Oxidative stress: the vulnerable β-cell

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Abstract
Antioxidative defence mechanisms of pancreatic β-cells are particularly weak and can be overwhelmed by redox imbalance arising from overproduction of reactive oxygen and reactive nitrogen species. The consequences of this redox imbalance are lipid peroxidation, oxidation of proteins, DNA damage and interference of reactive species with signal transduction pathways, which contribute significantly to β-cell dysfunction and death in Type 1 and Type 2 diabetes mellitus. Reactive oxygen species, superoxide radicals (O2•−), hydrogen peroxide (H2O2), and, in a final iron-catalysed reaction step, the most reactive and toxic hydroxyl radicals (OH•) are produced during both pro-inflammatory cytokine-mediated β-cell attack in Type 1 diabetes and glucolipotoxicity-mediated β-cell dysfunction in Type 2 diabetes. In combination with NO*, which is toxic in itself, as well as through its reaction with the O2•− and subsequent formation of peroxynitrite, reactive species play a central role in β-cell death during the deterioration of glucose tolerance in the development of diabetes.

Introduction
The pancreatic β-cell is in an extraordinary situation. It uses glucose catabolism to meet its energy needs, which also provides the energy required for insulin biosynthesis and exocytosis. At the same time, the β-cell generates within this metabolism the signal for glucose tolerance and insulin secretion. The integration of these two tasks requires a constellation in which the needs for adequate energy supply of the cell do not hamper the signal-generating function of glucose metabolism for insulin secretion. This creates an extraordinary challenge which represents a matter of life and death for the β-cell.

For its function as glucose sensor and insulin producer, the β-cell requires an intracellular milieu rich in oxygen and glucose in order to generate the signal for insulin secretion in its glucose metabolism and to supply adequately the target tissues in the periphery with this indispensable hormone for the regulation of intermediary metabolism. The β-cell is particularly weakly protected against the toxicity of free radicals [1,2], limiting the capacity for ROS (reactive oxygen species) inactivation by SOD (superoxide dismutase), catalase and GPx (glutathione peroxidase) (Figures 1, i–iii, and 2). Therefore the β-cell has a long-term chance of survival without damage only when a redox imbalance due to overproduction of ROS and RNS (reactive nitrogen species) can be prevented. In this short review, I will look into the reasons for the great sensitivity of the pancreatic β-cell to free radical toxicity and will consider the question of whether this particular vulnerability and sensitivity to the toxicity of reactive species is the price the β-cell has to pay in order to allow it to fulfil this dual function.

In order to understand with which target structures reactive species interact, an integrated view must take into account the physicochemical properties of the different reactive species, namely chemical reactivity, lifespan and permeability properties, as well as their intracellular sites of generation.

Properties of reactive species
Reactive species are small molecules with an oxygen or nitrogen atom in their structure [3]. These ROS and RNS can be free radicals, with an unpaired electron [e.g. NO* (nitric oxide radical), O2•− (superoxide radical) and OH• (hydroxyl radical)] or non-radicals (e.g. H2O2); they can be anions [e.g. O2− (superoxide) and ONOO− (peroxynitrite)] or non-ions (e.g. H2O2, NO* or OH*). Their reactivity is vastly different.

OH* is the most reactive oxygen radical known, reacting instantaneously with molecules in its immediate vicinity, which explains its great destructive power.

O2•− itself is far less reactive than OH* and does not readily react with most biological molecules. It does react quickly, however, with some other radicals, such as NO*, as well as iron clusters in some enzymes. Since O2•− does not readily cross membranes, a prompt reaction with another radical requires that the particular radical for such a reaction is formed in the same subcellular compartment or travels to this compartment. In quantitative terms, the mitochondrial respiratory chain is the most important site of O2•− generation [4].

H2O2 is produced continuously in all cells. It diffuses within and in between cells. H2O2 is not very reactive. It does not readily oxidize most proteins, lipids or DNA. Nevertheless, it can be cytotoxic at micromolar
concentrations. Since it is only a weak oxidizing or reducing agent, it can play a role in signal transduction. For β-cells, this has also been proposed [5], but the evidence, in contrast, for example, with plants [6], is only circumstantial so far.

