Effect of quercetin on paracetamol-induced rat liver mitochondria dysfunction

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The precise molecular mechanism of paracetamol-induced hepatotoxicity is still not well understood but it is believed that mitochondria may play an important role in this process. In this work we investigated the effects of paracetamol and quercetin on mitochondrial oxygen consumption. Moreover, we also studied the effects of both compounds, alone or in combination, on activities of mitochondrial enzyme such as ATP-ase, glutathione peroxidase (GPx) and glutathione reductase (GR). A single dose of paracetamol (500.0 mg kg−1) significantly decreased oxygen consumption in state 3. This effect of paracetamol was prevented by co-administration of quercetin. Actitivity of ATP-ase was significantly increased after paracetamol application. Pretreatment with quercetin completely prevented this increase. Activities of GPx and GR were not significantly influenced by paracetamol. On the other hand, quercetin caused either significant increase (GPx) or decrease (GR) in the enzyme activity. In conclusion, our data indicate that quercetin may be useful to mitigate paracetamol-induced liver mitochondria dysfunction.

Key words: mitochondria, paracetamol, quercetin, respiration, enzyme activity.

Abbreviations: GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; NAPQI, N-acetyl-p-benzoquinoneimine; RCR, respiratory control ratio.

Introduction

Paracetamol is an extensively used analgesic and antipyretic drug and, though safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose (RAMSAY et al., 1989). Under normal conditions, paracetamol is primarily metabolized in the liver by glucuronidation and sulfation. A small proportion of the

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drug is metabolized by several of the cytochrome P450 enzymes into the reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which is normally detoxified by glutathione (GSH) both non-enzymatically and enzymatically. In overdose, sulfation and glucuronidation become saturated and GSH is depleted by NAPQI. Excess of NAPQI causes oxidative stress and binds covalently to liver proteins (NELSON, 1995). Although the precise mechanism by which paracetamol causes cell injury is still unknown, it is suggested that mitochondria may play an important role in the paracetamol-induced liver cells death (FERRET et al., 2001).

Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most widely distributed bioflavonoids, which are abundant in red wine, tea, and onions. It has been reported that quercetin possesses many biological effects including cardioprotective (FORMICA & REGELSON, 1995), anticancer (KNEKT et al., 2002), gastroprotective (MOJZIS et al., 2001) and antimicrobial (RAUHA et al., 2000) effects. Furthermore, strong antioxidative effect of quercetin has also been documented (HOŁLMAN et al., 1997; TERAO, 1999; MIROSSAY et al., 2001; KOSTYUK et al., 2003). Characteristic feature of all the antioxidants is their ability to prevent oxidation of other compounds by reactive metabolite in a way that it oxidizes itself. The product of the reaction of an oxidant with an antioxidant should not induce further radical reactions when new free radicals or reactive metabolites of oxygen and oxidized substrates would be produced (ĐURACKOVA, 1998). From the point of view of the complexity of the effects of flavonoids in relation to free radicals, it is necessary to state that in some cases the flavonoids have also pro-oxidant effect (LAUGHTON et al., 1989).

Mitochondria play an important role in cell death induced by many drugs including hepatotoxicity as a result of paracetamol overdose. Our aim was to investigate the potential of paracetamol to affect certain mitochondrial parameters in rats. To that end we studied the effect of paracetamol on mitochondrial respiration, ATP-ase, glutathione peroxidase and glutathion reductase activities. Furthermore, we also studied the ability of quercetin to influence paracetamol-induced changes in selected mitochondrial parameters.

Material and methods

Male Wistar rats weighing 200–350 g were used. They had free access to a commercial balanced stock diet and water. The experimental animals were randomized into four groups (n = 6) which ran simultane-ously: 1 – treated with 0.1 mL kg⁻¹ saline (control animals, C); 2 – treated with single-dose of paracetamol (500.0 mg kg⁻¹ perorally, P); 3 – treated intra-gastrically with 50.0 mg kg⁻¹ of quercetin. The second dose of quercetin (50.0 mg kg⁻¹) was administered 5 hours after the first one (Q); 4 – treated intra-gastrically with paracetamol in dose 500.0 mg kg⁻¹; sixty minutes prior to paracetamol application, quercetin in dose 50.0 mg kg⁻¹ was given intra-gastrically. The second dose of quercetin was given four hours after paracetamol administration (P+Q). The rats were sacrificed by decapitation 24 hours after the paracetamol application, their livers were quickly removed and mitochondria were isolated by the method by JOHNSON & LARDY (1967). Protein concentration was determined according to HARTREE (1972).

