Protective effect of inducible nitric oxide synthase inhibitor on pancreas transplantation in rats

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AIM: To investigate the effect of inducible nitric oxide synthase inhibitor, aminoguanidine, on pancreas transplantation in rats.

METHODS: A model of pancreas transplantation was established in rats. Streptozotocin-induced diabetic male Wistar rats were randomly assigned to sham-operation control group (n = 6), transplant control group (n = 6), and aminoguanidine (AG) treatment group (n = 18). In the AG group, aminoguanidine was added to intravenous infusion as the onset of reperfusion at the dose of 60 mg/kg, 80 mg/kg, 100 mg/kg body weight, respectively. Serum nitric oxide (NO) level, blood sugar and amylase activity were detected. Nitric oxide synthase (NOS) test kit was used to detect the pancreas cNOS and inducible NOS (iNOS) activity. Pancreas sections stained with HE and immunohistochemistry were evaluated under a light microscope.

RESULTS: As compared with the transplant control group, the serum NO level and amylase activity decreased obviously and the evidence for pancreas injury was much less in the AG group. The AG (80 mg/kg body weight) group showed the most significant difference in NO and amylase: 14.2 ± 0.9 vs 1426 ± 177, P < 0.01 and blood sugar: 14.2 ± 0.9 vs 16.8 ± 1.1, P < 0.01.

CONCLUSION: Selective iNOS inhibitor, aminoguanidine as a free radical, has a protective effect on pancreas transplantation in rats by inhibiting NO and reducing its toxicity.

INTRODUCTION

Pancreas transplantation is frequently complicated by acute pancreatitis, largely due to ischemia/reperfusion injury secondary to cold preservation[1,2]. During the reperfusion period, oxygen-derived free radicals can lead to a severe impairment. Nitric oxide (NO) is a free radical with a strong reactivity, and has a fierce cytotoxicity. However, NO can significantly dilate blood vessels and remit vasospasm of grafts. Therefore, NO plays an ambivalent role in ischemia/reperfusion during pancreas transplantation. In this study, we established a model of pancreas transplantation in rats to investigate the expression of nitric oxide synthase (NOS) isoforms, and the effect of inducible nitric oxide synthase (iNOS) inhibitor (aminoguanidine) on pancreas transplantation.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250-300 g (Experimental Animal Center, China Medical University, China) were used as donors and recipients. The animals were kept in standard conditions with free access to water and rodent chow. Diabetes was induced by intravenous injection of streptozotocin at a single dose of 55 mg/kg body weight. Only rats with non-fasting plasma glucose levels of more than 22 mmol/L were used as recipients. We performed recipient transplantation surgery on days 14 and 15 after the injection of streptozocin. A total of 30 recipient animals were randomly assigned to the sham-operation group (n = 6) in which animals underwent midline laparotomy only, transplant control group (n = 6) in which animals underwent transplantation and received a bolus injection of saline instead of aminoguanidine, and aminoguanidine treatment group (n = 18) in which animals...
underwent transplantation. Before reperfusion, a bolus injection of aminoguanidine (60 mg/kg, 80 mg/kg or 100 mg/kg body weight) was given via the vena dorsalis penis.

Transplantation and collection of specimens
Synergetic pancreaticoduodenal transplantation was performed in diabetic recipients to assess islet cell functions. After overnight fasting with free access to water, the rats were anesthetized and underwent heterotopic pancreaticoduodenal transplantation as previously described with certain modifications. After shaving and disinfecting the abdomen with 75% alcohol, a midline incision was made. The donor pancreas was isolated on an aortic segment branching off the celiac axis and the superior mesenteric artery. The venous outflow was provided by the portal vein. Pancreas grafts were flushed with and stored in cold (4°C) heparinized lactate Ringer's solution. Heterotopic intra-abdominal transplantation was performed by end-to-side anastomosis of the aortic segment of the graft and the recipient infrarenal aorta. The graft portal vein was anastomosed to the recipient vena cava using the same technique. Enteric diversion of exocrine graft secretion was accomplished by end-to-side duodenojejunostomy. The abdomen was closed in two layers with 2-0 silk suture. After a single intramuscular injection of 5 mg cefamandole post-operation, the rats were kept under warming lamps until they became active. The warming and cooling ischemic time was less than 15 min and 25 min, respectively. The animals were killed after 4 h of reperfusion. The pancreas was harvested and divided into two segments with one fixed in 10% PBS formalin and the other preserved at -70°C. The blood was withdrawn without anticoagulant and centrifuged at 2000 r/min for 10 min. The serum was preserved at -20°C.

