The Role of Nitric Oxide Synthase in Post-Operative Hyperglycaemia

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Abstract: Post-operative hyperglycaemia is important with regard to outcomes of surgical operations. It affects post-operative morbidity, length of hospital stay, and mortality. Poor peri-operative blood glucose control leads to a higher risk of post-operative complication. Insulin resistance as a cause of post-operative hyperglycaemia has been blamed for some time. Nitric Oxide (NO) is produced by nitric oxide synthase (NOS) isoenzymes. Inducible nitric oxide synthase (iNOS) is not a normal cellular constitute. It is expressed by cytokines and non-cytokines e.g. fasting, trauma, intravenous glucose, and lipid infusion, which are encountered in surgical operations. Review of current published data on postoperative hyperglycaemia was completed. Our studies and others were explored for the possible role of NO in this scenario. Induction and expression of iNOS enzyme in pancreatic islet cells is included in the chaotic postoperative blood glucose control. The high concentrations of iNOS derived NO are toxic to pancreatic β-cells and may inhibit insulin secretion postoperatively. Hence, current peri-operative management is questionable regarding post-operative hyperglycaemia and necessitates development of a new strategy.

Key words: NO, glucotoxicity, lipotoxicity, post-operative hyperglycaemia, pancreatic islets.

Post-operative hyperglycaemia: A real problem

Post-traumatic hyperglycaemia is commonly encountered after surgery and in patients treated in intensive care units (ICU). It carries a higher risk for post-operative complications, prolonged recovery periods, and increased length of stay (LOS) [1,2].

Poor post-operative blood glucose control in diabetic [1] and non-diabetic patients [2] leads to a higher risk of complications. Many studies (3, 4) blame insulin resistance as a cause for post-operative hyperglycaemia. Cytokines [5,6], fasting [7,8], peri-operative feeding [9,10] and immobilization were reported to lead to insulin resistance. Different regimens postulated to overcome the outcome of elective operations [11,12]. Emergency traumatic surgery in conditions e.g. high velocity missile injury and traffic accidents carry an additional risk for post-operative hyperglycaemia because of double trauma.

To decrease post-operative morbidity and mortality, it is essential to explore the molecular mechanism of post-operative hyperglycaemia and its relation to trauma.

Pancreatic function during trauma has not been thoroughly studied. It is very important to comprehend post-operative hyperglycaemia and evaluate peri-operative management to improve surgical outcomes.

Review of currently published data on post-operative hyperglycaemia was conducted and the role of nitric oxide in this scenario was investigated.

Nitric oxide and nitric oxide synthase system

Nitric oxide (NO) was described in 1989. NO is the smallest synthetic molecule. It is produced by a family of enzymes known as nitric oxide synthase (NOS) in almost all mammalian cells e.g. vascular endothelium, neurons of the central and enteric nervous system, and cells of the immune system [13,14]. NO is a free radical and an extremely reactive gas [15]. It has a short half life of about 10 seconds. It acts as a signalling molecule, neurotransmitter, and macrophage mediated immunity that can heal or kill. Under conditions of high NO production, a number of enzymes can be inhibited by NO-enzyme interaction [16-18].

According to their expression, activity, and dependence on calcium, NOS isoenzymes are divided into 2 major functional classes:

- Constitutive nitric oxide synthase (cNOS); ncNOS, ecNOS
- Inducible nitric oxide synthase (iNOS).

Nitric oxide and insulin secretion

cNOS and iNOS can be expressed and/or induced by different stimuli in various tissue including pancreatic β-cell [17,19-24]. ncNOS derived NO is recognized as an important signalling molecule in a variety of cellular processes e.g. insulin secretion [19,21,22,24].

Our laboratory [23,27,29-30] and others [25,31-33] presented biochemical and immuno-cytochemical evidence for occurrence of ncNOS in mouse and rat pancreatic β-cells. When cNOS is activated, it produces a pulsatile low amount of NO for a short period of time [29,31,34]. Although the effect of ncNOS derived NO on insulin secretion is highly controversial, the results from rat and mouse pancreatic islets suggests that it acts as a negative modulator for glucose-stimulated insulin secretion (GSIS) [27,29].

