Protection of insulin-secreting INS-1 cells against oxidative stress through adeno-viral-mediated glutathione peroxidase overexpression

C Moriscot¹, MJ Richard¹, MC Favrot¹, PY Benhamou¹, ²

SUMMARY

Objectives: A large fraction of an islet graft can be lost early following allotransplantation from various non specific mechanisms including oxidative stress. Overexpression of antioxidant enzymes could confer a beneficial effect on islets exposed to reactive oxygen and nitrogen species. We examined the viability of beta cells driven to overexpress glutathione peroxidase (GPx) and exposed to a superoxide donor (hypoxanthine/xanthine oxidase HX/XO) and a nitric oxide donor (3-morpholinosydnonimine SIN-1).

Methods: Cultured INS-1 rat-derived insulin-secreting cells were transfected by an E1-deleted adenovirus carrying GPx cDNA (AdGPx). Additional experiments were performed with an adenovector carrying Cu/Zn superoxide dismutase cDNA (AdSOD). Cellular viability was tested by the WST-1 colorimetric assay and functionality by static incubation.

Results: AdGPx increased GPx activity within 48 hours from 0 (untransfected cells) to 60 ± 11 U/g (cells transfected at an MOI of 25:1). GPx overexpression significantly reduced cytotoxicity induced by HX/XO from 10.81 ± 1.41 to 5.42 ± 2.62% at 10 mU/ml and from 61.19 ± 4.17 to 52.9 ± 4.39% at 20 mU/ml (p=0.0002, transfected cells vs control cells). Doses of SIN-1 from 600 to 1,000 µmol/l resulted in cytotoxicity ranging from 17.66 ± 3.48 to 45.97 ± 6.48% in control cells and from 5.65 ± 1.37 to 35.80 ± 5.59% in AdGPx transfected cells (p = 0.015). The combination of AdGPx and AdSOD did not exhibit any synergistic cytoprotective effect. Control cells exposed to a HX/XO stress exhibited a reduction in glucose-theophylline stimulated insulin secretion by half, while stressed GPx overexpressing-cells maintained the same insulin secretion level than non-stressed cells.

Conclusions: Adenoviral-induced overexpression of GPx enhances the resistance of a rat beta cell line to both reactive oxygen (ROS) and reactive nitrogen species (RNS) cytotoxicity. Transposition of these findings to human islet transplantation with a clinically-relevant procedure deserves further investigations.

Key-words: Islet Transplantation · Glutathione Peroxidase · Nitric Oxide · Antioxidant Enzymes · Reactive Oxygen and Nitrogen Species · Adenovirus · Gene Transfer.


RÉSUMÉ

Protection de cellules insulino-sécrétrices INS-1 contre le stress oxydant par surexpression de glutathion peroxydase médiée par adénovirus

Objectifs : Une part importante des îlots greffés peut être perdue précocement après allo- greffe par plusieurs mécanismes non spécifiques dont le stress oxydant. La surexpression d’enzymes antioxydantes pourrait être bénéfique pour les îlots exposés à des radicaux libres oxygénés ou nitrés. Nous avons exploré la viabilité de cellules beta surexprimant la glutathion peroxydase (GPx) et exposées à un donneur de superoxide (hypoxanthine/xanthine oxidase HX/XO) et à un donneur de monoxyde d’azote (3-morpholinosydnonimine SIN-1).

Méthodes : Des cellules insulinosécrétrices de rat INS-1 ont été transfectées par un adénovirus déleté en E1 véhiculant le cDNA de la GPx (AdGPx). Des expériences complémentaires ont fait appel à un adénovecteur véhiculant le cDNA de la Cu/Zn superoxide dismutase (AdSOD). La viabilité cellulaire a été évaluée par le test colorimétrique WST-1 et la fonctionnalité par incubation statique.

