LABORATORY STUDY

Influence of subconjunctival steroid injection on blood glucose profile in diabetic rats

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Abstract

Purpose **The influence of subconjunctival steroid injection on blood glucose concentration was investigated in rats with streptozotocin (STZ)-induced diabetes.**
Methods **Adult male Wistar rats with STZ-induced diabetes (n = 10) and normal controls (n = 10) received subconjunctival injections of 0.1 ml of 0.4% dexamethasone or saline. Blood glucose concentrations were measured before and 3, 6, 12, 18 and 24 h after treatment.**
Results **In the STZ group, subconjunctival injection of steroids (p = 0.0013) or saline (p = 0.0037) significantly increased the blood glucose level 3 h after treatment. In the control group, the blood glucose concentration was not elevated by subconjunctival injection. In both STZ and control groups, the blood glucose concentration was significantly higher after steroid injection than after saline injection throughout the 24 h study period.**
Conclusions **Subconjunctival steroid injection induced a significant blood glucose increase in both diabetic and control rats. For diabetic rats, the subconjunctival injection itself constituted stress that resulted in glucose elevation.**

Key words Blood glucose concentration, Diabetes mellitus, Rat, Steroid, Streptozotocin (STZ), Subconjunctival injection

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Control of post-operative inflammation is clearly the most common indication for steroid use. In cataract surgery, subconjunctival steroid injection has been widely used in the belief that it will reduce post-operative inflammation.[1–5] Subconjunctival steroids can be absorbed into the systemic circulation,[6] which may raise blood glucose levels in diabetic patients. Little is known, however, about the glycaemic profile following subconjunctival steroid injection. The aim of this study was to assess the effect of subconjunctival steroid injection on blood glucose concentrations in rats with streptozotocin (STZ)-induced diabetes.

Materials and methods

Experimental animals

Twenty male 6-week-old Wistar rats (weighing 140–160 g) were used in this study. All rats were housed in a quiet room with constant temperature (23–25 °C), humidity (55–65%) and light (illumination from 0600 to 1800 hours). They were allowed free access to regular food (Nippon Bio Supp Center, Tokyo, Japan) and tap water. The current experiments were performed in accordance with institutional guidelines for animal experiments at the University of Tokyo.

Induction of diabetes

Rats were randomly divided into a diabetic group (STZ group) and a non-diabetic group (control group). After a 12 h fast, the rats in the STZ group (n = 10) were anaesthetised with intramuscular ketamine hydrochloride and xylazine hydrochloride. The STZ (Wako Pure Chemical Industries, Osaka, Japan) was freshly dissolved in 0.1 ml of 50 mM citrate buffer, pH 4.5, and the 45 mg/kg solution injected into the tail vein. The rats in the control group (n = 10) received the same anaesthesia and venous injection of an equivalent volume of citrate buffer. Blood glucose levels were checked 7 days after STZ administration using a glucose meter (Sanwa Kagaku Kenkyusho, Nagoya, Japan). The STZ-treated rats displayed mild hyperglycaemia (165.9 ± 34.3 mg/dl) compared with the control group (124.9 ± 33.3 mg/dl).

Subconjunctival injection

Seven days after STZ administration, rats were fasted for 12 h and anaesthetised with ketamine hydrochloride and xylazine hydrochloride. In both the STZ group and the control group, half the rats received a subconjunctival injection of 0.1 ml of 0.4% dexamethasone sodium phosphate, and the other half a subconjunctival injection of 0.1 ml saline. The injections were into the right eye. One week later, the steroid

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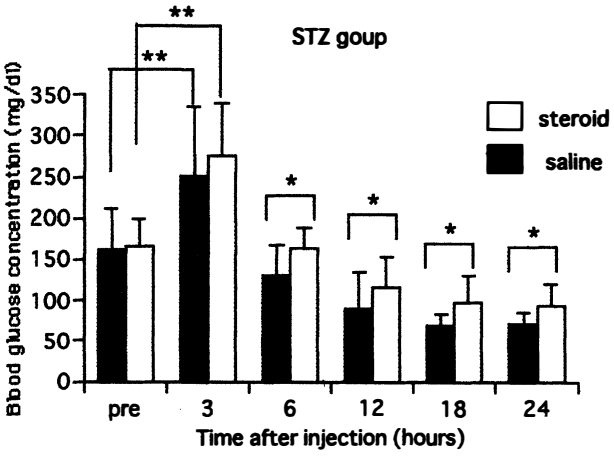

Fig. 1. *Blood glucose concentration after subconjunctival steroid or saline injection in the STZ group. *p < 0.05 (unpaired t-test); **repeated measures ANOVA and post-hoc test.*

