THE IMPLICATION OF THE OXIDATIVE STRESS IN THE PANCREATIC BETA CELL DISTRUCTION

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Abstract: The beta cell is unique in that it is unusually susceptible to the damaging role of ROS and this may be why alloxan and streptozotocin induce selective destruction of the beta cell. One of the aims of the present study is the demonstration of oxidative stress implication in the beta cell destruction and in the pathogenesis of diabetes mellitus. We also intend to evaluate some antioxidant substances in the effectiveness of beta cell protection against the oxidative stress. At the positive diabetic lot, the destruction of beta cells with manifest diabetes was obtained by administrating the streptozotocin. All the rats were intensely manifesting the clinical signs of diabetes with intense glucosuria, ketonuria and high glycemia. Diabetes was not achieved in all the cases of the lots pretreated with antioxidants, the glycemia average was smaller than that of the diabetic lot. The clinical and biochemical parameters were not as intensive at the antioxidative pretreated lots as at the diabetic lot. The destruction of Langerhans islets was less intensive at the rats antioxidants pretreated as was revealed by the morphology exam of the pancreas; the best protective effect being ensured by the N-acetyl L-cysteine and organic selenium administration. In conclusion the antioxidants used can attenuate in different grades the oxidative stress at the B cells level depending on the type of antioxidant.

INTRODUCTION

Transference of electrons between oxygen species (cellular respiration) allows each of us to survive on this planet, not only at the cellular level but also as an organism. Redox cycling thus implies a homeostatic balance between ROS production and antioxidant capacity, and is also termed redox homeostasis. In contrast, oxidative stress (redox imbalance) implies a loss of this unique homeostasis as well as an excess production of ROS, through the process of reduction or that of oxidation (1, 16). Oxidative stress implies a loss of homeostasis with an excess of ROS.

The beta cell is unique in that it is unusually susceptible to the damaging role of ROS and this may be why alloxan and streptozotocin induce selective destruction of the beta cell with ensuing type 1 diabetes mellitus in various animal models (6, 7, 8, 10). Alloxan has been shown to induce $O_2^-$ (9, 19) and streptozotocin inducible nitric oxide (iNO) (10), which results in the selective destruction of the beta cells within the islets. This susceptibility makes the beta cell a unique target and vulnerable for selective destruction by the ROS and RNS (5, 11, 17).

An aim of the present study is the demonstration of oxidative stress implication in the beta cell destruction and in the pathogenesis of diabetes mellitus. We also proposed to evaluate some antioxidant substances in the effectiveness of beta cell protection against the oxidative stress. The present study opens multiple perspectives in the diabetes research. First, there will be demonstrated which of the tested antioxidants has the most effectiveness in the B cell protection.
MATERIAL AND METHODS

Experimental subjects: 50 female Whistar rats, age of approx. 150 days. The rats of every lot were housed five in a cage, on sawdust, kept 12 hours light and 12 hours dark, with water at discretion. They were fed with standard rat chow once a day.

The experimental rats were divided into 5 groups:

- Lot 1 (n=10) – normal witness;
- Lot 2 (n=10) – positive diabetic – it was induced diabetes by streptozotocin;
- Lot 3 (n=10) – it received ascorbic acid preventively;
- Lot 4 (n=10) – it received astaxanthine preventively;
- Lot 5 (n=10) – received N-acetyl-L-cysteine and organic Se preventively.

Materials: streptozotocin (STZ); antioxidants: N-acetyl cystein, astaxanthine, organic selenium and ascorbic acid; insulin (Insuman); urinary strip for glucosuria, ketones and ascorbic acid determination; the glycemia was determined with enzyme method.

Method of administration of the substances:

- Streptozotocin (Sigma Aldrich) was administered in a 0.1 M sodium citrate solution 2% (pH=4.5), in a dose of 50 mg/kg intraperitoneal at the groups 2, 3, 4 and 5.
- The ascorbic acid (Sicomed) was administered to the lot 3 per oral in a dose of 30 mg/rat/day during two weeks before the streptozotocin injection.
- The astaxantine (Sigma Aldrich) was administered at the lot 4 per oral in an oleos suspension in a dose of 100 µg/rat/day (aprox. 600 µg/kg/day) during two weeks before the streptozotocin injection.
- N-acetyl L-cysteine (Sigma Aldrich) was administered at the lot 5 i.p. in a dose of 200 mg/kg during consecutive three days before the streptozotocin injection.
- Organic selenium (Alltech) was administered at the lot 5 per oral during two weeks before the streptozotocin injection. The organic bind selenium is obtained with growing Saccharomyces cerevisiae on a sodium selenite enriched substrate, the product contains 0.1% selenium and it was administered in a dose of 0.15 mg selenium/ rat/ day.