NO• reacts slowly with most biological molecules, but is highly reactive with other free radicals. Of particular importance for the toxicity of NO• is the reaction with O2•−, forming the more toxic ONOO•. In contrast, the reaction with other radicals is often rather beneficial. NO• can cross membranes and diffuse readily between and within cells. NO• reacts faster with O2•− than with haem compounds or even than the reaction of O2•− with SOD. The reaction of NO• with O2•− is important because the biological actions of NO• and O2•− are prevented and because ONOO• is formed, which is in itself significantly toxic (Figure 1, iv).

**Intracellular generation of reactive species**

Many toxic chemical compounds are reactive in themselves or can generate reactive species through xenobiotic metabolism. This group includes the diabetogenic agent alloxan [7]. The reactive species which play an important role in the pathogenesis of pancreatic β-cell loss in diabetes mellitus are generated intracellularly when the β-cells are under autoimmune attack through pro-inflammatory cytokines in T1DM (Type 1 diabetes mellitus) or when exposed to a β-cell toxic hyperglycaemic and hyperlipidaemic milieu in T2DM (Type 2 diabetes mellitus). As will be explained below, there is convincing evidence for the participation of both RNS and ROS in the pathogenesis of β-cell destruction in T1DM, whereas, for β-cell dysfunction in T2DM, only the participation of ROS can be depicted convincingly at present.

**Reactive species in pancreatic β-cell dysfunction and death in T1DM**

Pro-inflammatory cytokines released from immune cells infiltrating the endocrine pancreas in autoimmune T1DM interact in a paracrine fashion with the β-cells via specific receptors [8]. Via different signalling cascades, they induce a variety of effects and actions. In these cascades, signals are generated which induce an apoptotic programme of β-cell death [9]. A number of steps in this chain of events affect the rate of generation of RNS and ROS.

It is evident from studies in patients with diabetes and in animal models of T1DM, that IL-1β (interleukin 1β) is the key pro-inflammatory cytokine in the pathogenesis of T1DM, which significantly contributes to β-cell dysfunction and death. A pro-inflammatory cytokine with major additional cytotoxicity is TNFα (tumour necrosis factor α). If it is released from the infiltrating immune cells in addition to IL-1β, the speed of β-cell loss is significantly increased, resulting in a rapid progression of the disease with rapid loss of the entire pancreatic β-cell mass. The production and release of IFNγ (interferon γ), the third major pro-inflammatory cytokine in autoimmune diabetes, by infiltrating immune cells is much less prominent in the diabetic pancreas [8], so this cytokine may contribute less to β-cell destruction in T1DM. But it potentiates the effects of IL-1β, thereby contributing to its toxicity. This appears to be particularly true for the ER (endoplasmic reticulum) stress, in which a combination of the two cytokines has an additive effect [10].

IL-1β potently induces iNOS (inducible nitric oxide synthase), and the resultant production of the free radical NO• is a central factor in the toxicity of this pro-inflammatory cytokine [9]. TNFα is significantly weaker in this respect, but, in combination with IL-1β, it potentiates its effect so that 10-fold lower IL-1β concentrations become equally toxic. IFNγ potentiates the effects on iNOS and NO• production only at high concentrations in combination with IL-1β [10a].