Résultats : AdGPx augmente l’activité GPx passant en 48h de 0 (cellules non transfectées) à 60 ± 11 U/g (cellules transfectées à une MOI de 25:1). La surexpression de GPx réduit significativement la cytotoxicité induite par HX/XO passant de 10,81 ± 1,41 à 5,42 ± 2,62 % à 10 mU/ml et de 61,19 ± 4,17 à 52,9 ± 4,39 % à 20 mU/ml (p = 0,0002, cellules transfectées vs cellules témoins). Des doses de SIN-1 de 600 à 1 000 µmol/l conduisent à une cytotoxicité allant de 17,66 ± 3,48 à 45,97 ± 6,48 % dans les cellules témoins et de 5,65 ± 1,37 à 35,80 ± 5,59 % dans les cellules transfectées par AdGPx (p = 0,015). La combinaison de AdGPx et AdSOD n’a pas d’effet cytoprotecteur synergique. Les cellules témoins stressées par HX/XO ont une diminution de moitié de l’insulinosécrétion stimulée par glucose-theophylline, tandis que les cellules stressées surexprimant GPx maintiennent le même niveau d’insulinosécrétion que les cellules non stressées.

Conclusions : La surexpression de GPx par adénovirus augmente la résistance d’une lignée cellulaire beta de rat à des radicaux libres oxygénés et nitrés. Des études complémentaires sont justifiées pour envisager l’application de ces faits à la transplantation d’îlots chez l’homme.

Mots-clés : Transplantation d’îlots · Glutathion peroxydase · Monoxyde d’azote · Enzymes antioxydantes · Radicaux libres oxygénés et nitrés · Adénovirus · Transfert de gène.

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Despite recent improvements achieved in clinical islet transplantation outcome, several obstacles remain to be solved before this procedure can stand as a reliable therapeutic procedure for type 1, insulin-dependent diabetes mellitus. A major issue is the islet mass needed to restore a normal glucose metabolism, as current protocols usually require two pancreatic organs per recipient [1]. A large proportion of the graft is believed to be lost in the early days following transplantation, from non immune, non specific mechanisms including thrombosis, apoptosis, ischemic injury and oxidative stress. Mediators of beta-cell oxidative damage include nitric oxide and peroxynitrite (generated by the reaction of nitric oxide with the superoxide anion).

All aerobic cells contain a set of defenses against reactive oxygen species (ROS) and reactive nitrogen species (RNS). Among the primary enzymatic defenses, superoxide dismutases (SOD) catalyse the conversion of superoxide radicals into H$_2$O$_2$ while catalase (CAT) and glutathione peroxidase (GPx) both destroy H$_2$O$_2$ (Fig 1). GPx is an important seleno-protein for cellular antioxidative defense [2]. Cellular GPx acts as an intracellular NO scavenger (or as a chain-breaking antioxidant) in cells, along with its intrinsic action as a preventive antioxidant to reduce the concentration of ROS [3]. GPx has the advantage over catalase to act also as a peroxynitrite reductase, preventing both oxidation and nitration reactions caused by peroxynitrite [4].

The rationale behind the use of antioxidant enzymes in islet transplantation is the relative deficiency of human islets in these enzymes, as compared to other tissues [5-6]. The beneficial impact of overexpressing these enzymes in an islet graft was suggested by several lines of evidence. Thus, adenoviral-driven catalase overexpression reduced the cytotoxicity of superoxide donors on human, porcine and rat islets [7]. Adenoviral-mediated SOD gene transfer can be beneficial to human islet endocrine function and resistance to nitric oxide cytotoxicity [8]. Results obtained with GPx were variable so far. A protection of the rat islet β cell line RINm5F from menadione, which generates superoxide intracellularly, was reported [9], while no protection was observed from H$_2$O$_2$ nor hypoxanthine/xanthine oxidase, an extracellular generator of superoxide [9-10]. In the present study, we aimed at clarifying the role of GPx in the cytoprotection of the rat β cell line INS-1. We examined whether adenovirus-driven overexpression of glutathione peroxidase can provide protection for these cells towards ROS and RNS by exposing them to hypoxanthine/xanthine oxidase (superoxide radicals and H$_2$O$_2$, cytotoxicity) and SIN-1 (NO cytotoxicity). In addition, we compared protection given by overexpressing GPx alone and protection conferred by simultaneous overexpression of GPx plus SOD against generators of oxygen free radicals and NO.