Determination of serum NO and NOS levels
Nitrate reductase was used to detect the serum NO level and NOS test kit was used to detect the cNOS and iNOS activity in the pancreas.

Determination of serum blood and amylase levels
Serum glucose concentration was measured with an Exac Tech blood glucose meter in samples collected from the cut tip of the tail. Serum amylase concentration was measured with a multianalyzer (Clinilizer, CL-7150, Nippon Denshi, Tokyo, Japan).

Histopathology examination
One pancreas segment was fixed in 10% PBS formalin, dehydrated through a grade ethanol series, washed in xylene and embedded in paraffin. The segment was cut into 4 μm-thick sections. The sections were stained with haematoxylin and eosin and evaluated using light microscope.

Immunohistology
Primary antibody and anti-iNOS polyclonal antibody were produced in rabbits. Strept avidin-biotin complex immunoperoxidase staining system was used, and the positive staining was reddish-brown in color.

Statistical analysis
The data were presented as mean ± SD. All statistical analyses were performed using the SPSS 10.0 software. Differences in groups were tested by analysis of variance (ANVOA). P < 0.05 was considered statistically significant.

RESULTS

Serum NO level
The NO level increased significantly in transplant control group and deceased in the sham-operation group (P < 0.01) 4 h after reperfusion. After administration of aminoguanidine (AG), a selective iNOS inhibitor, NO level decreased significantly (P < 0.01). The effect of AG (80 mg/kg body weight) was obviously better than that of AG (60 mg/kg body weight) (P < 0.01). However, the effect of AG (100 mg/kg body weight) was not better than that of AG (80 mg/kg body weight) (P > 0.05) (Table 1).

Serum amylase activity
The amylase activity was higher in the transplant control group than in sham-operation group (P < 0.01). After administration of AG, the amylase activity decreased markedly, and the effect of AG (80 mg/kg body weight) was better (P < 0.01) (Table 1).

Blood sugar level
The blood sugar level decreased after pancreas transplantation, and was the lowest in the AG (80 mg/kg body weight) group (P < 0.01) (Table 2).

Activity of NOS isoforms in pancreas tissue
Four hours after reperfusion, the iNOS activity in pancreas tissue increased significantly (P < 0.01), but the cNOS activity had no change (P > 0.05). After administration of AG (80 mg/kg body weight), the iNOS activity decreased obviously (P < 0.01) while the cNOS activity remained normal (Table 3).

Histology
The pancreas was enlarged and swollen in the transplant control group, and appeared relatively normal in all animals of the sham-operation and AG (80 mg/kg body weight) groups. The microscopic pancreatic injury, as indicated by intracytoplasmic vacuoles, interstitial oedema, polymorphonuclear cell infiltrate, venous congestion, and

Table 1 Serum NO level and amylase activity 4 h after transplantation (mean ± SD)

| Group               | n | NO (μmol/L) | Amylase (U/dL) |
|---------------------|---|-------------|----------------|
| Sham-operation group| 6 | 30.0 ± 3.5  | 342 ± 73       |
| Transplant control group | 6 | 192.3 ± 60.0 | 4477 ± 630    |
| AG-60 mg/kg body weight | 6 | 137.3 ± 21.1 | 2848 ± 354    |
| AG-80 mg/kg body weight | 6 | 67.9 ± 19.5  | 1494 ± 263    |
| AG-100 mg/kg body weight | 6 | 66.0 ± 16.6 | 1426 ± 177    |

<sup>1</sup>P < 0.01, <sup>2</sup>P < 0.01 vs sham-operation group; <sup>3</sup>P < 0.01, <sup>4</sup>P < 0.01 vs transplant control group.
Table 2 Blood sugar level 4 h after transplantation (mean ± SD)

| Group                        | n  | Pretransplantation (mmol/L) | Posttransplantation (mmol/L) |
|------------------------------|----|-----------------------------|------------------------------|
| Sham-operated control        | 6  | 19.6 ± 1.4                  | -                            |
| Transplant control group     | 6  | 20.1 ± 2.0                  | 16.9 ± 2.0                   |
| AG-60 mg/kg body weight      | 6  | 19.9 ± 1.5                  | 16.8 ± 1.1                   |
| AG-80 mg/kg body weight      | 6  | 19.8 ± 1.7                  | 14.2 ± 0.9                   |
| AG-100 mg/kg body weight     | 6  | 20.5 ± 1.6                  | 15.1 ± 1.8                   |

\*P > 0.05, \*P < 0.01 vs AG-60 mg/kg body weight post transplantation; \*P > 0.05 vs AG-80 mg/kg body weight post transplantation.