IL-1β also induces the MnSOD and this results in an increased rate of conversion of O2•− into H2O2 in the mitochondria through this SOD isoenzyme [11]. Cu/ZnSOD, the cytoplasmic isoenzyme, is not affected by

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**Figure 1** | Chemical reactions of reactive species and actions of cytoprotective enzymes

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\begin{align*}
2\text{H}^+ + 2\text{O}_2^• \xrightarrow{\text{SOD}} & \text{H}_2\text{O}_2 + \text{O}_2 & (i) \\
2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} & \text{O}_2 + 2\text{H}_2\text{O} & (ii) \\
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{Gpx}} & 2\text{H}_2\text{O} + \text{GSSG} & (iii) \\
\text{NO}^• + \text{O}_2^• \xrightarrow{} & \text{ONOO}^- & (iv) \\
\text{H}_2\text{O}_2 + \text{e}^- \xrightarrow{} & \text{HO}^• + \text{HO}^- & (v) \\
\text{Fe}^{II} + \text{H}_2\text{O}_2 \xrightarrow{} & \text{Fe}^{III} + \text{OH}^• + \text{OH}^- & (vi) \\
\text{Net} : \text{O}_2^• + \text{H}_2\text{O}_2 \xrightarrow{\text{metal catalyst}} & \text{O}_2 + \text{OH}^• + \text{OH}^- & (vii)
\end{align*}
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IL-1β. The profile of the effects of TNFα and IFNγ alone and in combination with IL-1β on MnSOD is comparable with that for the effects on iNOS. The effects on the generation of both radicals are not only important in themselves, but also affect the balance between NO• and O2•−, and this can have significant effects on β-cell toxicity.

A decrease in O2•− through MnSOD may present as a protective signal through a reduction of NF-κB (nuclear factor κB) activation and other components of the IL-1β signalling pathway [12]. On the other hand, an increased conversion rate of O2•− into H2O2 by SOD is likely to increase toxicity to the β-cell with its poor enzymatic capacity for H2O2 inactivation [1,2].

Since mitochondria lack catalase and, in the β-cell, mitochondria contain an extremely low GPx activity, it is very important to prevent any excessive oxidative stress that may overwhelm the limited antioxidative defence capacity of the β-cell mitochondria. The capacity for inactivation of H2O2 in order to prevent its contribution, in the presence of transition metals, to the generation of highly cytotoxic OH• (Figure 1, v–vii) is sufficient under normal circumstances; however, during oxidative metabolism, a constant level of O2•− is generated. However, this low defence can be easily overwhelmed in pathological situations of increased oxidative stress. This is the case in the cytokine-mediated autoimmune attack on the β-cell.

Although IL-1β increases the generation of reactive species, which most β-cells do not survive in the long-term, TNFα is the aggravating factor which results in the destruction of β-cells in the T1DM pancreas. TNFα potentiates the effects of IL-1β on iNOS and MnSOD. In addition, ceramide is likely to play a significant role as a mediator of O2•− formation in TNFα-mediated toxicity as discussed previously [13], thereby explaining the dominance of ROS in the case of TNFα when compared with IL-1β. Thus, with a significant contribution of TNFα produced by the infiltrating immune cells in T1DM, the resulting greater cytotoxicity is the result of the more pronounced ROS component of TNFα toxicity. Through experiments aiming to distinguish the relative importance of RNS- and ROS-mediated toxicity, we were able to show that, although both RNS and ROS contribute equally in the case of IL-1β, in the case of TNFα, ROS dominate (E. Gurgul-Convey and S. Lenzen, unpublished work).

That the ROS-mediated component of cytokine toxicity primarily targets the mitochondria is shown by the fact that exposure of insulin-producing cells to IL-1β or to a cytokine mixture containing IFNγ and TNFα in addition, causes mitochondrial damage, while other subcellular structures remain intact. This damage can be prevented by expression of high levels of catalase or GPx in the mitochondria, but not in the cytosol [13].

The RNS component of IL-1β toxicity, mediated through NO• and potentiated by IFNγ and TNFα, is likely to focus its effects in the cytoplasm. This component will presumably contribute to ER stress, which plays a significant role in dysfunction of β-cells under cytokine attack [13a].

Reactive species in β-cell dysfunction and death in T2DM

β-Cell loss in T2DM is slower than in T1DM, typically with a long phase of β-cell dysfunction, characterized by defective insulin secretion in response to the physiological stimulus glucose. Although the causes of β-cell dysfunction are not fully understood, glucolipotoxicity has been considered to be an important contributing factor to β-cell dysfunction in T2DM [14–17]. Pro-inflammatory cytokines are not involved to any significant extent in the pathogenesis of T2DM [18,19].

