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Mini Review

The role of iNOS in beta cell destruction in diabetes

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Abstract
Nitric oxide (NO) is a signaling molecule which can play a role in pancreatic pathophysiology and is involved in regulation of beta cell mass. Nitric oxide is produced by the enzyme NO synthase (NOS). Nitric oxide synthase has three major isoforms; one of them, inducible NOS (iNOS) is expressed in almost every cell type. In healthy pancreatic tissues, iNOS immunoreactivity was demonstrated in islet of Langerhans cells, centroacinar cells and ductal cells. In addition, induction of islet iNOS was also demonstrated in acute pancreatitis, and type 1 and type 2 diabetes mellitus. When pancreatic beta cells come up against inflammatory cytokines, lipid stress or hyperglycemia, iNOS activation increases and the resulting high concentrations of NO produced causes cell dysfunction or death and therefore the insulin secretion is inhibited. However, the precise mechanism of iNOS expression and the occurrence of resulting beta-cell damage have not been truly clarified yet. Diabetogenesis cannot be completely prevented with iNOS inhibitors. Physiological and pathological limits of iNOS expression should be established and the mechanism of the damage on beta cells by over-expression should be defined and prevented.

Nitric oxide (NO), which is a free radical with certain biological effects on cells, is a gaseous signaling molecule that is required for pancreatic physiology, but can also play a role in pathophysiological processes of the pancreas and involve in β-cell mass regulation [1-3]. Nitric oxide is produced by the enzyme NO synthase (NOS) which has three major isoforms; *i.e.* neuronal NOS (nNOS or NOS1), endothelial NOS (eNOS or NOS3), and inducible NOS (iNOS or NOS2) [4]. Neuronal NOS and eNOS, also known as constitutive NOS (cNOS), are Ca(2+)/calmodulin-dependent isoforms and have protective roles in the gastrointestinal system. The Ca(2+)/calmodulin independent isoform iNOS is held responsible for various physiological and pathological processes [5]. Inducible NOS is expressed in healthy tissue cells whereas it is also detected in various pathological tissue cells such as hyperplasia, cyst and granuloma [6, 7]. It is believed that iNOS expression increases in the inflammatory process in tissues and in some malign transformations [4]. Inducible NOS, like other NOS isoforms, is involved in NO production by catalysing transformation of L-arginine to L-citrulline [8, 9].

Today, it is argued that iNOS is expressed in almost every cell type [3]. In a previous study, we investigated iNOS immunoreactivity in islet of Langerhans cells, acinar cells, centroacinar cells and ductal cells in the healthy pancreatic tissues of rats and we detected iNOS immunoreactivity in all cell groups analyzed, except the acinar cells [4]. Beta cell iNOS is similar to the one expressed in other cells and is encoded by the same gene in chromosome 17 like others. Nitric oxide induces a negative feedback on iNOS expression [8].

Insulin which is secreted by β-cells and has an effect especially on carbohydrate, leptin and protein metabolisms provides rapid penetration of glucose through the cell membrane [10]. Insulin-dependent diabetes mellitus (IDDM, type 1 diabetes mellitus) is an autoimmune disease characterized by selective destruction of insulin-secreting β-cells**.** Inducible NOS expression is observed in various autoimmune diseases [11].

High levels of NO production in pancreatic islets may negatively affect β-cell function. Induction of islet iNOS was demonstrated in both type 1 and type 2

diabetes mellitus (DM) [12]. Additionally, it was shown that iNOS is expressed in β-cells in acute pancreatitis [13]. Increased expression of islet iNOS causes excessive NO production and contributes to the dysfunction of β- and α-cells and inhibits insulin secretion [12, 14]. When pancreatic β-cells come up against inflammatory cytokines, lipid stress or hyperglycemia, iNOS activation increases and the resulting high concentrations of NO produced causes cell dysfunction or death [2, 12]. Other NOS isoforms do not cause the same result on β-cells. Cytokine-induced iNOS contributes to the development of type 1 DM whereas cNOS is related to the physiological regulation of insulin secretion [14]. Low concentrations of NO, which are constitutively produced by eNOS, promote cell survival [2].

Several pro-inflammatory cytokines, like interleukin (IL)-1β, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ, have cytotoxic effects on pancreatic β-cells and are also an important mediator of β-cell destruction in IDDM [15, 16). In islets, IFN- γ and IL-1β combinations induce iNOS expression, which causes incresed production of NO, resulting in islet cell destruction [17- 23].

Cytokines, as in the most of the cell types, also activate nuclear factor (NF)-κB in human pancreatic islets [3, 24]. Nuclear factor-κB induces iNOS gene expression [25]. Again, in most cell types, the major modulators of iNOS expression induced by pro-inflammatory cytokines are the members of the mitogen-activated protein kinases (MAPK) family, such as extracellular signal regulated kinases (ERK1 and ERK2), c-Jun N-terminal kinases (JNK1 and JNK2) or p38 [3].