Table 3 Activity of NOS isoforms in pancreatic tissue 4 h after transplantation (mean ± SD)

| Group                        | n  | cNOS (U/mL) | iNOS (U/mL) |
|------------------------------|----|-------------|-------------|
| Sham-operation group         | 6  | 5.35 ± 1.01 | 1.87 ± 0.19 |
| Transplant control group     | 6  | 5.91 ± 0.71 | 26.59 ± 5.78 |
| (AG-80 mg/kg body weight)    | 6  | 5.64 ± 0.97 | 2.01 ± 0.23 |

\*P > 0.05, \*P < 0.01 vs sham-operation group; \*P < 0.01 vs transplant control group.

local tissue hemorrhage and necrosis occurred 4 h after transplantation (Figure 1A and B). However, none of the samples from the AG (80 mg/kg body weight) group revealed histological evidence of pancreatic injury (Figure 1C and D).

**Immunohistochemistry**

Four hours after reperfusion, heavily stained specimens from transplant control group were positive for anti-iNOS, while iNOS staining was mainly localized on the endothelium, vascular smooth muscle, and islet cells (Figure 1E and F). No stained anti-iNOS antibody was detected in all specimens from the AG (80 mg/kg body weight) group (Figure 1G and H).

**DISCUSSION**

A model of pancreas transplantation in rats was established. Four hours after pancreas graft reperfusion, the expression and activity of iNOS on pancreas increased significantly, serum NO level and amylase activity, leading to severe pancreatitis, whereas cNOS remained normal. After administration of AG, the iNOS activity and NO concentration decreased, the toxicity of NO as free radicals was reduced, and the amylase activity decreased markedly. The severity of ischemia/reperfusion injury and postgraft pancreatitis was reduced, protecting the pancreas graft against ischemia/reperfusion injury.

Ischemia/reperfusion injury remains a major problem in pancreas transplantation. During the reperfusion period, endothelial dysfunction, activation of endogenous enzymes, leucocyte recruitment and activation all lead to generation of oxygen-derived free radicals, promote lipid peroxidation and deplete glutathione and other antioxidant compounds, leading to pancreatitis [13]. Contradictory results about the role of NO in pancreatic ischemia/reperfusion have been reported [14]. NO may lose an electron to form nitrosonium cation (NO+), which can combine with the superoxide radicals to form peroxynitrite (ONOO·), a highly active free radical with fierce cytotoxicity [15]. In pathological conditions, significant activation of iNOS by the release of inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), can increase NO concentration [16]. It was reported that endogenous NO is involved in formation of pancreatic edema in L-arginine-induced acute pancreatitis by increasing the vascular permeability and protein extravasation [17]. Treatment with L-NAME significantly reduces amylase activity and edema formation in the pancreas. A study about severe acute pancreatitis has shown a positive correlation between serum NO level and the number of adherent leucocytes [18]. The expression of iNOS is correlated to changes in the pancreatic histomorphology [19-21]. The expression of iNOS during reperfusion following pancreatic ischaemia contributes significantly to the development of acute pancreatitis [22]. Vasoactive mediators, such as bradykinin, platelet activating factor, endothelin and NO, participate in the development of pancreatic microcirculatory failure. Recently, in drug-induced pancreatitis models, some researchers found that there is a correlation among NF-kappaB activation, serum amylase, reactive oxygen species level and tissue damage, suggesting that NF-kappaB and iNOS play a key role in the pathogenesis of acute pancreatitis [23-25]. After treatment with antioxidants or NOS inhibitors, the levels of myeloperoxidase, serum amylase and NO, as well as iNOS activities are decreased significantly, and the pancreatic inflammation is improved [26,27]. Ma et al. [27] found that the expression of NF-kappaB and iNOS in peritoneal macrophages is significantly higher in rats with severe acute pancreatitis, and anti-inflammatory agents decrease the expression of TNF-alpha, IL-1 and NO in peritoneal macrophages, reducing the severity of pancreatitis. In Foch-Puy’s experiment, infusion of a contrast medium into the pancreatic duct could result in an inflammatory process characterized by increased lipase levels in plasma and edema as well as increased myeloperoxidase activity in pancreas, suggesting that activation of NF-kappaB is correlated with iNOS expression in pancreatic cells [28,29]. It was reported that ischemia/reperfusion provokes severe acute necrotizing pancreatitis with a high mortality rate and leads to systemic inflammatory reaction due to the activation of cytokine cascade and iNOS, indicating that NO overproduction by iNOS corresponds with the apoptotic process in the pancreas and the lung [30,31]. In a study on ischemia/reperfusion injury, Duchen found that calcium overload is associated with NO generation, and their combination leads
to collapse of mitochondrial membrane potential followed by cell death\textsuperscript{[20]}. It was reported that after administration of selective iNOS inhibitors, the iNOS activity and NO concentration are decreased significantly and the severity of pancreatitis is reduced\textsuperscript{[21]}. However, some experiments indicate that NO could activate guanylate cyclase, reduce the activity of platelets and inflammatory cells, relax smooth muscle, and dilate blood vessels\textsuperscript{[22,23]}. Therefore, NO can remit the vasospasm of grafts and decrease the occurrence of vascular crisis. Supplement of NO for donors during reperfusion of pancreatic isografts seems to prevent organ injury because NO attenuates leukocyte-dependent tissue injury\textsuperscript{[26]}. Thus, it remains debatable whether the increased production of NO due to pancreas transplantation is beneficial or detrimental to the tissue.

