rats (untreated), a decrease in myoinositol concentration in the nerve was found while the serum level remained unchanged. A special diet including myoinositol given to diabetic rats restored myoinositol concentration in the peripheral nerves and normalised the nerve conduction velocity [24]. However, studies on the influence of dietary myoinositol on diabetic peripheral neuropathies in humans showed no clear effect [20]. In selected structures of the rat brain, 4 and 12 weeks after STZ injection, a high (mostly in the adult rats) increase in the content of Ca2+/Calmudulin dependent protein kinase II (CaM kinase II) was observed [8]. CaM kinase II was found to be one of the main constituents of post-synaptic densities in glutamergic synapses and may influence NMDA receptor function and synaptic plasticity, which was shown in STZ-induced diabetes [13].

**Neuropsychological and electrophysiological observations**

Neuropsychological and electrophysiological studies show the adequacy of STZ-induced diabetes model and diabetes in human. In diabetic patients, cognitive impairments have been noted [25]. In STZ-induced diabetic rats, deficits in water maze learning were demonstrated [10]. In diabetic patients, increased latencies of auditory, visual and somatosensory evoked potentials have been reported [18]. Diabetic rats showed changes in hippocampal long-term potentiation (LTP), which is a form of synaptic plasticity [12]. Stone et al. [54] reported a correlation of high blood glucose values with poorer memory in old but not young rats. In elderly humans, high peak levels of glucose were associated with poor memory performance [39, 14], whereas, in young ones, higher levels of blood glucose were associated with better performance in a verbal memory test.
The influence of ageing on the development of diabetic complications requires more research and may be a factor that influences comparisons between clinical and experimental diabetes.

Reassessing the above cited observations, it seems that, at least to some degree, pathophysiology of STZ-induced diabetes in animals and human diabetes is similar. Moreover, there are important biochemical and clinical features, which are almost identical in both experimental and human diabetes. However, morphological findings are not so similar. There are probably at least several reasons. One of the most obvious may be the influence of uncontrolled variables in humans — genetics, age, diet, general health, behaviour, social conditions, drugs, duration of disease and life, control of euglycaemia, etc., which can be controlled and/or regulated under laboratory conditions. Another reason may be differences related to species, which may result in differences in metabolism and defensive activities, e.g., antioxidative defence and/or resistance against oxidative stress. Yet another reason may be the treatment or lack of treatment of diabetes. Generally, clinical studies are observations made on patients who control their glycaemia with drugs or at least with diet. Hence, they tend to maintain euglycaemia or slight hyperglycaemia. In patients, diabetes is commonly accompanied by other diseases, including infections, cardiovascular disorders, and nutritional disorders. Side effects of treatment should also be taken into consideration when analysing clinical data.

In fact, treatment with oral antidiabetic drugs or more frequently with insulin is also used in some animal studies.

It has to be remembered that in humans, diabetes develops and lasts usually for years. On the contrary, in animals, diabetes or hyperglycaemia lasts for weeks or months. This may be too short time for the development of cardiovascular complications. Instead, we can observe rather a direct unbiased effect of hyperglycaemia as a metabolic disorder, even if treated with insulin. Therefore, the morphological changes found in the CNS of diabetic animals should differ from those observed in humans. From this point of view, animal models of diabetes, including STZ-induced diabetes are valuable source of data but do not directly reflect changes developing in human brains. But, the fact that clear pathological findings using „pure” diabetic models have been presented shows the role of hyperglycaemia alone in damaging the CNS. Oxidative stress, resulting in neuronal cell damage and/or death, may be one of the most adequate explanations [45].

CONCLUSION

It seems that uncontrolled, and prolonged hyperglycaemia cannot be directly compared with neither experimental diabetes treated, with antidiabetic agents nor with diabetes in humans. However, animal studies, including untreated STZ-induced diabetes, may bring valuable data concerning the effects of hyperglycaemia alone.

REFERENCES


