Some aspects of cardiac antioxidant defence: Ebselen (PZ 51) treatment increases glutathione peroxidase activity in the rat heart

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Ebselen [PZ 51: 2-phenyl-1,2-benziselenazol-3-(2H)-one] is a synthetic organoselenium compound with anti-inflammatory activity [1, 2], which exhibits glutathione peroxidase (GSH-Px)-like activity, catalysing the reduction of hydrogen peroxide as well as other organic peroxides [3-5]. Its anti-inflammatory effect may be mediated by either the GSH-Px activity, the inhibition of leukotriene B4 formation [6], the antioxidant capacity, or a combination of all of them. Many attempts have been made to increase the antioxidant capacity of the myocardium, since free radical generation has been demonstrated in ischaemia-reperfusion damage [7, 8]; superoxide dismutase (SOD) and catalase have been used to decrease myocardial reperfusion injury [9]. Moreover, glutathione (GSH) decreases in the myocardium under ischaemic conditions [10] and a possible involvement of the myocardial mitochondrial pool of GSH has also been recently discussed [11]. This evidence and hypothesis led us to investigate some aspects of the cardiac antioxidant defence, as well as the effect of Ebselen treatment on the peroxidase activity of the myocardium, which are preliminary results necessary for a rationale of the use of Ebselen to prevent or ameliorate ischaemic or reperfusion damage. Materials and methods

Wistar rats weighing 240-260 g which were fed a standard laboratory diet and with free access to water were used for the experiments. Ebselen was administered intraperitoneally at a dose of 40 mg/kg body weight, as an olive oil suspension, where controls received the appropriate amount of oil. All animals were sacrificed 2 h after injection since the highest plasma concentration of total selenium after oral administration of 77Se-Ebselen to a healthy volunteer has been reported at this time [12]. Hearts were homogenized in 0.1 M potassium phosphate buffer pH 7.0, and the enzymatic activities and protein content determinations done on the cytosolic fraction (100 000 g supernatant). In order to determine the Ebselen-dependent GSH-Px-like activity, hearts were homogenized in 2% perchloric acid containing 1 mM EDTA, and the peroxidase activity studied in the neutralized supernatant. Different animals were used for these purposes to minimize the further biotransformation of Ebselen in the fresh cardiac homogenate [13]. GSH-Px activity [14], using H2O2 and t-butyldihydroperoxide, glutathione S-transferase activity, using 1-chlor-2,4-dinitrobenzene (CDNB) [15] and 4-hydroxy-2,3-trans-nonenal [16], glutathione reductase activity [17], SOD activity [18], DT-diaphorase activity [19] and protein content [20], were determined according to the references cited. The results are expressed as nmol of substrate modified/min per mg of protein, except for SOD where they are expressed as the nmol of adrenaline prevented from autoxidation.

Results and discussion

Table 1 shows the activity of different antioxidant and glutathione-related enzyme activity of the rat heart in control conditions. The enzymatic activities tested in control myocardium are directly or indirectly related to the antioxidant defence, i.e. by either reducing the activated oxygen species formed (SOD, GSH-Px), maintaining the reduced state of the substrates of the previous enzymes (glutathione reductase), preventing the redox cycling of different quinones (DT-diaphorase), or conjugating xenobiotics [15] or toxic products of lipid peroxidation [16] (glutathione S-transferases). The importance of these enzymatic activities in the myocardium has been discussed, with regard to the cardiac toxicity of different quinones [21] and the conjugation of the toxic product of lipid peroxidation [22] in myocardium [23]. Table 1 also shows the GSH-Px activity in the myocardial cytosolic fraction of rats treated intraperitoneally with 40 mg/kg Ebselen. A significant increase in peroxidase activity was found in the cytosolic fraction of hearts treated with Ebselen. To elucidate whether this increase was due to an effect of Ebselen or the Se derived from it on the GSH-Px of the heart, and not to a direct increase due to the myocardial tissue content of Ebselen itself or any of its metabolites, Ebselen-dependent GSH-Px-like activity was determined in the acid extract. It is not possible to establish if this activity observed in the neutralized acid extract of the heart is dependent on unmodified Ebselen, or if any of the hepatic metabolites [13] retains some GSH-Px-like activity and may also accumulate in the myocardium. In any case, there is an effective increase in the myocardial GSH-Px activity 2 h after Ebselen treatment. These results are of interest since the heart might become the target of different subjects were 3.5, 4.1, 4.6 and 3.2 pools/day, respectively. In the present steady state conditions FSR must equal the fractional catabolic rate. The present data indicate apo B turnover is 3.8±0.5 pools/day in healthy normolipidaemic subjects.

