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EDITORIAL

Islet transplantation and antioxidant management: A comprehensive review

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Abstract

Islet transplantation as a promising treatment for type 1 diabetes has received widespread attention. Oxidative stress plays an essential role in cell injury during islet isolation and transplantation procedures. Antioxidants have been used in various studies to improve islet transplantation procedures. The present study reviews the role of oxidative stress and the benefits of antioxidants in islet transplantation procedures. The bibliographical databases Pubmed and Scopus were searched up to November 2008. All relevant human and animal *in-vivo* and *in-vitro* studies, which investigated antioxidants on islets, were included. Almost all the tested antioxidants used in the *in-vitro* studies enhanced islet viability and insulin secretion. Better control of blood glucose after transplantation was the major outcome of antioxidant therapy in all *in-vivo* studies. The data also indicated that antioxidants improved islet transplantation procedures. Although there is still insufficient evidence to draw definitive conclusions about the efficacy of individual supplements, the benefits of antioxidants in islet isolation procedures cannot be ignored.

Key words: Antioxidant; Diabetes; Free radical; Islet; Transplant

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INTRODUCTION

Diabetes mellitus which is characterized by hyperglycemia has become an important disorder with major costs and complications worldwide. In a genetically susceptible person with an environmental trigger such as viruses and toxins, autoimmune destruction of β cells can occur causing type 1 diabetes usually in childhood and in young adults[1]. Type 2 diabetes usually results from dysfunction of β cells and peripheral insulin resistance. It is accepted that oxidative stress is increased in both type 1 and type 2 diabetes, and it has been shown in many studies that biochemical markers of oxidative stress are higher in tissue samples and in the pancreas of diabetic patients[2]. However, secondary complications comprising micro- and macro-vascular disorders which result in frequent amputation, end-stage renal failure, and blindness have motivated various investigators to identify new therapeutic approaches to cure diabetes. With this aim, whole pancreas transplantation was first carried out in 1966 by Kelly and Lillehei at the University of Minnesota and was then performed worldwide. Marked morbidity following pancreas transplantation prompted researchers to find other possible ways of curing this disease. As reviewed by Fontaine *et al*[3], since 1974 when the first human islet transplantation was conducted by Sutherland and his colleagues, and then up to 1999, among approximately 1000 patients who received islet allotransplantations, most of the results were disappointing and only 10% remained insulin-independent for longer than one year.

In 1999, use of the Edmonton protocol with its steroid-free immunosuppressive regimen was an impressive leap in achieving insulin-independence after islet transplantation[4]. Although the Edmonton protocol

succeeded in achieving insulin-independence, two or more donors are still needed to achieve normoglycemia following islet transplantation.

GREAT EFFORTS TO OPTIMIZE ISLET TRANSPLANTATION

Recent years, many studies have been carried out to optimize the Edmonton protocol to obtain the final goal of one-donor islet transplantation. Donor characteristics and pancreas procurement are the first steps. The quality of the donor pancreas depends largely on donor factors, such as age, body mass index, serum glucose levels, and hemodynamic stability[5]. In the procurement phase, improved surgical techniques such as *in-vivo* atraumatic dissection and *in-situ* separation have made sufficient advancements in minimizing warm ischemia before isolation. Moreover, using perfluorocarbon (PFC) and oxygen in the University of Wisconsin (UW) solution during cold ischemic preservation, which is identified as the Two layer method (TLM), has enhanced the final quality of islets[6].

One of the most important areas of research in islet transplantation is the isolation procedure, which remains a major topic of islet transplant investigations. At present, the maximum rate of purified islets at leading centers is about 50% to 70%[7]. The largest reduction in islet yield occurs during the islet isolation phase. Below we will look in detail at the islet isolation phase, which has been the focus of a large number of studies.

Other efforts have focused on new approaches relating to the best site of transplantation, better revascularization of islet-grafts, visualization after engraftment, and further anti-rejection strategies which are not covered in this review. For the latest progress in these aspects of islet transplantation, readers are referred to two recently published review articles[8,9].