**Material and methods**

**INS-1 cells**

Rat insulinoma cell line INS-1 (courtesy of W. P. Pralong, Lausanne, Switzerland) were cultured in RPMI 1640 medium as described previously [11], except for AdGPx infection where selenium (sodium selenite, 5 µg/l = 28.9 nM), as an essential element for GPx, was added to the culture medium just before and during all time of infection.

**Adenoviral vectors**

We used E1-deleted, replication-deficient recombinant adenovirus containing bovine glutathione peroxidase cDNA under the control of the Rous Sarcoma Virus (RSV), AdGPx (provided by M. Barkats, Paris, France) constructed as previously described [12]. Additional experiments used a similar adenoviral vector containing human Cu/Zn superoxide dismutase cDNA under the control of a cytomegalovirus (CMV) promoter, AdSOD (provided by P. Lemarchand, INSERM U25, Necker School of Medicine, Paris, France). Control experiments were conducted with a similar adenoviral vector containing no cDNA (AdNull).

The recombinant adenovirus were propagated in 293 cells and were purified by CaCl density gradient purification. The preparations were dialyzed and stored in the dialysis buffer (10 mmol/l TRIS-Cl pH 7.8, 15 mmol/l NaCl, 10 mmol/l MgCl$_2$, 10% glycerol) at −70°C until use. The titre of each viral stock was determined by plaque assay on 293 cells, and the titres consistently ranged between 1-2 × 10$^{11}$ plaque-forming units (pfu)/ml for AdSOD and around 5 × 10$^{10}$ plaque-forming units (pfu)/ml for AdGPx. Absence of replication-competent particles in adenovirus preparations was checked by PCR using primers specific for the AdE1A region. The concentration of recombinant adenovirus was also quantified by optical absorbance, and the ratio of particles to pfu consistently ranged between 50 and 80 [13].

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AdCAT</td>
<td>adenovirus-driven catalase</td>
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<tr>
<td>AdGPx</td>
<td>adenovirus-driven glutathione peroxidase</td>
</tr>
<tr>
<td>AdSOD</td>
<td>adenovirus-driven superoxide dismutase</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>GPx</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>INS-1</td>
<td>rat insulinoma cell line</td>
</tr>
<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
</tr>
<tr>
<td>MTT</td>
<td>3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>Pfu</td>
<td>plaque forming unit</td>
</tr>
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<td>RIA</td>
<td>radio immuno assay</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
</tr>
<tr>
<td>SIN-1</td>
<td>3-morpholinosydnonimine.HCl</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
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</table>

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Adenoviral cell infection

INS-1 cells were plated at an initial density of 3.10^4 cells per well in 96-well plates 48 h to 72 h before adenoviral infection.

Adenovirus was added at different multiplicity of infection ratios (MOI) in 100 µl culture medium, and cells were incubated at 37°C in 95% air and 5% CO₂ for 1 h. Then, medium containing adenovirus was removed, replaced with fresh culture medium, and cells cultured in multiwell plates for 72 h before stress.

Enzymatic activity of GPx

Cells were pelleted by centrifugation and lysed by five freezing and thawing cycles in hypotonic 8.75 mM Tris-HCl buffer pH 7.4. GPx activity was assayed in the supernatant obtained after centrifugation (10 min, 2,600 g, 4°C) of the cell homogenate. The supernatants containing soluble proteins were immediately used or stored at −80°C until analysis. GPx activity was measured as previously described [14] using tertbutyl hydroperoxide (Sigma Chemical Co, St Louis, MO, USA). Results were expressed as µmoles of nicotinamide adenine dinucleotide phosphate, NADPH (Boehringer Mannheim, Germany), oxidized per minute. Enzyme activity was normalized to the soluble cell protein content.