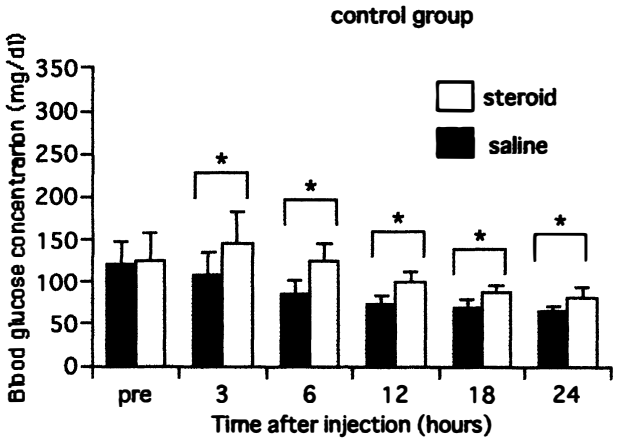

Fig. 2. *Blood glucose concentration after subconjunctival steroid or saline injection in the control group. *p < 0.05 (unpaired t-test).*

and saline groups were exchanged and the same procedures were performed on the left eye of each animal.

Measurement of blood glucose concentration

The blood glucose concentration was measured before and 3, 6, 12, 18 and 24 h after subconjunctival injection. All rats were fasted during the study.

Statistical analysis

Statistical significance was tested using an unpaired *t*-test (two-tailed), repeated measures ANOVA and a post-hoc test.

Results

In the STZ group, subconjunctival injections of steroids ($p = 0.0013$) and saline ($p = 0.0037$) induced significant increases in blood glucose 3 h after treatment (Fig. 1). In the control group, the blood glucose concentration did not rise in response to the subconjunctival injection of either dexamethasone or saline (Fig. 2).

In the STZ group, the blood glucose concentration between 3 and 24 h after treatment was significantly higher after steroid injection than after saline injection (Fig. 1). Among controls, the difference between the steroid and saline injection groups was statistically significant between 3 and 24 h after treatment (Fig. 2).

Discussion

Weijtens *et al.*[7] investigated the concentration of dexamethasone in the aqueous, vitreous and serum after subconjunctival injection. They reported a mean maximum serum concentration of 32.4 ng/ml approximately 30 min after subconjunctival injection of 2.5 mg dexamethasone disodium phosphate, which was higher than the level obtained with oral administration of dexamethasone. The serum dexamethasone concentration curve was best described by log-linear regression. The titre of 0.3 ml of 0.4% dexamethasone sodium phosphate (= 1.2 mg of dexamethasone) is equivalent to 7.5–9 mg of prednisolone. Steroids absorbed into the systemic circulation may increase the blood glucose level by stimulating hepatic glucose metabolism[8] and peripheral glucose production.[9] However, the blood glucose concentration profile after subconjunctival steroid injection has not been reported. We assessed the effect of subconjunctival steroid injection on the blood glucose level using rats with STZ-induced moderate diabetes.

Subconjunctival injection of saline significantly elevated blood glucose in the STZ group, but not in the control group. These results indicate that subconjunctival injection itself places considerable stress on rats, leading to hyperglycaemia in the diabetic rats. Activation of the sympathetic nervous system and the consequent release of catecholamines can contribute to the development of hyperglycaemia. Catecholamines directly stimulate glucose production by the liver[10,11] and also interfere with insulin-mediated glucose uptake.[10,12] Unless pancreatic islet function is simultaneously diminished, hyperglycaemia strongly stimulates insulin secretion and inhibits the release of glucagon. These effects of hyperglycaemia on pancreatic islet function usually work to maintain the glucose level during stressful situations in normal subjects. However, diabetic patients who suffer from impaired islet responses to glucose will be particularly prone to the development of marked hyperglycaemia under stressful conditions.[13] In the present study, the stress of subconjunctival injection induced hyperglycaemia in diabetic rats. On the other hand, the control rats were able to handle the stress of subconjunctival injection without significant fluctuations in glucose level.

The blood glucose concentration was significantly higher after subconjunctival steroid injection than after saline injection in both the control group and the STZ group. The difference between the steroid and saline groups remained statistically significant throughout the 24 h study period. Blood glucose concentration peaked 3 h after subconjunctival injection, followed by a gradual

decline due to fasting during the study. These data indicate that subconjunctival steroid injection exerts a hyperglycaemic effect which lasts for at least 24 h. However, the relative volume of chemicals injected in this study was 100 μl (670 μl/kg), which is more than 100 times the volume usually used in humans (6 μl/kg). Thus, the current results cannot simply be extrapolated to diabetic patients. Nevertheless, we recently found that subconjunctival steroid injection at the completion of cataract surgery induced a transient but significant blood glucose increase in diabetic patients on the day of surgery (unpublished data). Although subconjunctival steroid injection at the completion of cataract surgery is believed to reduce post-operative inflammation,[1–5] recent studies have demonstrated no such suppression in uncomplicated cases[14,15] or in diabetic eyes with neither proliferative retinopathy nor intraoperative complications.[16] We advocate that the usefulness and necessity of subconjunctival steroid injection be carefully reconsidered in light of the possible adverse effects, as shown herein, especially in diabetic patients.

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