iNOS is not a normal cellular constituent and can only be expressed in pathophysiological conditions in a response to inflammatory cytokines e.g. IL-1β, TNF-α, and lipopolysaccharide. Under such conditions, pancreatic β-cells produce huge amounts of NO in a more sustained manner [27,34-36] through induction of iNOS, comparing to the cNOS isoforms [15,37-38]. Non-cytokine induction of iNOS in pancreatic islets has also been reported. One hour in vitro incubation of healthy rat and mouse islets with high glucose concentrations [10-20 mmol/L) induced iNOS and ncNOS [27,30,34]. However, the activation of ncNOS was rapid, within minutes. It is at least in part, associated with the glucose-stimulated influx of extracellular Ca2+ into β-cells [27,33]. Glucose activation

of iNOS was slower and detectable after approximately 60 minutes [27]. The mechanism behind glucose-stimulated iNOS expression and activity is poorly understood. It has been suggested that glucose metabolism generates NADPH through the pentose shunt, which is an important stimulus in IL-1β induction of iNOS [39,40]. NADPH is an obligatory substrate for iNOS synthesis of NO [20-21]. This is of great interest since high amounts of iNOS derived NO is detrimental to β-cells [38-39]. Moreover, we showed that 24 hour intravenous (IV) glucose administration induced marked expression and activity of islets iNOS [41].

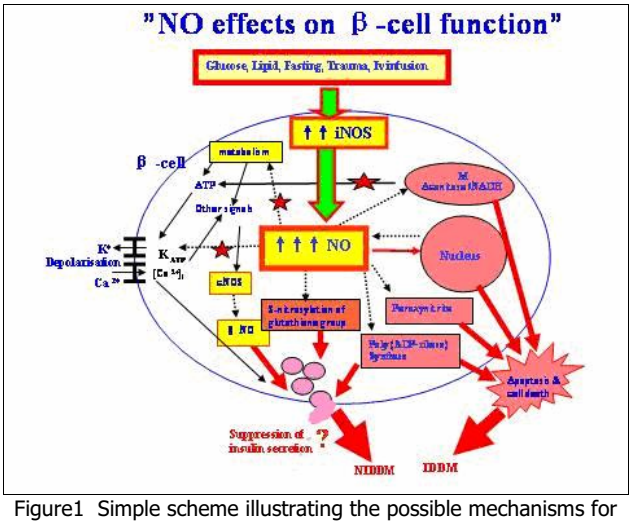

Figure1 Simple scheme illustrating the possible mechanisms for the toxic effects of NOS-derived NO on β -cell function

The inhibitory effect of increased NO production on insulin secretion, due to either enhanced activity of cNOS (physiological) or induction of iNOS (inflammatory condition) [29,38,42]. NO is widely accepted as a mediator of β-cell dysfunction and apoptosis [29,30,34,43-45]. A clear role of iNOS in the pathogenethesis of type 1 diabetes mellitus has been reported [38-39].

In addition, the extremely low level of NO metabolizing enzymes, e.g. catalase and glutathione peroxidase, makes pancreatic β-cells extremely susceptible to high levels of intracellular NO (46). High concentration of NO may interact with vital sites in the β-cell such as Kreb's cycle enzyme aconitase [47], ion channels [48], or other enzymes of importance for β-cell function [30,34,43,48] (Figure 1). Indeed, several studies demonstrated that inhibition of NOS isoenzymes activity by specific inhibitors was accompanied by enhanced GSIS, both in vitro and in vivo [27,29,30,34].

Initial inhibition of insulin release is exerted by ncNOS-derived NO, when the islets are exposed to high glucose concentration [26-27].

Glucotoxicity

Chronic hyperglycaemia is detrimental to pancreatic β-cells. It could be implicated in the pathogenesis of type 2 diabetes mellitus (DM) in a process called glucotoxicity [50-51].

We showed that the route of nutrient administration is important in pancreatic function by both biochemical and immuno-cytochemical evidences. Hyperglycaemia induced by IV glucose administration, or hyperlipidemia by IV intralipid infusion, caused marked induction and expression of iNOS in rat β-cells [41]. This is consistent with previous reports, that plasma insulin response was much greater following glucose ingestion than IV glucose administration despite an equivalent increase in plasma glucose concentration. This is explained by the release of incretin hormones from endocrine cells in the gastrointestinal tract e.g. glucagon-like peptide-1 (GLP-1) [52-53]. The expression of iNOS after IV infusion of glucose could be explained by suppression of release of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) whose secretion is dependant upon ingestion of carbohydrates (for GLP-1) or FFA (for GIP).

The relative importance of glucotoxicity versus lipotoxicity in inducing β-cell dysfunction and apoptosis remains controversial. Although it has been reported that lipotoxicity alone will not affect β-cell function without signs of glucotoxicity [54], we showed that glucose or intralipid infusion for 24h induced marked expression and activity of iNOS [41]. This is in line with results of cultured cell lines exposed to high glucose or FFA for 24-48 hours [55-56]. In this context, it is conceivable that iNOS derived NO might be a contributing factor in this process [55].