Induction and monitoring of diabetes:

- Streptozotocin was administered to the groups 2, 3, 4 and 5 at the day 0 of the experiment. At the same time the normal lot 1 received a placebo 0.4 ml 0.1 M sodium citrate solution without streptozotocin i.p. The groups with antioxidants received, as above described, the corresponding antioxidants as preventing treatment for the streptozotocin induced oxidative stress.
- After the streptozotocin injection the rats received 5% glucose drink water over the next 12 hours in order to prevent the severe hypoglycemia due to the massive insulin liberation from the destroyed beta cells. Lent insulin was injected i.m. 1 IU/rat at 48 and 72 hours in order to prevent the death from severe diabetic ketoacidosis.
- The diagnosis of diabetes induction was made by glucose and ketones determination from the urine. The glycemia was determined from the blood obtained in the moment of euthanasia, being considered diabetics the animals with glycemia over 180 mg/dl.

The pathology exam:

- The rats were sacrificed with exsanguinations under profound narcosis (Phenobarbital) at 3 (5 rats from each lot) and 6 (5 rats from each lot) days from the moment of streptozotocin injection.
Samples from the pancreas were collected at the dissection, then fixed in 10% buffered formalin solution and embedded in paraffin. Sections of 4 µm were stained H&E and Tricrom Masson.

Identification of B cells was attempted by the tricrom Mallory staining method.

There were counted and measured the followings for the each subject of the experiment at the histology exam: main dimension of islet cut surface of 20 islets, main number of intact endocrine cells in 20 islets, main number of death endocrine cells (necrotic and apoptotic) in 20 islets and main number of leukocyte infiltrate in 20 islets.

Statistical comparison of the values was made, using the Student-t test in the Microsoft Excel program. The differences of values with p<0.01 were considered relevant.

RESULTS AND DISCUSSION

At witness lot was not registered elevated values of the glycemia (average 69.5 mg/dl), it were negative for glucosuria and ketonuria. At positive diabetic lot it was obtained the destruction of beta cells with manifest diabetes. The average of glycemia was of 480 mg/dl at 48 hours after streptozotocin administration. All the rats were intensely manifesting the clinical signs of diabetes with intense glucosuria and ketonuria.

At the ascorbic acid pretreated lot diabetes was achieved in 7 cases from the ten. The average of glycemia was smaller then that of the diabetic lot but with no statistical significance (Fig.1).

At the astaxantine pretreated lot diabetes was achieved in 7 cases from the ten. The average of glycemia was smaller then that of the diabetic lot but with no statistical significance. The clinical and biochemical parameters were not as intensive as that of the diabetic lot.

At the lot 5 (pretreated with NAC and Se) diabetes was achieved in 6 cases and 4 rats remained healthy. The average of glycemia was smaller with statistical significance (p<0.01) then that of the diabetic lot. In all the antioxidative pretreated lots the clinical and biochemical parameters were not as intensive as that of the diabetic lot.

Massive B cell destruction was observed in the pancreas of each animal from the diabetic lot. The destruction of B cells was characterized by vacuolization, shadowed nuclei, swollen nuclei and cytoplasm, low tinctoriality or by apoptotic figures. The necrosis affected mainly the central part of the islets, being the part where the B cells are located preponderantly. The presence of inflammatory cells was observed in the endocrine islets and
around of the islets with central necrosis. An interesting aspect was the “constipation” of the
exocrine acinar cells around the islets with central necrosis, the cytoplasm being fulfilled with
zymogen granules.

The morphology parameters of the B cell destruction were slighter in the antioxidant-
pretreated animals. The main number of intact endocrine cells/islet is the highest in the NAC
+ Se treated lot, meaning a very good protective effect of these antioxidants against
streptozotocin induced oxidative stress. The aspects of B cells destruction were present in all
the animals that received streptozotocin, but in the antioxidant pretreated once these aspects
were more discrete and in a less number of islets. The morphology parameters of the
endocrine islet destruction are presented in the table 1 and fig 2.

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Lot 1 N</th>
<th>Lot 2 DM</th>
<th>Lot 3 vit. C</th>
<th>Lot 4 astaxantin</th>
<th>Lot 5 NAC + Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average 106.7</td>
<td>57.9</td>
<td>80.5</td>
<td>91.3</td>
<td>96.8</td>
<td></td>
</tr>
<tr>
<td>stand Dev</td>
<td>25.63</td>
<td>15.9</td>
<td>19.66</td>
<td>33.23</td>
<td>12.33</td>
</tr>
<tr>
<td>p- DM</td>
<td>&lt; 0,01</td>
<td>&lt; 0,01</td>
<td>&lt; 0,01</td>
<td>&lt; 0,01</td>
<td>n.d.</td>
</tr>
<tr>
<td>p-normal</td>
<td>* &lt; 0,01</td>
<td>* &lt; 0,01</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The main number of intact endocrine cells in 20 islets for each rat. It was considered statistically
significance the differences of values with p < 0,01, * in negative sense, n.d. – with no difference, p-DM
comparing to the diabetic lot, p-normal comparing to the whiteness lot. There is statistical significance between
the antioxidant pretreated lots and the diabetic lot (p < 0,01) in the number of unaffected endocrine cells, being
lesser in the diabetic lot (58/islet).

