Antioxidant action of novel derivatives of the apple-derived flavonoid phloridzin compared to oestrogen: relevance to potential cardioprotective action

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Novel derivatives of the apple-derived flavonoid phloridzin have been found to be potent antioxidants in vitro [1]. The antioxidant action of oestrogens such as 17β-oestradiol may contribute to their cardioprotective effects [2]. Phloretin the aglycone form of phloridzin has been reported to be oestrogenic [3] and its 3-hydroxy derivative may have enhanced weak oestrogen activity that could enable it to act in a similar cardioprotective manner to the antioxidant anti-oestrogen/weak oestrogen drug tamoxifen [4]. We have compared the potency of phloridzin and its derivatives as inhibitors of liposomal and microsomal lipid peroxidation to that of 17β-oestradiol, and their potential as antioxidant/oestrogenic cardioprotective agents is discussed.

Ox-brain phospholipid was suspended in phosphate buffered saline (140mM NaCl, 2.7mM KCl, 16mM Na2HPO4, 2.9mM KH2PO4) at pH 7.4 at a final concentration of 10mg/ml, followed by sonication. The resulting milky suspension of liposomes was allowed to stand in at 4 °C for 24h prior to use [5]. Rat liver microsomes were prepared from the livers of adult male rats by standard differential-centrifugal techniques as described in [5].

Liposomal or microsomal lipid peroxidation in the presence of Fe(III) and ascorbate was measured by the formation of thiobarbituric acid reactive substances as described previously [5]. The reaction mixture (final volume 1.0ml) contained either liposomes (1.0mg in 0.1ml phosphate buffered saline at pH 7.4), or microsomes (0.25mg of microsomal protein) phosphate buffered saline pH 7.4 (0.5ml) was used for the liposomal assays and 10mM KH2PO4-KOH pH 7.4 for the microsomal assays, 10μl of ethanol or test compound dissolved in the same volume of ethanol was added.

Peroxidation was started by adding aqueous solutions of FeCl3 (0.1ml) and ascorbate (0.1ml) to give final concentrations of 100μM of each. The reactions were started by the addition of the ascorbate and incubations were carried out at 37 °C for 20 min (unless stated otherwise). The reactions were terminated by the addition of 100μl of butylated hydroxytoluene (0.2% w/v) dissolved in ethanol to suppress further peroxidation during the heating stage of the thiobarbituric acid reaction.

The amount of lipid peroxidation was determined by the thiobarbituric acid test [5]. HCl (0.5ml, 25% v/v) was added to each reaction mixture, followed by 0.5ml of thiobarbituric acid solution (1% w/v in 50mM sodium hydroxide) and heating at 80°C for 30 min.

The chromogen was extracted with 2ml of butanol-1-ol and and the A532 of the upper (organic) layer was measured. All results were the mean ± SD of 6 separate experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Systems</th>
<th>IC50 (μM)</th>
<th>IC50 (μM)</th>
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<tbody>
<tr>
<td></td>
<td>Liposomal</td>
<td>Microsomal</td>
<td></td>
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<tr>
<td>17β-Oestradiol</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Phloridzin</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Phloretin</td>
<td>27</td>
<td>12</td>
<td></td>
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<tr>
<td>3-Hydroxyphloridzin</td>
<td>7</td>
<td>17</td>
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<tr>
<td>3-Hydroxyphloretin</td>
<td>3</td>
<td>1.5</td>
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The results in table 1 show that in the liposomal model membrane system, only the hydroxylated phloridzin derivatives, 3-hydroxyphloridzin and 3-hydroxyphloretin were more potent than inhibitors of lipid peroxidation than 17β-oestradiol (by 2-fold and ~5-fold respectively). In the microsomal model membrane system only 3-hydroxyphloretin was more effective than 17β-oestradiol and the extent of enhanced potency increased from ~5-fold to ~9-fold.

Phloretin was ~2-fold less potent than 17β-oestradiol in the liposomal system and was approximately equipotent in the microsomal system, whereas an IC50 value for phloridzin was not reached in either system.

These results suggest that although phloridzin itself was not an effective inhibitor of lipid peroxidation in the systems tested, its aglycone form phloretin was as effective as 17β-oestradiol in the microsomal system and only 2-fold less potent in the liposomal system. This suggests that dietary phloridzin/phloretin may confer similar antioxidant cardioprotective effects to 17β-oestradiol.

3-Hydroxyphloridzin was markedly more potent as an inhibitor of lipid peroxidation than 17β-oestradiol, in both liposomal and microsomal systems. This suggests it may have a potential use as a food additive or therapeutic agent, which could confer useful health benefits by combining potent antioxidant ability with weak oestrogenic properties. The enhanced antioxidant properties (and potentially enhanced weak oestrogenic/antioestrogenic properties) of 3-hydroxyphloridzin compared to phloretin could thus enable it to mimic the action of the cardioprotective antioestrogen drug tamoxifen (and in particular its more potent 4-hydroxy metabolite) [4].

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