Oxidative stress

Oxidative stress was performed 72 to 96 h after adenoviral infection. Xanthine oxidase (XO, grade III from buttermilk) and hypoxanthine (HX, 6-hydroxypurine), obtained from Sigma (Saint Louis, MO, USA) were dissolved freshly before use in RPMI culture medium. The cells were stressed 72 h after infection by adenovirus. SIN-1 dissolved immediately before use in RPMI culture medium was added to the wells containing INS-1 cells (3.10^4 cells per well) in 200 µl at final concentrations ranging from 600 to 1,000 µmol/l. Control wells received medium alone. Stressed cells were then cultured for 16 h before viability tests.

In vitro islet viability

INS-1 cells mitochondrial activity was assessed by WST-1 assay (Roche, Indianapolis, Ind., USA). This colorimetric assay is derived and simplified from the more commonly known MTT test that was previously established as a valid method for islet viability assessment [16]. It is based on the cleavage of a tetrazolium salt (+[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3 benzenedisulfonate) to formazan by the mitochondrial succinate-tetrazolium reductase. The formazan dye produced by viable cells is quantified in a multiwell spectrophotometer by measurement of the optical density (OD) at 440 nm. Each experiment was carried out with 6 wells per experimental group. Cytotoxicity of NO donors or prooxidant agents and protective activity of AdSOD or antioxidant activity of AdGPx (% protection) were defined according to the following formula:

\[
\text{Protection} = \frac{\text{OD of treated wells} - \text{OD of control wells}}{\text{OD of control wells}} \times 100
\]

Cytotoxic challenge

We used SIN-1 (3-Morpholinosydnonimine.HCl, Alexis, San Diego, Calif., USA) as source of nitric oxide in our study [15]. Both NO and superoxide are generated by SIN-1 in an equimolar manner resulting in the formation of peroxynitrite. Cells were stressed 72 h after infection by adenovirus. SIN-1 dissolved immediately before use in RPMI culture medium was added to the wells containing INS-1 cells (3.10^4 cells per well) in 200 µl at final concentrations ranging from 600 to 1,000 µmol/l. Control wells received medium alone. Stressed cells were then cultured for 16 h before viability tests.

Figure 1

A model of cellular injury by ROS and RNS and defense mechanisms by antioxidant enzymes.

| Glutathione peroxidase gene transfer and beta-cell viability |

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Cytotoxicity on control cells \( C = \frac{OD_C - OD_{CS}}{OD_C} \times 100 \), where \( OD_C \) = OD of control cells and \( OD_{CS} \) = OD of control cells exposed to cytotoxic stress;

Cytotoxicity on transfected cells \( T = \frac{OD_T - OD_{TS}}{OD_T} \times 100 \), where \( OD_T \) = OD of transfected cells and \( OD_{TS} \) = OD of transfected cells exposed to cytotoxic stress;

Percentage of protection \( = \frac{C - T}{C} \times 100 \).

In vitro cell function

Glucose-stimulated insulin secretion was determined by static incubation. Aliquots of 2.10⁵ cells were plated in triplicate on 3 mm Millicell inserts (Millipore, Bedford, Mass., USA) in a 24-well plate and consecutively stimulated for four different 1-h periods in Krebs buffer containing 0.5% BSA and 3.3 mmol/l glucose (period one and two), 27.5 mmol/l glucose + 10 mmol/l theophylline (period three), 3.3 mmol/l glucose (period four). Medium samples were frozen at −20°C for further insulin determination by RIA (CIS bio, Gif-sur-Yvette, France). A stimulation index was obtained as the ratio of insulin released during period three to the average of insulin released during periods two and four. Tissue insulin content was determined at the end of the 4th period. Acid alcohol was added to the cells, vortexed and incubated for 18 h at 4°C to extract insulin.