ISLET ISOLATION AND ROLE OF OXIDATIVE STRESS

Successful islet transplantation would enable patients to live without tedious multiple insulin injections and reduce the risk of hypoglycemia. As previously mentioned, the main restriction in this procedure is the loss of healthy islets at the end of the operation due to the inevitable prolonged time required for islet isolation. In the clinical setting, the isolated islets are transplanted immediately or within a few days after harvesting from donors. Isolated islets are avascular and are therefore ischemic from the time of isolation through to the period required for revascularization. Prolonged ischemia has profound deleterious effects on the islets, resulting in a significant loss of islet cells. Two major factors which are expected to cause potential cell damage include hypoxia and enzymatic/mechanical trauma related to the experimental procedures.

Reactive oxygen species (ROS) in physiological concentrations provide normal conditions to protect cells, for instance they are important within white cells to allow effective defense against infection. Nevertheless, whenever ROS are accumulated in excess and for long periods they can destroy cells. Free radicals cause damage to cellular proteins, membrane lipids and nuclear nucleic acids. The only protective mechanism present in the body to protect cells against excessive free radicals is the antioxidant enzyme system. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are the main antioxidant enzymes. Hypoxia which occurs during the islet transplantation procedure initiates a cascade of biochemical reactions which results in the production of ROS causing necrosis and apoptosis *via* intracellular pathways.

Another important point which can lead to worsening of this condition is that β cells contain low levels of antioxidative enzymes such as CAT, SOD and GPx and thus they can only weakly defend against oxidative stress[10-12]. These findings have demonstrated the major destructive role of oxidative stress in islet transplantation and have encouraged investigators to use antioxidants during the isolation phase and the entire transplantation process to overcome the final lack of healthy islets.

Various strategies such as modifications in enzymatic digestion[13], purification with iodixanol instead of ficoll[14], incubation of purified islets in culture medium, genetic manipulation for overexpression[15,16] or silencing[17] of specific genes, employment of different anti-inflammatory or other supplements such as small intestinal submucosa[18] and serecin[19] have been considered in an attempt to increase islet quality and yield. In a previous publication we hypothesized that using phototherapy could improve islet function before transplantation[20].

This review focuses on antioxidant management in islet transplantation procedures and evaluated both *in-vitro* and *in-vivo* studies.

ANTIOXIDANT RECRUITMENT IN ISLET TRANSPLANTATION STUDIES

To perform a comprehensive survey and obtain all related studies we searched Pubmed and Scopus databases up to November 2008. Search terms were "antioxidant", "islet", "transplant" and "oxidative stress". Individual antioxidants such as vitamin E or C were also searched with the term "islet".

There were many studies in which various methods other than utilizing antioxidants were used to increase function and viability of islets which were not included in this review. Table 1 lists the various antioxidant agents which have been added to islets during the isolation procedure or to the culture medium. Table 2 shows the *in-vivo* studies.

Metabolite and vitamin antioxidants

Vitamin E or tocopherol was the supplement most used to enhance the viability of islets. This lipid-soluble

