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MINIREVIEW

This article is dedicated to Professor Vratislav Schreiber, the founder of Czechoslovak experimental endocrinology, on the occasion of his 75th birthday

The Role of Nitric Oxide in the Development of Streptozotocin-Induced Diabetes Mellitus: Experimental and Clinical Implications

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Summary

The overproduction of the free radical nitric oxide (NO) by activated immunocompetent cells with subsequent development of local oxidative stress is supposed to be one of the possible pathophysiological mechanisms of β-cell damage during streptozotocin-induced diabetes. The blockade of increased NO production by simultaneous administration of NO-synthase inhibitors partially suppresses the hyperglycemia and the increase of glycated hemoglobin concentration. Here we summarize the current state of knowledge concerning the modulation of streptozotocin-induced diabetes development by treatment with NO-synthase inhibitors including the partial inhibition of the changes in serum leptin levels. The differences in the reaction to streptozotocin administration between wild type mice and inducible NO-synthase knockout mice are also discussed. The overproduction of NO during the development of streptozotocin-induced diabetes is probably an important part of the complex autoimmune reaction which leads to the destruction of pancreatic β-cells. Further clarification of the role of nitric oxide in streptozotocin-induced diabetes development could have important clinical implications.

Key words

Streptozotocin • Diabetes • Nitric oxide • Oxidative stress • Leptin • Rat

Introduction

Nitric oxide is an extremely reactive gas with chemical properties of a free radical. It fulfills a series of different functions in a number of physiological and pathophysiological processes in the human body (for review see Haluzík 1998). The production of nitric oxide by endothelial cells is necessary for the normal regulation of vascular tone. Its overproduction, on the other hand, plays a crucial role in the development of

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catecholamine-resistant hypotension during septic shock (Furchgott and Zawadski 1980). The endothelial dysfunction in patients with atherosclerosis, essential hypertension and diabetes is accompanied by decreased basal endothelial production of nitric oxide (Forte *et al.* 1997). The complex host response reaction of human immunocompetent cells involves the local overproduction of NO that leads to the destruction of bacterial or viral agents (Vallance *et al.* 1997). A similar mechanism, however, could be responsible for the damage of some cells and tissues in a series of autoimmune diseases including insulin-dependent diabetes mellitus (Corbett *et al.* 1992).

Oxidative stress is supposed to play a role in the development of insulin-dependent diabetes mellitus and subsequent diabetic complications (Wierusz-Wysocka *et al.* 1997). The immunological effector molecules of this process are cytokines, namely interleukin-1 (Mandrup-Poulsen *et al.* 1985). Interleukin-1 was demonstrated both to inhibit insulin secretion from pancreatic β-cells and to induce β-cell destruction. The increased levels of interleukin-1 and other cytokines activate the expression of the inducible isoform of NO-synthase that produces high amounts of free radical nitric oxide. High local NO concentrations induce oxidative stress followed by the destruction of pancreatic β-cells and subsequent development of insulin-dependent diabetes mellitus (Corbett *et al.* 1993).

The influence of NO-synthase inhibitors on streptozotocin-induced diabetes

Kolb *et al.* (1991) reported partial attenuation of streptozotocin-induced hyperglycemia in mice by simultaneous treatment with the NO-synthase inhibitor N^G-nitro-L-arginine-methyl ester (L-NAME). The importance of local nitric oxide overproduction in the development of streptozotocin-induced diabetes was later supported by Lukic *et al.* (1991) and Catanzaro *et al.* (1994) who found similar effects of another NO-synthase inhibitor L-N^G-monomethyl-arginine in C57BL/6J and C57BL/KS-mdb mice. Papaccio *et al.* (1995) reported that the effect of simultaneous L-NAME administration on diabetes induced by a low dose of streptozotocin in C57BL/6J mice was only transient and diminished after the withdrawal of L-NAME. They found that L-NAME administration for 15 days partially decreased the hyperglycemia in diabetic mice, but the blood glucose values returned to those of diabetic rats 14 days after L-NAME withdrawal. Moreover, morphological findings demonstrated that the degree of infiltration and islet β-cell damage were not inhibited by L-NAME treatment.