Statistical analysis

Comparisons of cytotoxicity levels between control and adenotransfected cells were done using an ANOVA test. Significance was set at \( P < 0.05 \). Data are shown as means ± SEM.

Results

Enzymatic activities of GPx in INS-1 insulin-secreting cells

Cells were cultivated for 5 days with different amounts of selenium added as sodium selenite to the culture medium. We tested 3 different concentrations: 5, 50 and 100 µg/l.

GPx activity levels were maximal with 5 µg/l selenite in AdGPx transfected cells (MOI 25: 1 and 40: 1) whereas control non transfected cells did not show any enzymatic activity, whatever selenium concentrations. The 5 µg/l selenite concentration was chosen for the further experiments, as higher concentrations were detrimental on INS-1 cells. GPx activity reached 60 ± 11 and 58 ± 10 U/g of proteins for cells transfected with an MOI of 25: 1 or 40: 1, respectively.

The efficiency of the AdSOD vector was previously published by our group showing a 2- to 3-fold increase in Cu/Zn SOD activity in INS-1 cells, using an MOI of 10 to 25: 1 [8].

Effects of overexpression of antioxidant enzymes on the toxicity of HX/XO

Following exposure of INS-1 cells to increasing doses of XO in the presence of 0.5 mM HX, a dose-dependent cytotoxic effect was measured by WST1 assay. Dose of 10, 12, 14, 16, 18 and 20 mU/ml resulted in a cytotoxicity of 10.81 ± 1.41%, 22.43 ± 3.14%, 33.97 ± 3.03%, 44.82 ± 2.72%, 52.39 ± 3.56% and 61.19 ± 4.17% respectively in control cells (Fig 2).

Adenoviral-driven GPx overexpression (MOI of 25: 1, 72 h culture prior to stress) significantly reduced cytotoxicity induced by XO from 10.81 ± 1.41% (control untransfected cells) to 5.42 ± 2.62% (AdGPx-transfected cells) at 10 mU/ml and from 61.19 ± 4.17% (control untransfected cells) to 34.04 ± 4.21% at 20 mU/ml (Fig 2).
52.9 ± 4.39% (AdGPx-transfected cells) at 20 mU/ml (p = 0.0002, AdGPx-transfected cells vs control cells at every XO dose). The protection conferred by AdGPx was 56.99 ± 17.58% and 20.53 ± 8.72% at 10 mU/ml and 20 mU/ml, respectively. Control experiments conducted with a similar adenoviral vector containing no cDNA (AdNull) did not show any difference between control cells and AdNull-transfected cells regarding the cytotoxicity of HX/XO (data not shown) as previously reported [7].

Additional experiments were conducted using a combination of AdGPx and AdSOD at an MOI of 20:1 each (Fig 3). When INS-1 cells were simultaneously transfected with AdGPx and AdSOD, a nearly 45% protection was observed against HX/XO cytotoxicity at a dose of 10 mU/ml with cytotoxicity values of 32.85 ± 6.39% in control cells vs 18.39 ± 5.06% in AdGPx/AdSOD transfected cells (p < 0.0001). With doses ranging from 10 to 20 mU/ml XO, the protection conferred by the double infection (AdGPx + AdSOD) was maintained at a similar level than the protection measured in INS-1 cells transfected with AdGPx alone.

