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Mechanism underlying resistance of human pancreatic beta cells against toxicity of streptozotocin and alloxan

To the Editor: The diabetogenic agents, streptozotocin and alloxan induce insulin deficiency due to their selective pancreatic beta-cell toxicity [1]. However, there are significant species differences in the diabetogenicity of streptozotocin and alloxan [1]. While rodents are particularly prone to the diabetogenic action of these two diabetogens, humans are considered to be resistant [1]. Human pancreatic beta cells, in contrast to rodent beta cells, have been shown to be resistant to the toxic action of streptozotocin and alloxan in vitro [2, 3, 4].

RINm5F insulin-producing cells which do not constitutively express the GLUT2 glucose transporter are virtually resistant to the toxic action of streptozotocin and alloxan [5, 6]. Expression of the rat GLUT2 glucose transporter isoform rendered these cells sensitive and the toxicity of both streptozotocin and alloxan increased in parallel with the increase of the level of GLUT2 glucose transporter expression in this rat insulin-producing tissue culture cell line [5, 6].

Recently, it has been reported that after transplantation of human pancreatic islets into nude mice, beta cells are not damaged even after injection of high doses of streptozotocin [4] or alloxan [2, 3], while the beta cells of rat islets trans-planted concomitantly into the same animal are destroyed [2, 3, 4].

There are different potential explanations for this observation. Human beta cells could be more resistant to the toxic action of streptozotocin and alloxan either because they express the GLUT2 glucose transporter only to a very low extent [7, 8] (1–2% of the level expression in rat beta cells), or, alternatively, because streptozotocin and alloxan, in contrast to the rat GLUT2 glucose transporter isoform, are not taken up through the human GLUT2 glucose transporter isoform into the intracellular compartment where the toxins exert their cell-death action.

We therefore cloned and expressed the human GLUT2 glucose transporter isoform, using a known methodology [5], in RINm5F insulin-producing tissue culture cells which constitutively do not express the GLUT2 glucose transporter isoform [5, 6].

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In an experimental protocol using the MTT cytotoxicity assay [5] we could show that the toxicity of both streptozotocin and alloxan increased in dependence upon the human GLUT2 glucose transporter gene expression level in six different RINm5F cell clones (A–F), as measured by quantitative real-time PCR analyses using the Opticon real-time PCR cycler (MJ Research, San Francisco, Calif., USA) (Table 1). PCR was carried out with primer sets specific for human GLUT2 using SYBRGreen (Biozym, Hessisch Oldendorf, Germany) for fluorescence detection. There was a significant correlation ($p<0.01$; F-test) both for streptozotocin (r=0.987) and alloxan (r=0.948) between the decrease of the half maximally effective concentrations (EC_{50}) for the toxins, at which 50% of the cells died, and the increase of the human GLUT2 glucose transporter expression level (Table 1).

A calculation of the number of human GLUT2 mRNA molecules which must be expressed in insulin-producing cells in order to reduce the EC_{50} value for toxicity by 1 mmol/l showed that less GLUT2 mRNA molecules are required for this purpose in the case of the toxicity of alloxan (3331±228) than in the case of streptozotocin (4670±164). Thus, it can be concluded that the human GLUT2 glucose transporter shows an apparently greater affinity for alloxan than for streptozotocin.

For purposes of comparison, we did a comparable calculation for the rat GLUT2 transporter using data which we published earlier [5, 6]. The result shows that also in the case of the rat GLUT2 transporter, alloxan (1213±96) requires less GLUT2 mRNA molecule expression than streptozotocin (3416±358) showing that not only the human GLUT2 glucose transporter but also the rat GLUT2 glucose transporter has a greater affinity for alloxan than for streptozotocin.

In addition, it is evident that the expression of fewer rat GLUT2 mRNA molecules is required both in the case of alloxan (factor 2.8) and of streptozotocin (factor 1.4) to reduce the EC_{50} value for their toxicity by 1 mmol/l than in the case of the expression of the human GLUT2 glucose transporter.