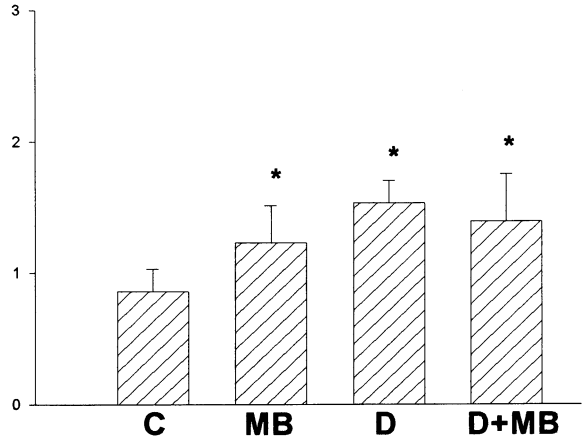

Fig. 1. *Erythrocyte superoxide dismutase activity (IU) in control (C), methylene blue (MB), diabetic (D) rats and diabetic rats treated simultaneously with methylene blue (D+MB). Data are expressed as means ± S.D. * significant difference from the control group (p<0.05).*

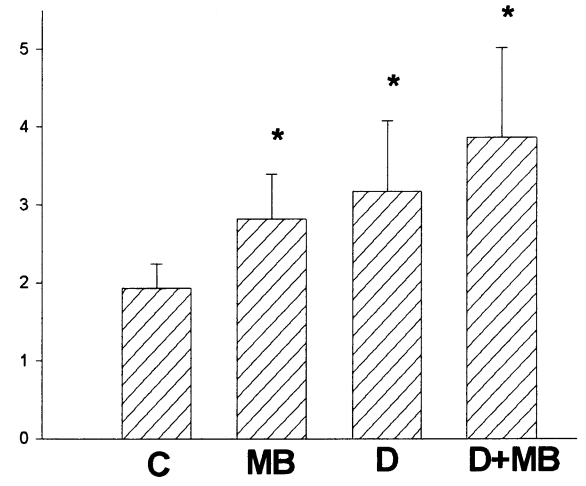

Fig. 2. *Serum malondialdehyde levels (mmol.l^{-1}) in control (C), methylene blue (MB), diabetic (D) rats and diabetic rats treated simultaneously with methylene blue (D+MB). Data are expressed as means ± S.D. * significant difference from the control group (p<0.05).*

In our experiments, we have studied the influence of two NO-synthase inhibiting compounds, methylene blue and L-NAME, on the development of streptozotocin-induced diabetes and parameters of global oxidative stress in Wistar male rats (Haluzík *et al.* 1998,

1999b, 1999c). While L-NAME is specific non-selective inhibitor of NO-synthase, methylene blue is rather unspecific substance which combines the properties of NO-synthase inhibitor with free oxygen radical scavenger (Haluzík *et al.* 1995a,b). We have found that both methylene blue and L-NAME significantly suppressed the development of streptozotocin-induced diabetes. While L-NAME treatment significantly decreased only glycated hemoglobin concentrations in diabetic rats at the end of the experiment, methylene blue treatment attenuated both the increase in glycated hemoglobin levels and blood glucose concentrations. Interestingly, the effect of methylene blue on diabetic parameters was more pronounced after three compared to six weeks of diabetes duration. Methylene blue alone increased both erythrocyte superoxide dismutase activity and serum malondialdehyde levels. Its administration to diabetic animals did not change the diabetes-induced increase in erythrocyte superoxide dismutase activity and serum malondialdehyde levels (Figs 1 and 2). L-NAME administration augmented erythrocyte superoxide dismutase activity without affecting serum malondialdehyde. The administration of this compound to diabetic animals did not change their global oxidative stress parameters (Figs 3 and 4).

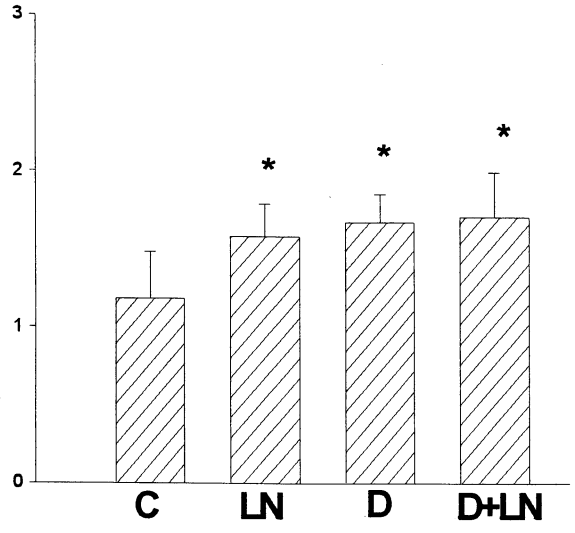

Fig. 3. *Erythrocyte superoxide dismutase activity (IU) in control (C), L-NAME (LN), diabetic (D) rats and diabetic rats treated simultaneously with L-NAME (D+LN). Data are expressed as means ± S.D. * significant difference from the control group (p<0.05).*