Table 1 ***In-vitro*** **effects of antioxidants on pancreatic islets**

Authors	Substance/Dose	Sample	Study design	Duration	Assessments
Campbell *et al*[21], 2008	Sodium selenite (30 nmol/L)	Rat	Islets were incubated with/without Na_2SeO_3	72 h	Insulin content ↑ Insulin secretion ↑ Glucose-stimulated insulin secretion[1]
Kanitkar *et al*[22], 2008	Curcumin (10 μmol/L)	Rat	Cryopreservation of islets with/without curcumin Assessments were performed after 24 h culture post-thawing	Cryopreserved	Intact islets ↑ ROS ↓ Insulin Secretion ↑ Expression of Hsp70 and HO-1 ↑
Thomas *et al*[23], 2007	Peptide SS-31 (1 nmol/L)	Mice	SS-31 was added to all reagents which were used for islet isolation	Isolation procedure	Islet apoptosis ↓ Islet yield ↑
		Human	Islets were incubated in culture medium with without SS-31	72 h	Islet apoptosis ↓
Hara *et al*[24], 2007	Epigallocatechin-3-gallate (EGCG) (36, 72, 360 μmol/L)	Rat	Islets cultured under normal or H/R condition with/ without EGCG	48 h	Islet apoptosis ↓ LDH ↓ Insulin secretion ↑
Xiong *et al*[25], 2006	Puerarin (10, 50, 100 μmol/L)	Rat	Islets cultured under normal or H_2O_2 stress conditions with/without Puerarin	24 h	Islet apoptosis ↓, Islet viability ↑ CAT & SOD activity ↑, ROS ↓ Insulin secretion ↑
Marzorati *et al*[26], 2006	Glutathione (1, 5, 10 mmol/L) Vitamin E (2×10^{-5} mmol/L) Ascorbic acid (0.3 mmol/L)	Human	Antioxidant agents were added to culture medium individually	48 h	Glutathione : CCL2/MCP-1 release ↓ Insulin secretion[1] Vitamin E: CCL2/MCP-1 release[1] Ascorbic acid: CCL2/MCP-1 release[1]
Amoli *et al*[27], 2006	Curcumin (10, 20 μmol/L)	Rat	Curcumin was added to culture medium	18 h + 24 h	MCP-1 release ↓
Rao *et al*[28], 2005	MCI – 186	Rat	Islets were treated with H_2O_2 in the presence or absence of MCI-186	18 h	Cell death ↓
Avila *et al*[29], 2005	L-glutamine (5 mmol/L)	Human	Pancreas were manually perfused through the main pancreatic duct with either the standard HBSS or with HBSS + L-glutamine	0 → 24 h	Islet yield ↑ Lipid peroxidation (MDA) ↓ Glutathione (GSH) ↑ Viability[1] Insulin secretion[1]
Brandhorst *et al*[30], 2005	Free L-glutamine (2.5 & 5 mmol/L/L) Stable L-glutamine [NALG] (2.5 & 5 mmol/L)	Pig	Islets pretreated with free L-glutamine or NALG for 24 h and then stressed with H_2O_2, ETA or cytokine mix	24 h	Viability: L-glutamine ↑ NALG[1]
Giovagnoli *et al*[31], 2005	Encapsulated enzymes (100 mg/35 mL)	NPCCs	NPCCs were co-cultured with/without entrapped enzymes	9 d	Viability ↑, Insulin secretion ↑ Insulin/DNA ratio ↑ mRNA expression of insulin and Glut-2 ↑
Bottino *et al*[32], 2004	MnTDE (34 μmol/L)	Human	MnTDE was added as a supplement to culture or isolation medium	60 h	Islet yield ↑, Insulin secretion ↑ NF-κB DNA-binding ↓ IL6 & MCP-1 ↓, IL8[1] PARP activation ↑
Arata *et al*[33], 2004	Ascorbic Acid-2 Glucoside (AA2G) (100 μg/mL)	Human	Cryopreservation of islets with UW solution or AA2G + UW solution	Cryopreserved	Viability ↑ Insulin secretion ↑ Insulin gene expression ↑
Luca *et al*[34], 2003	Free vitamin D3 (2 μmol/L) Encapsulated vitamin D3 (20 μmol/L)	Rat	Islets were treated with/ without vitamin D3	9 d	Insulin secretion ↑
Hardikar *et al*[35], 2001	Taurine (0.3 & 3 mmol/L)	Rat	Cryopreservation of islets with/without taurine	Cryopreserved	Viability ↑ Lipid peroxidation ↓
Luca *et al*[36], 2000	Vitamin D3 Vitamin E	NPCCs	NPCC cells were treated with/without VitD3 & VitE during their maturation and differentiation process	16 d	Large & intact islets ↑ Insulin secretion ↑
Tajiri *et al*[37], 1999	Vitamin E (50 μg/mL)	Rat	Islets were co-cultured with/ without vitamin E	1 d	Insulin secretion ↑
Shewade *et al*[38], 1999	Riboflavin	Rat	Inclusion of riboflavin in the cryopreserved medium	Cryopreserved	Viability ↑ Lipid peroxidation ↓ GSH ↑, Insulin secretion ↑
Jindal *et al*[39], 1996	Zinc (20 μmol/L)	Rat	Preservation of islets in UW and Hanks solution with/without Zinc	1, 3, 6 d	Viability[1]

↑: Significant increase compared with non-treated group; ↓: Significant decrease compared with non-treated group; [1]No significance difference between groups. Hsp70: Heat shock protein 70; HO-1: Heme oxygenase-1; H/R: Hypoxia/Reoxygenation; LDH: Lactate dehydrogenase; CCL2/MCP-1: Monocyte chemoattractant protein 1; HBSS: Hanks balanced salt solution; NALG: N-acetyl-L-alanyl-L-glutamine; MDA: Malondialdehyde; MnTDE: Manganese (Ⅲ) 5,10,15,20-tetrakis (1,3-diethyl-2imidazoyl) porphyrin; NF-κB: Nuclear factor-κB; PARP: Poly (ADP-ribose) Polymerase indicative of ongoing cell damage and death; UW: University of Wisconsin; NPCCs: Neonatal pancreatic porcine cell clusters.

