Available online at www.sciencedirect.com
ScienceDirect
Diabetes & Metabolism 33 (2007) 44–53
Diabetes & Metabolism
http://france.elsevier.com/direct/diabet

Original article

MnTMPyP, a metalloporphyrin-based superoxide dismutase/catalase mimetic, protects INS-1 cells and human pancreatic islets from an in vitro oxidative challenge

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Received 28 March 2006; accepted 2 September 2006
Available online 26 January 2007

Abstract

Aims. – Pancreatic islets can be lost early following allotransplantation from oxidative stress. Antioxidant enzyme overexpression could confer a beneficial effect on islets exposed to reactive oxygen species (ROS) and nitrogen species. Here, we tested the effect of MnTMPyP, a superoxide dismutase/catalase mimetic.

Methods. – INS-1 insulin-secreting cells or human islets were cultured with MnTMPyP and exposed to a superoxide donor (the hypoxanthine/xanthine oxidase (HX/XO) system), a nitric oxide donor [3-morpholinosydnonimine (SIN-1)] or menadione. Viability of INS-1 cells was assessed by WST-1 colorimetric assay and FACS analysis (Live/Dead® test). ROS production was determined using fluorescent probes. Islet viability was estimated by WST-1 assay and endocrine function by static incubation.

Results. – Following MnTMPyP treatment, ROS production in INS-1 cells was reduced by 4- to 20-fold upon HX/XO challenge and up to 2-fold upon SIN-1 stress. This phenomenon correlated with higher viability measured by WST-1 or Live/Dead® test. MnTMPyP preserved islet viability upon exposure to SIN-1 or menadione but not upon an HX/XO challenge. Similarly, decrease in insulin secretion tended to be less pronounced in MnTMPyP-treated islets than in control islet when exposed to SIN-1, but no changes were noticed during an HX/XO stress.

Conclusions. – MnTMPyP was able to improve the viability of INS-1 cells and human islets exposed to oxidative challenges in vitro. Protection of INS-1 cells could be as high as 90%. This agent is therefore potentially attractive in situations involving the overproduction of ROS, such as islet transplantation.

Résumé

Protection des cellules INS-1 et des îlots pancréatiques humains contre un stress oxydant in vitro par la MnTMPyP, métalloporphyrine à activité superoxyde dismutase et catalase.

Objectifs. – Le stress oxydant contribue à une perte précoce des îlots pancréatiques greffés. La surexpression d'enzymes antioxydantes protège les îlots des effets délétères des espèces radicalaires de l'oxygène (ROS) et du monoxyde d'azote (NO). Nous avons étudié ici les effets de MnTMPyP, qui possède une activité superoxyde dismutase et catalase.

Méthodes. – Des cellules insulinosécrétrices INS-1 et des îlots humains ont été cultivés en présence de MnTMPyP et exposés à un donneur d'anions superoxyde [hypoxanthine/xanthine oxydase (HX/XO)], de NO [3-morpholinosydnonimine (SIN-1)] ou à la ménadione. La viabilité des cellules INS-1 a été évaluée par le test colorimétrique WST-1 et par cytométrie de flux (test Live/Dead®), la production de ROS par sondes fluorescentes, la viabilité des îlots par le test WST-1 et la fonction endocrine par incubation statique.

Abbreviations: IEQ, equivalent number of islets; MnTMPyP, Mn(III) tetrakis (1-methyl-4-pyridyl) porphyrin; NO, nitric oxide; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine.HCl; SOD, superoxide dismutase.

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doi:10.1016/j.diabet.2006.09.004

Résultats. – Sous MnTMPyP, la production de ROS dans les cellules INS-1 a été réduite d'un facteur 4 à 20 lors d'un stress HX/XO et d'un facteur 2 lors d'un stress SIN-1. Cela était corrélé à une meilleure viabilité (tests WST-1 ou Live/Dead®). MnTMPyP a maintenu la viabilité des îlots exposés au SIN-1 ou à la ménadione, mais non au stress HX/XO. La diminution de l'insulinosécrétion tendait à être moins prononcée dans les îlots traités par MnTMPyP que dans les îlots témoins, en présence de SIN-1, mais aucun effet n'était noté lors d'un stress HX/XO.

Conclusions. – MnTMPyP a permis d'améliorer la viabilité de cellules INS-1 (jusqu'à 90 % de protection) et d'îlots humains exposés à un stress oxydant in vitro. Cette molécule semble intéressante dans les situations qui impliquent une surproduction de ROS, notamment la transplantation d'îlots.