Wu (1995) found that neither aminoguanidine nor N^{ω}-nitro-L-arginine methyl ester influenced the onset of diabetes in spontaneously diabetic BB rats. Series of studies concerning the influence of aminoguanidine on streptozotocin-induced diabetes has been performed by Kedziora-Kornatowska and coworkers. In the first experiment (Kedziora-Kornatowska *et al.* 1998a), these authors found that simultaneous aminoguanidine treatment did not affect the body weight, blood glucose and HbA1c concentration of diabetic rats. The induction of diabetes resulted in an increase of malondialdehyde concentration and a decrease of superoxide dismutase and catalase activities. The glutathione peroxidase activity in erythrocytes increased after six weeks of diabetes and returned to control levels after 12 weeks. The simultaneous aminoguanidine administration attenuated the increase of glutathione peroxidase activity in erythrocytes after six weeks of diabetes and augmented its activity after 12 weeks of diabetes duration. In another experiment, the influence of aminoguanidine on local oxidative stress in kidneys of diabetic animals was studied (Kedziora-Kornatowska *et al.* 1998b). Renal malondialdehyde concentrations were elevated, while the superoxide dismutase and catalase activities were decreased in the diabetic rats compared to the controls. Simultaneous aminoguanidine treatment attenuated the increase in renal malondialdehyde content and diminished superoxide dismutase and catalase activities.

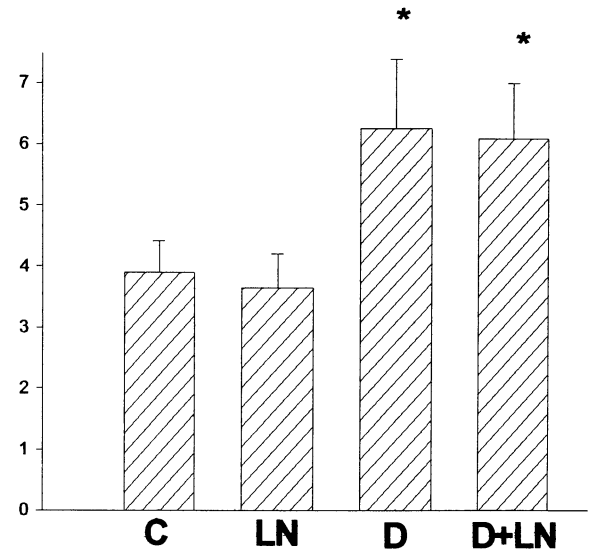

Fig. 4. *Serum malondialdehyde levels (mmol.l^{-1}) in control (C), L-NAME (LN), diabetic (D) rats and diabetic rats treated simultaneously with L-NAME (D+LN). Data are expressed as means ± S.D. * significant difference from the control group (p< 0.05).*

The same group of authors (Kedziora-Kornatowska *et al.* 1998b) reported that aminoguanidine treatment partially

suppressed the elevated production of both superoxide anion and nitric oxide by peripheral granulocytes of diabetic rats.

Flodström *et al.* (1999) studied the importance of nitric oxide generation by inducible NO-synthase in the development of streptozotocin- and/or interleukin-1β-induced diabetes in the model of inducible NO-synthase knockout mice. *In vivo* experiments demonstrated a significant reduction in the number of diabetic animals among inducible NO-synthase knockout mice compared to wild type mice after a single streptozotocin injection (23 % vs 100 % of diabetic animals 15 days after streptozotocin injection). *In vitro* experiments revealed that the exposure of pancreatic islets from wild type mice to interleukin-1β caused the induction of inducible NO-synthase with subsequent impairment of insulin synthesis and release. In inducible NO-synthase knockout mice the interleukin-1β administration had no effect on the function of pancreatic islets.

Taken together, the experimental results concerning the influence of NO-synthase inhibitors on the development of streptozotocin-induced experimental diabetes can be summarized as follows. The local overproduction of nitric oxide with subsequent development of oxidative stress in streptozotocin-treated rats undoubtedly plays an important role in β-cell damage and diabetes induction. The inhibition or total lack of inducible NO-synthase, however, is not able to block the development of diabetes completely, because in inducible NO-synthase knockout mice 23 % of animals developed diabetes after streptozotocin injection.

The administration of different NO-synthase inhibitors yielded very different results as far as the degree of attenuation of streptozotocin diabetes development was concerned. Aminoguanidine, the NO-synthase inhibitor with a relatively high selectivity for its inducible isoform, was much less effective in attenuating diabetes development than non-selective NO-synthase inhibitors or compounds that combine the NO-synthase inhibiting effects with the scavenging of free oxygen radicals (methylene blue). The global oxidative stress changes in diabetic animals treated simultaneously with NO-synthase inhibitors did not correlate precisely with the attenuation of diabetes development. The reason for these discrepancies is not clear. The mechanism of local nitric oxide overproduction is probably only a part of the complex immune reaction that leads to β-cell destruction in streptozotocin-treated rats. The differences in tissue penetration of different NO-synthase inhibiting compounds must also be taken into account when comparing their *in vivo* effects. Moreover, the local (but not only the global) oxidative stress and nitric oxide production should be measured in the pancreatic tissue of diabetic animals to clarify the above mentioned questions.