**Table I**

<table>
<thead>
<tr>
<th>Dose of SIN-1</th>
<th>Control</th>
<th>AdGPx</th>
<th>% Protection</th>
</tr>
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<tbody>
<tr>
<td>600 µmol/l</td>
<td>17.66 ± 3.48</td>
<td>5.65 ± 1.37</td>
<td>63.13 ± 12.52</td>
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<tr>
<td>800 µmol/l</td>
<td>30.48 ± 6.55</td>
<td>18.92 ± 6.66</td>
<td>41.40 ± 8.30</td>
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<tr>
<td>1000 µmol/l</td>
<td>45.97 ± 6.48</td>
<td>35.80 ± 5.59</td>
<td>21.87 ± 5.36</td>
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</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Dose of SIN-1</th>
<th>Control</th>
<th>AdGPx + AdSOD</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 µmol/l</td>
<td>34.95 ± 7.45</td>
<td>28.51 ± 3.28</td>
<td>16.63 ± 8.37</td>
</tr>
<tr>
<td>800 µmol/l</td>
<td>54.02 ± 9.89</td>
<td>40.96 ± 7.44</td>
<td>24.12 ± 0.08</td>
</tr>
<tr>
<td>1000 µmol/l</td>
<td>66.91 ± 7.40</td>
<td>56.74 ± 2.46</td>
<td>14.54 ± 5.76</td>
</tr>
</tbody>
</table>

**Figure 3**

Reduction in HX/XO-induced cytotoxicity on INS-1 cells by AdGPx + AdSOD.

INS-1 cells, 3.10^4 cells per well, were transfected with AdGPx + AdSOD at an MOI of 20:1 + 20:1, stressed 72 h later by increasing concentrations of XO and assessed for viability by WST-1 assay 16 h later. Data show means ±95% confidence interval, n=3. P<0.0001, AdGPx/AdSOD transfected cells vs control cells at every XO dose. Control AdGPx+AdSOD ■.

**Effects of overexpression of antioxidant enzymes on the toxicity of SIN-1**

Exposure of INS-1 cells to increasing amounts of SIN-1 resulted in a dose-dependent cytotoxic affect shown by WST-1 assay (Tab I). Doses of SIN-1, from 600 to 1,000 µmol/l, applied to control or AdGPx-transfected cells resulted in cytotoxicity ranging from 17.66 ± 3.48 to 45.97 ± 6.48 in control cells and from 5.65 ± 1.37 to 35.80 ± 5.59 in AdGPx-transfected cells (p = 0.015, control vs transfected cells at every SIN-1 dose). GPx overexpression was associated with a significant reduction in SIN-1 induced cytotoxicity. Protection was effective even at a high dose (1,000 µmol/l), reaching 21.87 ± 5.36%.

Control experiments were conducted with a similar adenoviral vector containing no cDNA (AdNull) and showed an aspecific reduction of SIN-1 induced cytotoxicity below 10% (data not shown).

Additional transfection experiments (Tab II) were conducted on INS-1 cells using a combination of AdGPx and AdSOD. Table II summarizes the grade of protection conferred by this co-infection towards SIN-1 cytotoxicity. Protection was reduced whatever the selected doses of SIN-1 and was not statistically significant, reaching only 16.63 ± 8.37% and 24.12 ± 0.08% at 600 µmol/l and 800 µmol/l in AdGPx/AdSOD transfected cells compared to control cells at every XO dose. Control AdGPx+AdSOD ■.

Additional transfection experiments (Tab II) were conducted on INS-1 cells using a combination of AdGPx and AdSOD. Table II summarizes the grade of protection conferred by this co-infection towards SIN-1 cytotoxicity. Protection was reduced whatever the selected doses of SIN-1 and was not statistically significant, reaching only 16.63 ± 8.37% and 24.12 ± 0.08% at 600 µmol/l and 800 µmol/l in AdGPx/AdSOD transfected cells compared to control cells at every XO dose. Control AdGPx+AdSOD ■.

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63.13 ± 12.52% and 41.40 ± 8.30% at the same doses in AdGPx alone-transfected cells.

**Effect of GPx gene transfer on cellular endocrine function**

HX/XO-stressed cells, AdGPx-transfected and controls, were tested for their insulin secretion function by static incubation.