Furthermore, IV infusion of nutrients is commonly prescribed as an important treatment model both pre- and post-operatively [6,57], in burn patients, and in some patients as a life long treatment when they can not take oral food. If IV nutrients induce NO-production in human β-cells, this may explains post-operative hyperglycaemia to some degree.

Lipotoxicity

Long term Total Parenteral Nutrition (TPN) in rats for 10 days resulted in increased iNOS and decreased ncNOS activity in pancreatic islets [30,34]. Infusion of lipids for 24 hours induced suppression of insulin secretion [41]. This is in agreement with previous studies [30,34,58]. Although the induction of iNOS could not account entirely for alteration in β-cell survival, it might negatively modulate the secretory function of β-cells. In addition, long term exposure of β-cells to FFA resulted in a marked production of reactive oxygen species e.g. superoxide anion (O-2) [59]. Combination of NO and O-2 resulted in the formation of peroxynitrite, which is a powerful oxidant and cytotoxic molecule. The increase in NO, O-2 and peroxinitrite concentrations were positively correlated with mitochondrial and DNA damage in β-cells [44]. It has been reported that an increased plasma FFA obtained by IV infusion of lipids resulted in decreased plasma levels of glucagon in humans [60]. The suppression of insulin secretion during TPN could partly be due to the absence of incretin hormone which may be normalized by injection of GLP-1 [61].

Fasting and pancreatic function

Cyclic AMP is a potent inhibitor of islet's NOS activity [24,26,30,34]. It is markedly suppressed in islets isolated from fasting mice and rats. Besides, GSIS was markedly impaired in islets isolated from fasting mice, and associated with a decreased production of CO and HO-2 expression. Hence two potent inhibitors of islet NO production; CO and cyclic AMP, were markedly suppressed

in islets isolated from fasting mice and rats. This may explain the increased iNOS activity in β-cells and suppression of insulin secretion in these animals.

Taken together, decreased CO production and increased iNOS-derived NO production is associated with a diabetic condition in islets β-cells [62].

Preoperative fasting or IV glucose infusion for 24 hours induced strong expression and activity of iNOS in rat pancreatic islets post-operatively (data not published), which was stronger than those seen in rats that received preoperative oral glucose or were freely fed. This is of significant clinical importance if the same thing happens in human pancreatic islets. Since, preoperative fasting and/or post-operative IV glucose infusions are applied in surgical patients, especially in abdominal operations. Hence both fasting and IV glucose administration play a role, at least partly, in suppression of insulin secretion and induction of post-operative hyperglycaemia. Although post-operative insulin resistance is still blamed, fasting and IV glucose may act as contributors to insulin suppression by inducing post-operative hyperglycaemia, which needs further study in humans.

Trauma and β-cell function

Trauma-induced iNOS expression and activity has been noted in rat pancreatic islets [63]. During trauma, the body responds with a series of reactions e.g. a change in metabolism, to a catabolic state, and an expression of insulin resistance [64]. The consequence of post-operative insulin resistance is that patients in the post-operative period are in a metabolic state similar to T2DM [13]. Insulin resistance persists for about 2-3 weeks after uncomplicated elective upper abdominal surgery [65]. It negatively affects the post-operative recovery, convalescent period, and LOS.

Surprisingly, in spite of insulin resistance and its role in post-operative hyperglycaemia, iNOS isoenzyme may be involved very early in the impairment of the insulin secretion. Hence, β-cells seem to be unable to respond adequately to a glucose challenge. It seems reasonable to assume that an improvement in the insulin secretory capacity of the pancreas may positively affect the post-operative glycemic state and ultimately the outcome of surgery.

The present findings may stir more debate in the explanation of post-operative hyperglycaemia.

Conclusions

1. Trauma, fasting, hyperglycaemia, hyperlipidemia and route of nutrient administration possibly are other factors contribute to post-operative hyperglycaemia.
2. It is recommended to investigate the molecular mechanism behind the pathophysiology of post-traumatic hyperglycaemia in human beings. The role of nitric oxide in this scenario should be appreciated.
3. New strategy should be developed regarding peri-operative management and postoperative hyperglycaemia.
4. A possible pharmacological target is to suppress iNOS activity in pancreatic islets with agents stimulating cyclic AMP/PKA pathway e.g. PACAP. This may be a hope to restore adequate insulin secretion post-operatively.

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