Table 2 ***In-vivo*** **effects of antioxidants on pancreatic islets**

Author	Substance/Dose	Sample	Procedure	Output
Thomas *et al*[23], 2007	Peptide SS-31 (3 mg/kg)	Mice → Mice	Injection of SS-31 to donor mouse + adding SS-31 during islet isolation	Better glucose control after transplant
Avila *et al*[29], 2005	L-glutamine (5 mmol/L)	Human → Rat	Transplantation of human islets which were treated before with/without intraductal L-glutamine to nude rats	Normoglycemia percentage ↑ (83% *vs* 26%) Time to reach normoglycemia ↓ (1.83 d *vs* 7.6 d)
Brown *et al*[40], 2005	Pyruvate Vitamin E Vitamin C	Rat → Rat	Oral administration of pyruvate, vitamin E and vitamin C during perioperative period	Pyruvate enhanced engraftment & functionality of suboptimal islet mass Vitamin E and vitamin C failed to enhance
Olcott *et al*[41], 2004	Salen-manganese [EUK-8] (100 mg/kg)	Mice → Mice	Daily treatment with/without EUK-8 (IP injection) to recipient NOD mice	Better glucose control until 20 d after transplant
Winter *et al*[42], 2002	Vitamin A (500 μg) Ascorbic Acid (40 mg) Vitamin E (10.6 mg) Selenium (2 μg)	Rat → Rat	Oral antioxidants, 24 h prior to transplantation	Slight better glucose control
Bottino *et al*[43], 2002	SOD mimetic compound (34 μmol/L)	Human → Mice	Culture of isolated islet in the presence of SOD mimetic for at least 2 h before transplantation	Better glucose control
Hardikar *et al*[35], 2001	Taurine (0.3 & 3 mmol/L)	Rat → Mice	Transplantation of islets which were previously cryopreserved with/without taurine	No significant difference was observed
Tajiri *et al*[37], 1999	Vitamin E (40 mg/kg)	Rat → Rat	Every other day IP injection of vitamin E to transplanted rats	Treatment for 2 not for 6 wk enhanced basal insulin release and arginine induced insulin release
Vajkoczy *et al*[44], 1997	Vitamin E (150, 8000 mg/kg)	Rat → Rat	Oral vitamin E after xenograft	Reduction of xenograft leukocyte-endothelium interaction at day 6 Adequate development of functional capillary density
Gembal *et al*[45], 1993	Allopurinol Vitamin E Chlorpromazine	Rat → Rat	Transplantation of islets which were pretreated with/without combination of allopurinol, alpha-tocopherol and chlorpromazine.	Better glucose control after 3 d

↑: Significant increase compared with non-treated group; ↓: Significant decrease compared with non-treated group.

vitamin protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction[46]. Luca *et al*[36] and Tajiri *et al*[37] have shown that vitamin E can increase secretion of insulin from islets when added *in-vitro* to the culture medium. Likewise, when vitamin E was administered before, after, or during the transplantation period[37,42,44], the islet transplantation outcome was better and glucose control was slightly superior compared with control groups. Another study demonstrated that digestion buffer which contained vitamin E, resulted in healthy isolated islets, however, this study was not designed to evaluate the effect of vitamin E and therefore a control group was absent. An investigation into the effect of a mixture of allopurinol, vitamin E and chlorpromazine on islet transplantation concluded that a combination of free radical scavengers, antioxidants and membrane stabilizing drugs may be used to increase the effectiveness of islet transplantation[45].

In contrast to the above-mentioned positive effects of vitamin E, Brown and colleagues[40] showed that vitamin E and C could not enhance the function of rat engrafted islets; although the authors did not specify the dose which they applied. In a different study[26], the use of vitamin E and ascorbic acid did not decrease the release of monocyte chemoattractant protein 1 (MCP-1) from human islets. MCP-1 is a chemokine secreted from pancreatic islets to recruit the immune system and plays an important role in the clinical outcome of islet transplantation because of its proinflammatory property[47,48]. Nevertheless, this review did not assess the secretion of insulin or the viability of islets following the use of vitamin E, thus we can not conclude that islets may or may not benefit from vitamin E.