Insulin-secretion by control cells during a glucose-theophylline stimulation decreased by 61% and 56% when cells were exposed to 14 and 16 mM/mL XO, respectively while stressed GPx overexpressing-cells maintained the same insulin secretion level than non-stressed cells.

**Discussion**

Nitric oxide and oxygen free radicals are believed to play an important role in the destruction of beta cells in autoimmune diabetes [17-21]. Our study focused on the protective potential of antioxidant enzymes against action of oxygen free radicals and NO using INS-1 cells overexpressing GPx, in combination with Cu/ZnSOD or not. Our results show that overexpression of GPx gave excellent protection against the toxic effects of HX/XO. Cells overexpressing GPx were also much more resistant than control cells when exposed to 3-morpholinosydnonimine (SIN-1), a chemical compound that generates NO and oxygen free radicals.

The mechanism and specificity of NO production vary among the different NO donors. SIN-1 spontaneously decomposes under aqueous conditions, generating superoxide and NO at comparable rates [22-23]. Superoxide reacts rapidly with NO, resulting mainly in peroxynitrite production and, at a lesser level, in hydrogen peroxide formation [24]. Superoxide, NO, peroxynitrite and hydrogen peroxide have all been detected in incubations of SIN-1 [25-27]. Sies et al. [28] have shown that the maintenance of protection by GPx against peroxynitrite reductase HX/XO generates both hydrogen peroxide and superoxide radical (Fig 1). In our study, the combined overexpression of GPx and SOD did not provide any additional protection against HX/XO nor SIN-1, and was even worse than overexpression of GPx alone. This finding may suggest that the main pathway of cell injury is peroxynitrite (Fig 1) in our model, and suggests an advantage of GPx over catalase, inasmuch as GPx can also interact with peroxynitrite. This is in agreement with reports showing that overexpression of GPx is more protective against the toxicity of SNAP and SNP (other NO donors) than SOD [27].

All aerobic cells contain a set of defenses against reactive oxygen species. It would be anticipated that cells containing low levels of one or more of these enzymes would be particularly vulnerable to oxidative damage. This appears to be the case in pancreatic β cells, which are readily destroyed by oxidants and in which there is an unusually low expression of antioxidant enzymes, particularly CAT and GPx [5-10]. Part of the cytotoxicity induced by proinflammatory cytokines on β cells also involves oxidative stress and can be reduced by overexpressing antioxidant enzymes [29]. Whether CAT, SOD or GPx should be preferentially used in clinical islet transplantation was not addressed in our study. Our major finding is that GPx protected against both ROS and RNS, as opposed to CAT or SOD [7-8].

Previous studies already reported improvements in cellular resistance to oxidative stress with GPx overexpression. Thus the RINm5F rat beta cell line, engineered to overexpress GPx, is protected against menadione, which generates superoxide intracellularly, while no protection was observed from H₂O₂ nor hypoxanthine/xanthine oxidase, which generates superoxide extracellularly [9]. This discrepancy may be explained by differences in antioxidant enzyme expression between RIN cells and INS-1 cells. GPx overexpression was also shown to be beneficial in other models, such as ischemia/reperfusion in neurons [30]. Ischemia/reperfusion is a relevant mechanism to explain the loss of islet following transplantation, in that this process can generate ROS and RNS.

As opposed to previous studies performed in insulin-secreting cells with GPx, our work used adenoviral-mediated overexpression, which is a straightforward method requiring little manipulation of the cells. This method can be applied to pancreatic human islets, although the efficiency of neogene expression throughout the islet is questionable. Other gene expression systems, if proven to be safe and more efficient, may be used in the clinical islet transplantation setting.

In conclusion, we have shown that a recombinant adenovirus allows a readily overexpression of an antioxidant enzyme, glutathione peroxidase, in the INS-1 insulin-secreting cell line. In this model, a significant reduction in the cytotoxicity of reactive oxygen species and reactive nitrogen species was observed. Transposition of these findings to human islet transplantation with a clinically-relevant procedure deserves further investigations.

**References**