The changes of serum leptin levels in experimental streptozotocin-induced diabetes and its modulation by NO-synthase inhibitors

Leptin is a recently discovered protein hormone produced predominantly by adipocytes. Its serum levels reflect the body fat content, i.e. they are increased in obese compared to lean subjects (for review see Mantzoros and Moschos 1998). Leptin was originally identified in *ob/ob* mice with nonsense mutation of the leptin gene and virtually no functional leptin (Zhang *et al.* 1994). With the exception of the body fat content, serum leptin levels are also regulated by a number of hormonal signals including cortisol, the growth hormone, testosterone, estrogens and in particular insulin (Kolaczynski *et al.* 1996, Nedvídková *et al.* 1997).

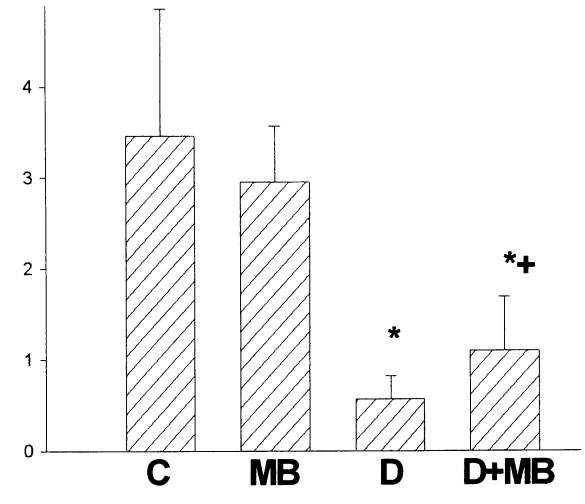

Fig. 5. *Serum leptin levels (ng.ml^{-1}) in control (C), methylene blue (MB), diabetic (D) rats and diabetic rats treated simultaneously with methylene blue (D+MB). Data are expressed as means ± S.D. * significant difference from the control group ($p<0.05$), $^{+}$ significant difference from the diabetic group ($p<0.05$).*

It has recently been found that leptin is significantly decreased in rats and mice with streptozotocin-induced diabetes (Havel *et al.* 1998, Sivitz *et al.* 1998). The drop of serum leptin levels in diabetic animals is very rapid and is relatively independent of the changes in the body fat content. Reports concerning changes of serum leptin levels in animals with streptozotocin-induced diabetes indicate that probably several mechanisms are responsible for the decrease in serum leptin levels. Firstly, there is a likely direct toxic effect of streptozotocin on the adipose tissue that decreases leptin production by adipocytes. Secondly, the rapid development of almost complete insulin deficiency and the subsequent lack of insulin stimulation of leptin synthesis is another mechanism of streptozotocin-induced hypoleptinemia. The crucial role of insulinopenia in the drop of serum leptin levels was further supported by experiments in which daily subcutaneous insulin administration significantly suppressed the drop of serum leptin levels in diabetic animals.

In our experiments, the influence of treatment with NO-synthase inhibitor and free radical scavenger, methylene blue, on the changes in serum leptin levels was studied in Wistar rats with streptozotocin-induced diabetes (Haluzík *et al.* 1999a,b). The treatment with methylene blue alone did not significantly affect either blood glucose, glycated hemoglobin levels or serum leptin levels. The simultaneous treatment of diabetic animals with methylene blue led to a partial inhibition of diabetes development (suppression of increased blood glucose and glycated hemoglobin levels) and, moreover, to a significant attenuation of the drop in serum leptin levels (Fig. 5). We suppose that the modulation of serum leptin levels by methylene blue is the result of partial prevention of streptozotocin-induced β-cell damage which in turn leads to higher resting insulin secretion in diabetic animals treated simultaneously with methylene blue.

Conclusions

The cytokine-induced overproduction of nitric oxide by inducible NO-synthase, with subsequent increase of local oxidative stress in the pancreatic Langerhans islets, is one of the most important pathogenetic mechanism of β-cell damage during the experimental streptozotocin-induced diabetes in rats and probably also in human insulin-dependent diabetes mellitus. The experiments with NO-synthase inhibitors and especially those performed using inducible NO-synthase knockout mice provide strong evidence for this statement. However, the above mentioned mechanism is probably only a part of the complex autoimmune reaction that leads to the β-cell damage and the development of diabetes. The experiments with NO-synthase inhibitors, which are able to attenuate partially the development of streptozotocin-induced diabetes, have provided an important piece of information necessary for the further elucidation of the precise pathogenetic mechanisms of human insulin-dependent diabetes mellitus and opens new potential perspectives in its prevention and treatment.

Acknowledgements

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