The exact mechanisms of iNOS expression and resulting β-cell damage have not been entirely clarified yet. It is reported that NF-κB activation is necessary but not sufficient alone for cytokine-induced iNOS expression in human Langerhans islets [26], However, in another article, it is claimed that cytokine-induced NOS activation in pancreatic β-cells is unaffected by inhibition of NF-κB activation [27]. It is also claimed that the effect of IFN-γ on iNOS expression cannot be explained by the increased NF-κB activation; instead, IFN-γ induces an increase in cellular interferon regulatory factor (IRF)-1, which may contribute to increased iNOS mRNA expression [28]. Actually, NF-κB and IRF-1, both being transcription factors, are activated in islets by cytokines. However there is no information indicating that these factors are regulating iNOS promoter in insulin producing cells. New approaches are necessary to explain cytokine-induced iNOS expression and the subsequent β-cell damage [24].

In the studies to be performed regarding this subject, there are some details that need to be paid particular attention. The primary ones are the characteristics of the species studied and the accurate assessment of the factors increasing and decreasing iNOS expression according to these species. Inducible NOS expression varies strain-dependently. These variations are related to IL-1 receptor type 1 (IL-1RI) or IRF-1, and/or caused differences in heat shock protein (HSP)70 expression [29]. While, iNOS mRNA in rodent insulin-producing cells is induced by only IL-1β, in human pancreatic islets combination of two (IL-1β + IFN-γ) or three (IL-1β + IFN-γ + TNF-α) cytokines are necessary for iNOS activation. There are significant differences between iNOS regulation in rodent and human pancreatic islets [8]. It has been shown in another research that in mouse islets, IFN-γ and TNF-α induce NO production and iNOS mRNA expression synergistically [30].

It is argued that not only expression, but also effect of iNOS may vary with species. Inducible NOS protein has been found in normal adult rat and pig islets, but not in fetal pig islets. In adult pig islet grafts, some correlation between iNOS protein presence and islet cell apoptosis and primary non-function has been found, however, in rat islets, despite the persistent presence of iNOS, no evidence has been found about its deleterious effect on rat islet viability or function [31].

Beta cells have some specific characteristics. For example, it is believed that they are endoderm-originated; however, also some evidences have been presented on their similarity with the neural crest-originated cells [32, 33]. It is not known yet that at what proportion these characteristics are effective on the expressions of proteins like iNOS in the cell. Inducible NOS expression is up-regulated by most of the insulin resistance inducers such as pro-inflammatory cytokines, obesity, free fatty acids, hyperglycemia, endotoxins and oxidative stress [3]. JNK1 and JNK2 activation is responsible for hyperglycemia-induced iNOS gene expression. On the other hand, dihydrochloride L-N6-(1-Iminoethyl)lysine (L-NIL), is the specific inhibitor of iNOS. Also, thiazolidinediones (synthetic peroxisome proliferator-activated receptor (PPAR)-γ agonists) inhibit the iNOS expression [3].

It has been investigated whether environmental factors like heavy metal salts are modulating iNOS expression in β-cells. In this research it has been observed that iNOS gene expression is increased by Pb(2+) and suppressed by Hg(2+) (34). It is also claimed that some cytokines, including IL-10 and transforming growth factor (TGF)-β, are suppressing iNOS expression [34].

It has been reported that long-term total parenteral nutrition treatment in rats causing glucose-stimulated insulin release disorder may be explained by iNOS

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expression in β-cells and an obvious iNOS-derived NO production [35]. Another observation is the inhibition of iNOS expression in insulin-containing cells by glucagon [23].

Although a consensus that cytokines increase the iNOS expression is made, there are different opinions regarding its effect on β-cell destruction. It is even claimed that the effect of IL-1β on insulin secretion in rat islets is not related to the enzyme activity of NOS and iNOS-related NO production [19, 36]. Although cytokines may induce NO-independent corruption, it can be concluded that iNOS inhibition may provide protection against cytokine-induced β-cell damage. It is found that suppression of iNOS in type 2 DM leads to increase in β-cell viability and regular secretory respond to glucose [12]. Research on inhibition of iNOS expression still continues. It has been claimed that the iNOS inhibitor ONO-1714, while completely inhibiting IL-1β-induced NO production, does not reduce islet iNOS mRNA expression [37]; that disruption of membrane lipid rafts (*e.g.*, with cyclodextrin) clearly reduces IL-1β-induced gene expression of iNOS and NO in β-cells [38]; that the selective iNOS inhibitor, 1400W, inhibits NO formation at the level of the pancreatic islets [39]; and that neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has a cytoprotective effect via inhibition of iNOS transcription in cytokine treated β-cells [40]. However, these inhibiting effects do not mean that diabetes can be completely prevented. For example, iNOS inhibitor aminoguanidine delays onset of diabetes but does not reduce the diabetes incidence [41].

In the presence of risk factors inducing iNOS over-expression, it should be tried to prevent β-cell destruction and diabetogenesis by iNOS inhibitors or the mechanism of the damage on the cells by iNOS expression should be defined and prevented. Therapeutic inhibition of an enzyme such iNOS, which can have both physiological and pathological effects, is of course very difficult. Furthermore, iNOS does not have a stable level of expression. As this enzyme may be induced by many factors and cause destruction of β-cells that can lead to diabetes, systemic use of its inhibitors has the potential of causing worse results. Therefore, no such treatment method has been yet developed.

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