Keywords: SOD mimics; Islet of Langerhans; INS-1 cells; Cytoprotection; Oxidative stress

Mots clés : SOD mimique ; Îlot de Langerhans ; Cellules INS-1 ; Cytoprotection ; Stress oxydant

1. Introduction

Despite improvements in clinical islet transplantation outcome, several obstacles remain to be solved before this procedure can stand as a reliable therapeutic procedure for type 1 diabetes mellitus. Current protocols usually require two pancreatic organs per recipient to restore a normal glucose metabolism. A large proportion of the graft is lost in the early days following transplantation, from non immune, non specific mechanisms. Among these mechanisms including thrombosis, apoptosis and ischemic injury, oxidative stress is very important [1]. The mediators of beta-cell oxidative damage include nitric oxide (NO) and peroxynitrite ($ONOO^-$). Their deleterious effects are reinforced by the low intracellular antioxidant status of pancreatic islets.

In previous works, adenoviral-mediated gene transfer of SOD or catalase was shown to prevent NO-induced beta-cells damage and HX/XO injury [2,3] but techniques using adenovirus are not clinically used because of their potential toxicity. Moreover, the large size and short in vivo life-span of these enzymes as proteins limit their clinical use.

Extensive studies have been carried out to find the suitable SOD-mimics to substitute superoxide dismutase. Several low-molecular-weight molecules have been developed and characterized. There are four main classes of SOD mimics: desferrioxamine, macrocyclics, salen compounds and mesoporphyrins. Like their native counterpart, the metal-dependent SOD-mimics could contain Zn, Fe or Mn but have relatively long metabolic half-life and are able to penetrate into the cells. The Mn porphyrins have a broad antioxidant specificity which includes scavenging superoxide anion (O_2^-) [4], hydrogen peroxide (H_2O_2) [5], $ONOO^-$ [6], NO [7] and diminishing lipid peroxidation [8].

In this study, we examined the effects of Mn(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) on INS-1 cells and human islets exposed to cytotoxic challenges. MnTMPyP is a SOD/catalase mimetic that possesses the catalytic activity of both enzymes and is permeable to cells. Thus this molecule can potentially exhibit the beneficial and synergistic effects of both SOD and catalase towards an excess of superoxide and hydrogen peroxide. We found that MnTMPyP conferred a significant protection against HX/XO or SIN-1 stress and induced a reduction of ROS production. The addition of MnTMPyP to menadione-treated INS-1 cells resulted in protection from zero to mild depending on the menadione concentrations used. Regarding human islets, MnTMPyP addition before and during the stress period showed quite an efficient protection against SIN-1 or menadione but not against XO. Likewise, preliminary data on endocrine function suggest that it was preserved in case of SIN-1 exposure but not with XO.

2. Material and methods

2.1. Materials

Antioxidant treatment. MnTMPyP (BioMol, TEBU, Le Perray-en-Yvelines, France), resuspended in water to obtain a 10 mmol/l stock solution was added at a final concentration of 25 μmol/l to INS-1 cells or human islets 30 min to 24 hours before stress application and during the period of stress. In each experiment, treatment and control conditions were compared.

INS-1 cells and human pancreatic islets. Rat insulinoma cell line INS-1 were cultured in RPMI 1640 medium as previously described [9]. Human pancreatic islets were isolated in the Cell Culture Core Laboratory of the University Hospital of Lille (France) or in UMTCT Grenoble Cell Therapy Unit (Saint-Ismier, France) and cultured in CMRL medium supplemented with 10% FCS as previously described [3].

2.2. Methods

2.2.1. Cytotoxic challenges

2.2.1.1. HX/XO challenge. Xanthine oxidase (XO) and hypoxanthine (HX) (Sigma, Saint Quentin Fallavier, France) were dissolved before use in RPMI medium, then added to INS-1 cells (96-wells plates, 5.10^4 cells per well) in 100 μl at 0.5 mmol/l HX final concentration, while concentrations of XO varied from 0 to 50 mU/ml. This XO/HX system produces superoxide anions, hydroxyl radicals and H_2O_2. Control cells received 0.5 mmol/l HX alone. HX/XO was left for 1.5 h, then removed and cells were incubated in 100 μl fresh medium for 16 h before viability determination.

2.2.1.2. SIN-1 challenge. We used SIN-1 ((3-Morpholino sydnonimine.HCl), Sigma, Saint Quentin Fallavier, France) as source of nitric oxide in our study. The nitrovasodilator SIN-1 slowly decomposes to release both nitric oxide and superoxide in an equimolar manner and thereby produces peroxynitrite, a powerful oxidant [10]. A 4 mmol/l stock solution dissolved in