Based on the findings of this study and present reports, it is very likely that NO plays a dual role in ischemia/reperfusion injury of pancreas. During the early reperfusion period, NO, under the charge of cNOS, can improve postischemic reperfusion. With the prolongation of reperfusion time, NO depletion results in failure of microcirculation, during which supplement of NO or NOS substrate could protect microcirculation against failure\textsuperscript{[24]}. When reperfusion is prolonged (more than 4 h), activation and excessive expression of iNOS due to the release of inflammatory agents such as TNF-\(\alpha\), IL1-\(\beta\), result in a considerable increase in NO concentration, and the toxic effect of NO as a free radical leads to the development of graft pancreatitis\textsuperscript{[25]}. Therefore, administration of selective iNOS inhibitors can not only reduce the toxicity of NO as a free radical, but also retain vasodilatation effect, and protect the graft against pancreatitis. Aminoguanidine (AG) is a mechanism-based inactivator of NOS isoforms and exhibits a marked specificity for the inactivation of its inducible isoform, which proceeds through multiple pathways of covalent modification of the iNOS protein and heme residue at the active site\textsuperscript{[26]}.

At present, some experiments demonstrated that in the transplanted islets, iNOS and toxic NO are produced due to infiltration of inflammatory cells into islets and production of proinflammatory cytokines (such as TNF-\(\alpha\), IL1-\(\beta\)), and an excessive production of NO is deleterious to pancreas \(\beta\)-cells\textsuperscript{[27-29]}.

In conclusion, selective iNOS inhibitor, aminoguanidine as a free radical, has a protective effect on pancreas transplantation in rats by inhibiting NO and reducing toxicity.
Background
Pancreas transplantation is frequently complicated by acute pancreatitis, largely due to ischemia/reperfusion injury. During the reperfusion period, nitric oxide (NO) may form peroxynitrite (ONOO·), a highly active free radical, and has a fierce cytotoxicity. However, NO can significantly dilate blood vessels and remit the vasospasm of grafts, protecting pancreatic graft from thrombosis due to transplantation. Therefore, NO plays an ambivalent role in ischemia/reperfusion injury during pancreas transplantation. However, contradictory results about the role of NO in pancreatic ischemia/reperfusion injury have been reported. It remains debatable whether the increased production of NO due to pancreas transplantation is beneficial or detrimental to the tissue.

Research frontiers
Based on the findings of this study and recent reports, it is very likely that NO plays a dual role in ischemia/reperfusion injury of pancreas. During the early reperfusion period, NO under the charge of cNOS, could improve pancreas perfusion. With the prolongation of reperfusion time, NO depletion could result in failure of microcirculation, during which supplement of NO or NOS substrate can protect microcirculation against failure. When reperfusion is prolonged, activation of iNOS due to the release of inflammatory agents, such as tumor necrosis factor α and interleukin-1β, can increase NO concentration. The toxic effect of NO as a free radical can lead to graft pancreatitis. Hence, administration of selective iNOS inhibitors can reduce the toxicity of NO, and protect graft against pancreatitis.