Oxygen consumption was determined in state 4 without addition of ADP and in state 3 after ADP using Clark type electrode made by WTW oxi 325 (Germany) at 25°C on the substrate succinate. The respiration medium contained 80 mM KCl, 15 mM Tris-HCl, pH 7.4, 6 mM MgCl₂, 10 mM K-phosphate buffer solution with pH = 7.4 and 1 mM EDTA. Results were calculated as oxygen consumption in mmol/min/kg proteins in both states of respiration. Respiratory control ratio (RCR) was determined as a ratio between oxygen consumption in state 3 and state 4.

ATP-ase activity (EC 3.6.1.3) was determined according to MEESNER (1974) in μmol Pi/min/kg protein. Activity of glutathione reductase (GR, EC 1.6.4.2) was determined according to the method by CALBERG & MANNERVICK (1985) and is given in kat/kg mitochondrial protein. The reaction was initiated by the addition of 10 μL mitochondria suspension to the reaction mixture and the decrease in absorbance at 340 nm was monitored at 37°C for 5 min. Activity of glutathione peroxidase (GPx, EC 1.11.1.9) was measured by consecutive glutathione reductase reaction. This reaction was monitored by oxidation of NADPH assayed at 366 nm (FLOHÉ et al. 1984) and is expressed in U/kg mitochondrial protein.

Student’s t-test was used for statistical analysis with p < 0.05 level considered as significant (indicated as an asterisk). Results were calculated as the relative percentage changes compared to the values of control groups that were taken as 100 per cent.

Results and discussion

The effects of both compounds studied on mitochondrial respiration are shown in Table 1.

The respiration in state 4 is significantly increased in groups Q and P+Q (p < 0.01 and p < 0.05, respectively). The mitochondrial respiration in state 3 in the paracetamol-treated animals decreased significantly in comparison with the control group (p < 0.01).

On the other hand, quercetin significantly increased oxygen consumption in state 3 as well (p < 0.01). Combination of both drugs (group P+Q)
Table 1. Respiration of rat liver mitochondria after application of paracetamol, quercetin and their combination.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Exp. group (P)</th>
<th>rel. ± %</th>
<th>Exp. group (Q)</th>
<th>rel. ± %</th>
<th>Exp. group (P+Q)</th>
<th>rel. ± %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumption $O_2$ in state 4 (mmol min(^{-1}) kg(_p)^{-1})</td>
<td>11.056 ± 1.09</td>
<td>13.169 ± 1.16</td>
<td>+19.0</td>
<td>19.419 ± 2.91</td>
<td>+75.6**</td>
<td>13.966 ± 1.98</td>
<td>+26.3*</td>
</tr>
<tr>
<td>Consumption $O_2$ in state 3 (mmol min(^{-1}) kg(_p)^{-1})</td>
<td>28.876 ± 3.48</td>
<td>16.668 ± 3.29</td>
<td>-42.3**</td>
<td>40.974 ± 5.68</td>
<td>+41.8**</td>
<td>27.761 ± 2.98</td>
<td>-3.9</td>
</tr>
<tr>
<td>RCR</td>
<td>2.61 ± 0.24</td>
<td>1.27 ± 0.21</td>
<td>-51.5**</td>
<td>2.11 ± 0.21</td>
<td>-19.2</td>
<td>1.99 ± 0.41</td>
<td>-23.8*</td>
</tr>
</tbody>
</table>

\textsuperscript{a} \textit{x} – arithmetic mean, \textit{s} – standard deviation, * – statistical significance \textit{p} < 0.05, ** – statistical significance \textit{p} < 0.01.