Ascorbic acid or vitamin C is a monosaccharide antioxidant. This water-soluble vitamin is a reducing agent and can neutralize oxygen species. This metabolite antioxidant in the form of ascorbic acid-2 glucoside (AA2G) supplies a stable form of vitamin C in the culture medium and cryopreservation solution and thus improves viability, secretion and expression of insulin in cryopreserved human islets[33]. In the study by Winter *et al*[42], the combined administration of vitamin C and E, but not vitamin A and selenium, led to a significant improvement in functional islet graft survival, associated with augmented islet engraftment. In contrast, Brown *et al*[40] and Marzorati *et al*[26] showed that the combination of vitamin E and C was unable to improve graft survival or decrease *in-vitro* MCP-1 release, respectively.

Glutathione (GSH), as a cysteine-containing peptide, is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other

metabolites and enzyme systems as well as reacting directly with oxidants in the cells. When GSH was added to culture medium, in contrast to vitamin E and C, it reduced MCP-1[26]. L-glutamine as a precursor of GSH was shown to increase islet yield when added intra-ductally during human pancreatic islet isolation. This study indicates that intra-cellular GSH levels can be increased by means of intra-ductal glutamine administration prior to the isolation procedure. Consequently, authors found lower cellular lipid peroxidation in islets isolated from glutamine-pre-treated pancreata, indicating less oxidative damage. Although they could not detect any differences in cell viability and islet function *in vitro*, the islets isolated from glutamine-pre-treated pancreata performed significantly better than controls after transplantation in diabetic nude mice[29]. Supporting this study, Brandhorst *et al*[30] demonstrated that pig islet culture will significantly improve if L-glutamine is administered in an unbound (free) form compared with the stable compound N-acetyl-L-alanyl-L-glutamine (NALG).

Taurine (2-amino ethanesulfonic acid) an end-product of sulfur amino acid metabolism, is one of the most abundant free amino acids in the body. The membrane stabilizing, free radical scavenging, and osmoregulatory roles of taurine have been well documented[49,50]. In one study, taurine enhanced viability and reduced lipid peroxidation in cryopreserved islets. However, no significant difference was observed in the islet insulin content between groups following cryopreservation, and taurine could not enhance glucose control following transplantation of treated islets[35].

1, 25 dihydroxyvitamin D3, the active form of vitamin D3 is a membrane antioxidant and can prevent lipid peroxidation at the cell membrane[51]. Luca and colleagues treated neonatal porcine cell clusters (NPCCs) with vitamin D3 at a certain time in their maturation and differentiation process. Insulin recovery showed that vitamin D3, unlike untreated controls, resulted in preservation of islet function for significantly long periods of time. Furthermore, this group exhibited sustained release vitamin D3 which entrapped vitamin D3 in microspheres. They showed that this form of vitamin D3 caused more insulin secretion compared with free vitamin D3 and both forms caused more insulin secretion than the untreated control group[34,36].

Another study evaluated the benefit of 1, 25 dihydroxyvitamin D3 (1, 25 D3) on cytokine-induced human pancreatic islets. Addition of 1, 25 D3 significantly reduced nitrite release, IL-6 production and MHC class I expression which were not significantly different from controls. The authors suggested that vitamin D3 could affect this *via* reduction of cytotoxic challenges. Hence, it might play a role in the prevention of islet allograft rejection. However, more *in-vivo* studies are required to affirm this suggestion.

Riboflavin (vitamin B2) although it is not a pure antioxidant, has been used in one study[38]. The authors showed that inclusion of this vitamin in the cryopreservation medium could protect islets comparable to healthy fresh isolated islets.

Trace elements

Selenium as an essential trace element plays an important role in the expression of some selenoproteins and selenoenzymes such as GPx. The antioxidative property of this element has been confirmed in many different cell types[52]. The insulin-mimetic effect of selenium has been found both *in-vitro* and *in-vivo*[53]. To ameliorate islet function, Campbell *et al*[21] showed that sodium selenite could increase islet content and secretion but not glucose-stimulated insulin secretion in culture media. In the study by Winter, when a combination of vitamin E, C, A and selenium were administered orally to transplanted mice, no significant improvement in functional islet graft survival was seen when compared with mice administered only vitamin C and E.