Innovations and breakthroughs
We established a model of pancreas transplantation in rats. Administration of selective iNOS inhibitors could not only reduce the toxicity of NO as a free radical, but also retain vasodilatation effect, and protect graft against pancreatitis. Aminoguanidine (AG) is a mechanism-based inactivator of NOS isoforms and exhibits a marked specificity for the inactivation of its inducible isoform, which proceeds through multiple pathways of the INOS protein and heme residue at the active site. Our data also suggest that blood sugar level in AG group was much lower than that in transplant control group, indicating that the selective iNOS inhibitor, AG, has a protective effect on pancreas transplantation.

Applications
Pancreas transplantation can give IDDM additional pancreas to take the place of its own, which has lost the function of insulin secreting. Pancreas regulates insulin secretion, and maintains the blood glucose level. At present, nothing else could achieve this object. Factors influencing pancreas functions following transplantation include graft pancreatitis and rejection which are difficult to treat with a poor prognosis. In this study, after administration of selective INOS inhibitor AG, the INOS and amylase activity and NO concentration were decreased, the toxicity of NO as a free radical was reduced. The severity of ischemia/reperfusion injury and postgraft pancreatitis was reduced, protecting the graft against pancreatitis.

Terminology
Inducible nitric oxide synthase (iNOS): NO is synthesized from L-arginine by inducible nitric oxide synthase (iNOS). iNOS does not express at normal conditions, and is activated in response to proinflammatory cytokines. iNOS plays a pathological role in the disease process of severe acute pancreatitis.

Peer review
This study investigated the effect of inducible nitric oxide synthase inhibitor, aminoguanidine, on pancreas transplantation, showing its scientific and clinical values.

REFERENCES
1 Sweeney JH, Mann GE. Role of oxidative stress in the pathogenesis of acute pancreatitis. Scand J Gastroenterol Suppl 1996; 219: 10-15
2 Benz S, Bergt S, Obermaier R, Wiessler R, Pfeffer F, Schareck W, Hopf UT. Impairment of microcirculation in the early reperfusion period predicts the degree of graft pancreatitis in clinical pancreas transplantation. Transplantation 2001; 71: 759-763
3 Lee S, Tung KS, Koopmans H, Chandler JG, Orloff MJ. Pancreaticoduodenal transplantation in the rat. Transplantation 1972; 13: 421-425
4 Schroeder RA, Gu JS, Kuo PC. Interleukin 1beta-stimulated production of nitric oxide in rat hepatocytes is mediated through endogenous synthesis of interferon gamma. Hepatology 1998; 27: 711-719
5 Butler AR, Flitney FW, Williams DL. NO, nitrosonium ions, nitrosamide and nitrosamine. Roles of mitochondria in health and disease. Tung KS, Koopmans H, Chandler JG, Orloff MJ. Trends Pharmacol Sci 1995; 16: 18-22
6 Mizutani A, Maki H, Torii Y, Hitomi K, Tsukagoshi N. Ascorbate-dependent enhancement of nitric oxide formation in activated macrophages. Nitric Oxide 1998; 2: 235-241
7 Takacs C, Czakó L, Morschel E, László F, Tiszlavicz Z, Rakoczay Z Jr, Lonovics J. The role of nitric oxide in edema formation in L-arginine-induced acute pancreatitis. Pancreas 2002; 25: 277-282
8 Chen HM, Shyr MH, Lau YT, Hwang TL, Chen MF. Leukocyte-endothelial adherence correlates with pancreatic nitric oxide production in early cerulein-induced pancreatitis in rats. Shock 1998; 10: 218-222
9 Rahman SH, Ammori BJ, Larvin M, McMahon MJ. Increased nitric oxide excretion in patients with severe acute pancreatitis: evidence of an endotoxin mediated inflammatory response? Gut 2003; 52: 270-274
10 Rau B, Bauer A, Wang A, Gansauge F, Weidenbach H, Löffler J, Beger HG, Nüssler AK. [Morphological disorders in endogenous nitric oxide synthesis in experimental acute pancreatitis: role of iNOS]. Virchows Arch 2001; 439: 195-203
11 Tomaszewska R, Dębinska A, Warzecha Z, Cenanovicz P, Stachura J. Morphological changes and morphological-functional correlations in acute experimental ischemia/reperfusion pancreatitis in rats. Pol J Pathol 2000; 51: 179-184
12 Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Gentorino T, Ciccolo A, Van de Loo FA, Britti D, Caputi AP, Thiemermann C. Inducible nitric oxide synthase-deficient mice exhibit resistance to the acute pancreatitis induced by cerulein. Shock 2002; 17: 416-422
13 Ayub K, Serracino-Inglott F, Williamson RC, Mathie RT. Expression of inducible nitric oxide synthase contributes to the development of pancreatitis following pancreatic ischaemia and reperfusion. Br J Surg 2001; 88: 1189-1193
14 Yoo BM, Oh TY, Kim YB, Yeo M, Lee JS, Surh YJ, Ahn BO, Kim WH, Sohn S, Kim JH, Hahm KB. Novel antioxidant ameliorates the fibrosis and inflammation of cerulein-induced chronic pancreatitis in a mouse model. Pancreatology 2005; 5: 165-176
15 Sugiyama Y, Kato S, Mitsufuji S, Okanoue T, Takeuchi K. Pathogenic role of endothelial nitric oxide synthase (eNOS/ NOS-III) in cerulein-induced rat acute pancreatitis. Dig Dis Sci 2001; 56: 1396-1403
16 Ma ZH, Ma QY, Wang LC, Sha HC, Wu SL, Zhang M. Expression of iNOS in human pancreatic tissue. Inflamm Res 2005; 54: 522-527
17 Folch-Puy E, Granell S, Iovanna JL, Barthet M, Closa D. Peroxisome proliferator-activated receptor gamma agonist reduces the severity of post-ERCP pancreatitis in rats. World J Gastroenterol 2006; 12: 6458-6463
18 Leindler L, Morschel E, László F, Mándi Y, Takács J, Jármá K, Farkas G. Importance of cytokines, nitric oxide, and apoptosis in the pathological process of necrotizing pancreatitis in rats. Pancreas 2004; 29: 157-161
19 Viola G, al-Mufti RA, Sohail M, Williamson RC, Mathie RT. Nitric oxide induction in a rat model of selective pancreatic ischemia and reperfusion. Hepatogastroenterology 2000; 47: 1250-1255
20 Duchen MR. Roles of mitochondria in health and disease. Diabetologia 2004; 53 Suppl 1: S96-S102
21 Li BF, Liu YF, Xia LP, Cheng Y, Cheng DH, Wang XD, Li
22 Benz S, Obermaier R, Wiessner R, Breitenbuch PV, Burska D, Weber H, Schnabel R, Mayer J, Pfoffer F, Nizze H, Hopt UT. Effect of nitric oxide in ischemia/reperfusion of the pancreas. J Surg Res 2002; 106: 46-53