Table 2. Activities of the enzymes after application of paracetamol, quercetin and their combination.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Exp. group (P)</th>
<th>rel. ± %</th>
<th>Exp. group (Q)</th>
<th>rel. ± %</th>
<th>Exp. group (P+Q)</th>
<th>rel. ± %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP-\textit{ase} (\textmu{}mol P(_i) min(^{-1}) mg(_p)^{-1})</td>
<td>0.162 ± 0.04</td>
<td>0.229 ± 0.02</td>
<td>+41.3**</td>
<td>0.171 ± 0.03</td>
<td>+5.6</td>
<td>0.162 ± 0.07</td>
<td>0</td>
</tr>
<tr>
<td>Glutathione reductase (kat kg(_p)^{-1})</td>
<td>86.0 ± 7.47</td>
<td>96.767 ± 5.95</td>
<td>+12.5</td>
<td>46.028 ± 15.76</td>
<td>-44.5**</td>
<td>69.083 ± 19.93</td>
<td>-19.7</td>
</tr>
<tr>
<td>Glutathione peroxidase (U mg(^{-1}))</td>
<td>0.433 ± 0.08</td>
<td>0.451 ± 0.05</td>
<td>+4.9</td>
<td>0.539 ± 0.07</td>
<td>+23.3*</td>
<td>0.579 ± 0.09</td>
<td>+32.6*</td>
</tr>
</tbody>
</table>
decreased oxygen consumption only slightly, non-significantly. Respiration control ratio was significantly decreased in paracetamol-treated animals \((p < 0.01)\). Quercetin partially inhibited paracetamol-induced decrease in RCR but the decrease is still significant in comparison with control value \((p < 0.05)\).

As presented in Table 2, paracetamol significantly increased ATP-ase activity when compared with the control value \((p < 0.01)\). After the application of Q or P+Q no significant changes in ATP-ase activity were observed. The activity of glutathione reductase was significantly changed only in quercetin-treated animals \((p < 0.01)\). Paracetamol caused only a slight increase in GR activity. Similarly, combination of both drug produced no significant changes in GR activity. GPx activity was not significantly influenced by paracetamol. On the other hand, quercetin and combination paracetamol+quercetin significantly increased the activity of GPx \((p < 0.05)\).

The cell death and liver injury produced by paracetamol have been studied extensively for more than 20 years. Some studies have implicated a role for mitochondrial damage in the toxic process initiated by paracetamol in hepatocytes (Vendemiale et al., 1996; Knight & Jaeschke, 2002; Zhao & Slattery, 2002). It is suggested that paracetamol may elicit a direct effect on the mitochondrial function before cell injury develops (Nazareth et al., 1991). The present study demonstrates that single-dose of paracetamol in a dose of 500.0 mg kg\(^{-1}\) significantly decreased oxygen consumption in state 3. This finding confirms the works of KATYARE & SAVAR (1989) and Padalko et al. (1996), who documented that toxic doses of paracetamol decreased respiration of liver mitochondria. Burnham & Harm (1991) documented that NAPQI had similar effect on mitochondrial respiration and their results indicated that attack on the respiratory chain by NAPQI causes a disruption of energy homeostasis in paracetamol hepatotoxicity.

It was well documented that NAPQI causes oxidative stress and several compounds with antioxidant properties have been studied to reduce free radical formation and prevent paracetamol hepatotoxicity (Chungoo et al., 1997; Kourounakis et al., 1997; Sumioka et al., 1998). According to this “free radical theory” we studied possible protective effect of quercetin on paracetamol-induced mitochondrial dysfunction. As mentioned above, quercetin is one of the most abundant natural flavonoids. Its antioxidant effect was documented in many in vitro and in vivo experimental studies (Chen et al., 1990). Furthermore, quercetin is known as an excellent metal chelator (Afanasev et al., 1989). Recently, it was confirmed that both antiradical and chelating effects are involved in the protective effect of quercetin (Cheng & Breen, 2000). In the present study, quercetin prevented paracetamol-induced decrease in oxygen consumption in state 3.

Our experiments confirmed the fact that paracetamol in toxic doses exhibits unfavourable effects on the respiration of mitochondria and on the activity of ATP-ase. No significant effects on GPx and GR activity were observed. Respirations in states 3 and 4 were increased after the application of quercetin but finally it had no significant effect on the value of RCR. Activity of ATP-ase in the presence of quercetin was slightly increased. Decrease of the activity was seen in the case of glutathione reductase, in contrast to glutathione peroxidase where increase in activity was observed.

In summary, paracetamol caused significant changes in mitochondrial respiration and the activity of mitochondrial ATP-ase. Quercetin reverted effects of paracetamol probably due to its antioxidant effects. We suggest that this compound offers a potential for ameliorating the hepatotoxicity of paracetamol.

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References


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403