It is evident from studies on β-cells exposed to a combination of high glucose and a saturated fatty acid that NO• generation through iNOS induction does not contribute to β-cell dysfunction [20]. MnSOD is also not induced.

Increased mitochondrial metabolic flux is required in the β-cell for generation of the ATP signal for glucose-induced insulin secretion [21] and its potentiation through fatty acids [15]. On the other hand, increased metabolic flux through the respiratory chain at high glucose and lipid concentrations should increase O2•− formation, thereby reducing the mitochondrial membrane potential via UCP2 (uncoupling protein 2) [14,22]. This should decrease metabolic flux through the respiratory chain and thus reduce O2•− production, thereby acting in a protective manner against ROS-induced damage, but, at the same time, attenuating nutrient-induced insulin secretion. This casts doubt on the concept that increased intramitochondrial generation of ROS crucially contributes to β-cell damage in T2DM.

We have not found any convincing evidence so far of a major contribution of UCP2-mediated mitochondrial membrane potential changes to the development of glucolipotoxicity-mediated damage to insulin-producing cells. The concept put forward by Brownlee [23,24] for glucose- and fatty-acid-induced tissue damage is not adequate in this context. It does not specify the reactive species which are generated intramitochondrially and which would then traverse the membrane into the cytosol and the nucleus inducing their effects which subsequently cause the vascular damage that is responsible for the development of diabetic complications as well as β-cell dysfunction. Considering the physicochemical aspect, it is unlikely that O2•− is this reactive species. On the other hand, we have observed that insulin-producing cells overexpressing H2O2-inactivating enzymes in the cytoplasmic compartment are better protected against glucolipotoxicity. The source of increased ROS generation in the cytosolic compartment is not clear at present, even though it is likely that the ultimately toxic reactive species generated is OH•. One such potential source for ROS generation in the cytoplasm might be NADPH oxidase [25].

This interpretation is supported by the results of morphological analyses showing that insulin-producing cells exposed to the fatty acid palmitate show no signs of mitochondrial damage, but very pronounced defects of the ER [26], confirming observations of increased ER stress in
response to glucolipotoxicity [20]. Thus one of the prominent targets of this free-radical-mediated toxicity might indeed be the ER.

Support for the concept from animal model studies

Recent evidence from studies in different animal models of diabetes provides interesting support for the contention that disturbances of the constitutively weak antioxidative defence of the β-cell can quickly result in an overwhelming of its limited defence capacity.

These animal models show that a decrease in the ROS-inactivating capacity in the β-cells owing to genetic variations, even without autoimmune background, results in defective insulin secretion and a deterioration in glucose tolerance.

In the DBA/2 mouse model [27], a constitutive overexpression of MnSOD, resulting in overproduction of H2O2, is responsible for damaging the β-cells through overriding the limited H2O2-inactivating capacity of the mitochondria.

In the C57/BL6J mouse model [28], a defective nicotinamide nucleotide transhydrogenase prevents the prompt regeneration of GSH from GSSG, thereby leaving GPx without sufficient amounts of GSH required for inactivation of H2O2. This shows that instantaneous regeneration of GSH is mandatory to protect β-cell mitochondria against oxidative damage.

Conclusions

The intracellular milieu of the pancreatic β-cell is rich in oxygen and glucose. This is not the case for most other mammalian cell types, but is true, for example, in leaves and other plant tissues [29]. This special internal milieu makes the β-cell, with its constitutively low enzymatic antioxidative defence equipment [1,2], particularly susceptible to oxidative stress and vulnerable during autoimmune attack and glucolipotoxic challenge. On the other hand, a more efficient inactivation of ROS in the β-cell mitochondria may hamper the signalling function of metabolism for nutrient-induced insulin secretion. Thus reactive species contribute to β-cell damage in T1DM and T2DM, even though the underlying mechanisms differ significantly [18].

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References


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