23 Obermaier R, von Dobschetz E, Muhs O, Keck T, Drognitz O, Jonas L, Schareck W, Hopt UT, Benz S. Influence of nitric oxide on microcirculation in pancreatic ischemia/reperfusion injury: an intravital microscopic study. Transpl Int 2004; 17: 208-214

24 Yuan CH, Liu YF, Cheng Y, Zhao N, Li GC, Liang J, He SG. Protective effects of L-arginine on reperfusion injury after pancreaticoduodenal transplantation in rats. Hepatobiliary Pancreat Dis Int 2004; 3: 349-354

25 Larsen CM, Wadt KA, Juhl LF, Andersen HU, Karlsen AE, Su MS, Seedorf K, Shapiro L, Dinarello CA, Mandrup-Poulsen T. Interleukin-1beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. J Biol Chem 1998; 273: 15294-15300

26 Bryk R, Wolff DJ. Mechanism of inducible nitric oxide synthase inactivation by aminoguanidine and L-N6-(1-iminomethyl)lysine. Biochemistry 1998; 37: 4844-4852

27 Kwon KB, Kim EK, Jeong ES, Lee YH, Lee YR, Park JW, Ryu DG, Park BH. Cortex cinnamomi extract prevents streptozotocin- and cytokine-induced beta-cell damage by inhibiting NF-kappaB. World J Gastroenterol 2006; 12: 4331-4337

28 Mosén H, Salehi A, Henningsson R, Lundquist I. Nitric oxide inhibits, and carbon monoxide activates, islet acid alpha-glucoside hydrolase activities in parallel with glucose-stimulated insulin secretion. J Endocrinol 2006; 190: 681-693

29 Arafat HA, Katakan AK, Chipitsyna G, Gong Q, Vancha AR, Gabbeta J, Dafoe DC. Osteopontin protects the islets and beta-cells from interleukin-1 beta-mediated cytotoxicity through negative feedback regulation of nitric oxide. Endocrinology 2007; 148: 575-584