Furthermore, selenium in the insulin-transferrin-selenium (ITS) compound is a commercially available media supplement for the culture of mammalian cells under serum-free or near serum-free conditions. In islet culture, when Fraga and colleagues used ITS supplement instead of FBS, ITS was capable of maintaining viable islets for up to two months[54].

Zinc, another trace element antioxidant has a role in the storage, synthesis, and secretion of insulin in islet cells. In one study[39] when investigators added zinc to cold preservation UW and Hanks solution, no difference in islet viability was seen between the groups. The authors concluded that despite the integral role of zinc in islet metabolism, they were unable to find a beneficial role for zinc in cold storage solutions for the purposes of islet preservation.

Manganese as a trace element is a component of antioxidant enzymes and is used in new SOD mimetic compounds which will be discussed later.

Herbs

Curcumin is a polyphenolic compound commonly found in the dietary spice turmeric[55,56]. Curcumin is an inhibitor of nuclear factor-κB (NF-κB) and has various biological activities such as anti inflammatory, antioxidant, antiseptic and anticancer effects[57,58]. In two different studies, curcumin was shown to decrease MCP-1 release from islets[27], decrease generation of ROS, increase secretion of insulin, and present more intact islets[22].

Puerarin, the main isoflavone glycoside found in the Chinese herb, Radix of Pueraria lobata (Willd) Ohwi, has been used for various medicinal purposes in traditional Chinese medicine. It has been shown that puerarin has antioxidant activities such as radical scavenging and increasing SOD activity as well as antihyperglycemic effects[59]. Xiong *et al*[25] found that if islets pretreated with puerarin for 48 h were exposed to H_2O_2, this did not result in loss of islet viability. They showed that this protective effect resulted from inhibition of free radical generation. Puerarin was also found to increase CAT and SOD activities.

Epigallocatechin-3-gallate (EGCG) is the main ingredient found in green tea. Anticarcinogenic,

antioxidant, and antiangiogenic activities have been attributed to EGCG as a constituent of green tea[60]. In the study by Hara and colleagues, EGCG was shown to protect islets from hypoxia/reoxygenation injury. Insulin secretion was increased and apoptosis was inhibited by EGCG[24].

Enzymes and new antioxidants

Two forms of enzymes, encapsulated slow release SOD and CAT and SOD-mimetic compounds were shown to increase viability, islet yield, and to decrease the release of MCP-1 and IL-6 from islets.

Encapsulated SOD and CAT in Poly D, L-lactide-co-glycolide (PLGA) microspheres were analyzed with NPCC cells. These powerful antioxidizing agents were shown to significantly improve morphology, viability and function, as assessed by microscopy, molecular, biochemical and functional studies on the incubated NPCCs[31].

SOD mimetics: There are four main classes of SOD mimetics including desferrioxamine, macrocyclics, salen compounds, and mesoporphyrins[61]. Two types of these compounds have been employed to possibly enhance islet survival by counteracting oxidative stress.

MnTDE is a manganese-porphyrin pentachloride, a synthetic porphyrin protein, which has been prepared as a SOD mimetic[62]. Synthetic probes with MnTDE (AEOL10113 or AEOL10150) were used in the culture medium of human islets to ameliorate islet yield and insulin secretion. When treated islets were transplanted into diabetic mice, glucose control was better than in the non-treated group[32,43].

MnTMPyP Mn (Ⅲ) tetrakis (1-methyl-4-pyridyl) porphyrin, is a composition of porphyrin SOD mimetics. MnTMPyP preserved islet viability upon exposure to a nitric oxide donor in culture medium[61].

EUK-8 a salen compound SOD mimetic is one of a new class of synthetic salen-manganese compounds with SOD, peroxidase, and CAT activities. EUK-8 treatment prolonged the survival of islet allografts in newly diabetic non-obese diabetic (NOD) mice[41].

Peptide SS-31 (D-Arg-2-, 6-dimethyltyrosine-Lys-Phe-NH2) is a novel peptide shown to target the inner mitochondrial membrane and prevent oxidative damage to cells. It has been shown to decrease islet apoptosis and increase islet yield[23].

MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5one; Edaravone) is a new free radical scavenger produced for use in some clinical conditions. It is a strong scavenger of hydroxyl radicals and was shown to have benefits in myocarditis and cerebral infarction. MCI-186 prevented islet cell death dose-dependently when cells were treated with H_2O_2[28].

DISCUSSION

Most studies on antioxidants and diabetes in the literature have evaluated the possible effects of antioxidants in preventing β cell glucose toxicity and cytokine-mediated cell damage in the field of type 1 diabetes pathophysiology. The role of antioxidants in type 2 diabetes is still undetermined due to scientific conflict. Vitamin E and C, selenium and the majority of antioxidant trace elements, as well as herbs and drugs have been used or are being used to try to manage diabetes and its complications[2,63]. Moreover, there are, as seen in this review, few studies available which used antioxidants to improve islet transplantation outcome. However, a review of the existing studies indicates that many antioxidants are able to enhance cellular defense mechanisms against oxidative stress in islet cells. With deeper inspection of the presented studies, the effects of these drug compounds can be divided into two separate sub-categories.

The first sub-category concerns the direct antioxidant effects of drug compounds on islet viability (direct inactivation of free oxygen species). For instance, vitamin E and C were shown to increase viability of islets but they failed to decrease MCP-1 release from the cells. This shows that these vitamins do not act *via* inflammatory pathways. Enzyme antioxidants also promote islet yield and activity by direct inactivation of free radicals. The role of nitric oxide (NO) in early islet transplantation rejection should not be overlooked. NO was shown in experimental studies to harm islet cells in culture medium early after transplantation. N (G)-monomethyl-L-arginine (NMA) (a reversible inhibitor of NO synthase) prevented the dysfunction or destruction of cultured islets and markedly decreased the time needed to restore euglycemia after intraportal transplantation of islets in diabetic rats[64]. Direct antioxidants such as vitamin E and salen-manganese compounds can prevent NO and nitrite radicals as well as ROS[65,66] thus may play a protective role.

The second sub-category of effects concerns the action of these drug compounds on the modulation of beta cell apoptosis initiation and signaling. One potential ROS-dependent target molecule is the nuclear transcriptional factor NF-κB. It is now known that NF-κB is a key transcription factor involved in regulating proinflammatory cytokines, chemokines, adhesion molecules, and inflammatory enzymes. Some antioxidants block the effects of NF-κB including curcumin[22,27], glutathione and MnTDE porphyrin[32] and have been shown to ameliorate islet yields by reducing MCP-1 release as a consequence of NF-κB blockade.

However, the two above-mentioned sub-categories may change following further investigations. Newer studies, especially those after 2004, have focused on the possible pathways by which antioxidants may improve the function of islets.

As previously stated, antioxidant enzymes are found in low levels in islets compared with other tissues. In addition, minimal amounts of GPx protein and mRNA expression as well as GPx activity in islets have been detected[67], showing that GPx is low compared with SOD and CAT in islets. It is important to develop methods to increase islet survival and the number of islets during isolation and transplantation, thus

the suggestion of using antioxidants to improve islet transplantation is reasonable.

However, based on current data from *in-vitro* studies, we can conclude that the addition of an antioxidant during islet isolation procedures would result in better islet function. Furthermore, the overexpression of intracellular antioxidant enzymes and proteins[68], as well as transgenic islets[15] has been found to improve the function of islets, which is in agreement with our conclusions.

Interestingly, all the *in-vivo* studies with the exception of one on taurine[35] showed better control of blood glucose using antioxidant supplementation mainly in the early stages of islet transplantation. However, similar to *in-vitro* studies, there was no significant advantage of any one antioxidant on glucose control following transplantation. Of course, the lack of human studies limits our conclusions regarding *in-vivo* research and necessitates further investigations to establish the benefits of antioxidants in human islet transplantation. Fortunately, there is sufficient evidence on the existence of oxidative stress in diabetes and the significant role of antioxidants in the reduction of diabetic complications[69-76].

CONCLUSION

The collective data reviewed here show that different antioxidants improve islet transplantation procedures both *in-vitro* and *in-vivo*. We recommend antioxidant supplementation in islet isolation protocols, however, there is still insufficient evidence to draw definitive conclusions about the efficacy of individual supplements and to support profitable antioxidant management *in-vivo* particularly in humans.

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