Fig.2. Main number of the dead endocrine cells and inflammatory cells per islet in 20 counted islets of each
animal. There is statistical significance between the antioxidant pretreated lots and the diabetic lot (p < 0,01)

Streptozotocin (STZ) is selectively toxic to insulin-secreting beta-cells of pancreatic
islets and induces impairment of islet glucose oxidation and of glucose-induced insulin
secretion. STZ, a methylthiosemicarbazone with a 2-substituted glucose, causes B cell necrosis and
insulin-dependent diabetes mellitus in many species (8, 10). The mechanism of action of STZ
on rodent beta cells is well known. The glucose moiety allows preferential uptake of STZ into
beta cells, probably via the glucose transporter (GLUT)-2. Intracellular metabolism of STZ
yields nitric oxide that precipitates additional DNA strand breaks. Within 48 h, severe insulin-
dependent diabetes ensues, characterized histologically by massive B cell necrosis and
macrophage infiltration (8, 10). This is also presumed to be the mechanism by which
diabetogenic doses of STZ induce diabetes in other susceptible species (10). Similar effects
are induced by Interleukin-1 (IL-1), and the deleterious effects of IL-1 on islets appear to be mediated by nitric oxide (NO) (10).

STZ contains a nitroso moiety and may liberate NO by processes analogous to those for the NO-releasing drug nitroprusside. NO is rapidly transformed to nitrite in aqueous solution, and NO activates heme-containing enzymes such as guanylyl cyclase and inhibits iron-sulfur enzymes such as mitochondrial aconitase (7).

The used antioxidants in this experiment seem to attenuate the diabetogenic effect of streptozotocin. In order of the effectiveness the combination of NAC and Se proved to be a good option for the B cell protection against oxidative and nitrosative stress.

Not only are ROS involved in the development of diabetes but they also play an important role in the long-term development of associated complications as demonstrated by many studies in the specialty literature (2, 3, 4, 12, 13, 18). ROS plays a major role in the B cell destruction, as well, as is demonstrated in this study and other similar works (8, 9, 14, 15). It seems that the antioxidative treatment is not only a complementary treatment to the diabetes treatment but it can not be left out in the correct management of the diabetic patient. It is well known that diabetes means an increased oxidative status and this fact may be the cause, at least in part, for the progressive B cell destruction in both type 1 and type 2 diabetes mellitus.

CONCLUSIONS

1. The specific destruction of the pancreatic B cells (apoptosis and necrosis) is provoked through the streptozotocin administration; the mechanism consist of the increasing of oxidative stress, a fact demonstrated in this experiment, by the possibility of preventing the effect of streptozotocin through antioxidant substances.
2. The oxidative sensible cell signal system plays an important role in the development, progressive nature (remodeling) and cell death of beta cells from the endocrine pancreas.
3. The used antioxidants can attenuate the oxidative stress at the B cells level in a different degree, depending on the type of the antioxidant.
4. The order of effectiveness is: N-acetyl L-cysteine and Se, the lipid soluble astaxanthin and the last one is the water soluble ascorbic acid, of the used antioxidants in the protection of B cells.
5. The differences of effectiveness in the protection of B cells against the ROS and RNS can be explained by different "penetration" to the level of insulin secreting cells, the different mode and effectiveness of reactive species neutralization as well.
6. A diet supplementation with antioxidants is recommended for protecting the B cell against reactive species, in thus preventing the diabetes mellitus (categories at high risk). An antioxidant treatment is indicated in pathologic states that involve high, acute or chronic oxidative stress (ex. pancreas or extra pancreatic inflammations, chronic internal diseases etc.) for protecting the beta cells, which are the most sensible cells of the organism to the oxidative stress.
7. The high level of ROS is demonstrated in diabetes mellitus type 1 and 2, and in all forms of impaired glucose tolerance (IGT, IFG, metabolic syndrome) as well, as a consequence of high glycemia level. So it is important to conduct an effective antioxidant treatment in case of these syndromes, in order to prevent the diabetes complications, but also to protect the remnant functional B cells and to prevent the progressive degradation of these cells, a characteristic phenomenon in all forms of diabetes.
8. Further studies are important for obtaining a perspective over different types of antioxidants, effective in the of B cell protection. There are many factors that influence the
efficiency of an antioxidant at a given level in the organism. In the case of the Langerhans islets, one of the impediments is the penetration inefficiency of the majority of classical antioxidants.

9. An interesting aspect was the “constipation” of the exocrine acinar cells around the islets with central necrosis, probably due to a local sympathetic over activity caused by the local liberation foci of the inflammation mediators in the necrotic.

BIBLIOGRAPHY