The results show that it is apparently the very low level of constitutive GLUT2 glucose transporter expression in the human beta cell [7, 8] rather than the inability of the human GLUT2 glucose transporter isoform to provide uptake of streptozotocin and alloxan into the intracellular compartment (Table 1) which is responsible for the extraordinarily high resistance of humans against the diabetogenic action of streptozotocin and alloxan. However, even if the human GLUT2 glucose transporter isoform would be more abundant in human pancreatic beta cells, the lower capacity for uptake of the toxins through the human GLUT2 glucose transporter isoform as compared to the rat transporter isoform would limit

Table 1. Half maximal concentrations (EC_{50}) for toxicity in the MTT assay of streptozotocin and alloxan RINm5F insulin-producing cell clones with different levels of human GLUT2 glucose transporter expression

Cell clone	Human GLUT2 expression GLUT2 mRNA molecules/ng total RNA	Streptozotocin EC_{50} (mmol/l)	Alloxan EC_{50} (mmol/l)
RINm5F-control	not detectable (6)	17.4±2.7 (4)	no inhibition up to 20 mmol/l (4)
RINm5F-GLUT2-A	3127±118 (6)	9.5±0.9 (4)	15.0±0.3 (4)
RINm5F-GLUT2-B	1762±85 (6)	8.8±0.8 (4)	14.9±0.3 (4)
RINm5F-GLUT2-C	8356±256 (6)	7.6±2.0 (4)	11.8±0.2 (4)
RINm5F-GLUT2-D	16907±722 (6)	6.0±1.3 (4)	10.3±0.3 (4)
RINm5F-GLUT2-E	20509±436 (6)	5.5±0.3 (4)	11.0±0.3 (4)
RINm5F-GLUT2-F	25384±335 (6)	4.2±0.8 (4)	7.9±0.2 (4)

After exposure of the cells to serial concentrations (0, 2.5, 5, 7.5, 10, 12.5, 15, 20 mmol/l) of streptozotocin or alloxan viability was determined in the MTT cytotoxicity assay. GLUT2 gene expression was quantified by RT-PCR analyses through interpolation against human GLUT2 cDNA standards. Data are means ± SEM with the numbers of experiments given in parentheses

the diabetogenicity of both alloxan and streptozotocin in humans.

Therefore it is also not surprising that development of Type 1 diabetes mellitus is not known to be a typical side effect when streptozotocin is used as a chemotherapeutic agent in human cancer treatment and that it is not a particularly efficient anti-cancer drug when used in the treatment of human insulinomas which usually do not express the GLUT2 glucose transporter [4].

Another element which might be considered to contribute to the lower sensitivity of human pancreatic beta cells towards these toxins could be a better antioxidative defence status or a protection by stress response proteins such as hsp70 [9].

However, the antioxidative enzyme equipment is apparently not vastly different in rat and human beta cells [9] and even high levels of antioxidative enzyme overexpression in insulin-producing cells have been shown not to provide significant protection [10]. It is also unlikely that stress response proteins are a component of major importance for protection [9] in the concentration range of streptozotocin and alloxan (up to 20 mmol/l) used in the present experiments. Thus, even if human beta cells have a somewhat better antioxidative defence status, this would by far be insufficient to provide a protection of a magnitude comparable to that provided by a low level of GLUT2 glucose transporter expression.

It is thus the exclusion of the toxins from the intracellular space due to a lack of GLUT2 glucose transporter expression which provides the common explanation for the resistance of these insulin-producing cells against toxicity of these two beta-cell toxins, which otherwise differ in their mechanisms of toxic action [5, 6, 11, 12].

Thus even though insulin-secreting cells are more susceptible to damage through oxidative stress than other cell types they are nevertheless fully protected against the toxic action of these two compounds in the absence of noticeable GLUT2 glucose transporter expression.

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