Table 1. Antioxidant and some GSH-related enzymic activities in myocardial cytosolic fraction of control rat heart and the effect of Ebselen treatment (40 mg/kg body weight) on the GSH-Px activity of the cytosol and the recovery of Ebselen-dependent GSH-Px activity in the acid extract of the heart

Data shown are the means ± S.E.M. of the number of observations in parentheses, of at least three different hearts, and are expressed as nmol/min per mg protein, or *nmol/min per g wet weight of tissue.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Enzyme (substrate)</th>
<th>Specific activity</th>
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<tbody>
<tr>
<td>Control cytosol</td>
<td>Superoxide dismutase</td>
<td>58.2 ± 6.5 (5)</td>
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<tr>
<td></td>
<td>DT-diaphorase</td>
<td>69.1 ± 1.8 (5)</td>
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<tr>
<td></td>
<td>Glutathione S-transferase (CDNB)</td>
<td>50.2 ± 8.5 (6)</td>
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<tr>
<td></td>
<td>Glutathione S-transferase (4-HNE)</td>
<td>64.1 ± 7.6 (6)</td>
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<tr>
<td></td>
<td>Glutathione reductase</td>
<td>15.9 ± 7.0 (6)</td>
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<tr>
<td></td>
<td>Glutathione peroxidase (H₂O₂)</td>
<td>77.5 ± 12.2 (6)</td>
</tr>
<tr>
<td></td>
<td>Glutathione peroxidase (t-BOOH)</td>
<td>102.6 ± 15.3 (6)</td>
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<tr>
<td></td>
<td>Acid extract Glutathione peroxidase (H₂O₂)</td>
<td>155.5 ± 10.7 (6)</td>
</tr>
<tr>
<td></td>
<td>Glutathione peroxidase (t-BOOH)</td>
<td>103.9 ± 14.3 (6)</td>
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<tr>
<td></td>
<td>Glutathione peroxidase (t-BOOH)</td>
<td>35.8 ± 4.0 (6)*</td>
</tr>
<tr>
<td></td>
<td>Acid extract Glutathione peroxidase (t-BOOH)</td>
<td>39.5 ± 2.2 (6)*</td>
</tr>
</tbody>
</table>

Abbreviations used: t-BOOH, t-butylhydroperoxide; 4-HNE, 4-hydroxy-2,3-trans-nonenal.

pathophysiological situations in which oxidative stress appears, i.e. quinone toxicity, ischaemia-reperfusion, etc. It is certainly of interest to be able to increase the GSH-Px of the myocardium, either as a preventive mechanism against oxidative stress, but also as possible pretreatment before surgical reperfusion of occluded coronary arteries. Very recently the hypothesis has been proposed of the accumulation of respiratory-deficient cells in the heart as a consequence of mitochondrial DNA mutation and as an explanation of the limitation of the life-span [24]. If this is true, the increase of the antioxidant capacity with Ebselen gains importance since mitochondria lack catalase [25] and rely exclusively on GSH-Px activity to reduce H₂O₂, and the mitochondrial GSH pool in the heart is very small [11].

This work was supported by a project No PB87-0986 from the D.G.I.C.Y.T. to F.J.R. We thank A. Nattermann & Cie. GmbH for the generous gift of Ebselen and Prof Dr H. Esterbauer for 4-hydroxy-nonenal. E.N. is a post-doctoral fellow of the D.A.A.11. Cultura, lnvestigació i Ciencia of the Generalitat Valenciana (Valencia, Spain).


Received 15 June 1990