Preclinical comparison of bis-diketopiperazine-propane (dexrazoxane) and bis-diketopiperazine-ethane (antimet) on the adriamycin-cardiotoxic effect

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A cardiotoxic effect induced by adriamycin (by repeated i.v. administration to experimental rats in 7-day intervals of administration) begins to be manifested in the ECG record by prolongation of the SαT segment between days 14 and 20, on day 30 it is statistically significant. By means of this index, the known protective effect of dexrazoxane (the preparation Cardioxan) against adriamycin cardiotoxicity has been successfully confirmed in a four-week experiment. A comparative study (using the identical frequency of the dosing scheme and SαT segment as the decisive parameter) has revealed that antimet - as another original substance of the diketopiperazines group - also involves (though less significantly) protective effects against the toxic action of adriamycin.

Key words: Adriamycin, bis-diketopiperazine derivatives, cardiotoxicity.

The cardiotoxicity is an undesirable effect limiting the therapeutic use of cytostatically effective antibiotics of the anthracyclines group, whose classic representative is adriamycin (ADR). The development of this toxic effect proceeds - according to some authors [8] - via a mechanism of the formation of hemiquinone structures with bivalent iron ions (an intervention into ionic balance with functional consequences primarily in the myocardial cells) and via a subsequent release of free oxygen radicals. This assumption opened one of the routes of development, aimed at the substances which would remove the cardiomyopathic effects of anthracyclines [8, 11]. For the performance of the search for them, CZARNECKI [2], SOLDANI [10], JENSEN [8] and DANESI [3] recommend the rat model, in which the decisive index of ADR-cardiotoxicity is the prolongation of the SαT segment in the ECG record. Using this parameter, we attempted to test the possible protective efficacy of bis-diketopiperazine-ethane (antimet, further referred to as BDPE), prepared by the present authors [5] and synthesized by JARY [7], against cardiotoxicity induced by a repeated dose of ADR. Bis-diketopiperazine-propane (dexrazoxane, further referred to as BDPP) [1, 6], already tested in therapeutical practice, was employed for the sake of comparison.

Material and methods

Substances used. Adriamycin: doxorubicin HCl for i.v. administration in the preparation Adriamycin RDF - Adriablastina RDF (Farmitalia Carlo Erba); BDPP: dexrazoxane in the preparation Cardioxan (Eurocetus Holland) was a gift from Medicom International (Brno, Czech Republic); BDPE: antimet synthesized by the laboratory JAKO (Kutná Hora, Czech Republic); Ether pro narcosi (Chirana, Czech Republic).

Experimental animals. Female rats, Wistar Han II (origin: VÚFB, Konárovice, Czech Republic), initial mass 160–170 g.

Experimental scheme. On the basis of preceding experiments comparing the cytostatic effects of BDPE and BDPP in rats [4] and preliminary toxicity pre-experiments (LD) with the combinations ADR - BDPE and ADR - BDPP, a dose of 100 mg/kg of BDPP were employed as a potentially cardioprotective dose in the first experimental stage. In the second stage the BDPE dose was increased to 200 mg/kg.

Both in the first and second stages the animals were divided into groups of 10:

Ist group (controls): in seven-day intervals the first always received a physiological solution of NaCl p.o. of 2 ml/100 g,
after 60 min a physiological solution of NaCl i.v. into the caudal vein (in the same volume units as ADR administered in other groups),

IInd group (adriamycin): a physiological solution of NaCl was administered p.o. in a similar manner as in the IInd group, ADR was administered i.v. (3 mg/kg in 0.2 ml/100 g) after 60 min,

IIId group (dexrazoxane-adriamycin): BDPP was administered p.o. in a methylcellulose suspension of 60 mg/kg, ADR was administered in a similar manner as in the IInd group,

IVth group (antimet-adriamycin): BDPE was administered p.o. in a methylcellulose suspension of 100 mg/kg (similarly as BDPP in the IIIrd group), ADR was administered in a similar manner as in the IInd group.

In the second stage the arrangement was similar to that in the first stage (only BDPE doses were increased to 200 mg/kg) and therefore the dexrazoxane-adriamycin group was not repeated.

On days 1, 14, 20 and 30 in all groups (always in light ether anaesthesia), ECG (a mingograph 7, Siemens ELE-MA, Sweden) was recorded from the leads from the right forelimb and the left hindlimb, five subsequent evolutions were measured, always \( S_{αT} \) in msec (Fig. 1). These intervals of measurement were selected on the basis of pre-experiments in which ECG records were made also on days 3, 8 and 17.

Heart rate (number of pulses/min) and the length of the \( S_{αT} \) segment were evaluated (both in msec and in percents, the control with saline = 100%). The means after adriamycin alone (against the controls with saline) and after protective administration of the substances under study (against the group with adriamycin alone) were statistically (t-test) compared. A value of \( p \) lower than 0.05 represents a statistically significant difference.

The study was terminated on day 30, the animals were killed with ether narcosis, and macroscopical autopsy and histopathological examination of the myocardium (after fixing with paraformaldehyde and staining with hematoxylin-eosin) were performed.

Table 1. Effects of BDPP and BDPE on adriamycin-induced cardiotoxicity in rats: length of the \( S_{αT} \) segment in the ECG record (in msec)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Groups</th>
<th>Days</th>
<th>1</th>
<th>MV ± SD</th>
<th>( p )</th>
<th>14</th>
<th>MV ± SD</th>
<th>( p )</th>
<th>20</th>
<th>MV ± SD</th>
<th>( p )</th>
<th>30</th>
<th>MV ± SD</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>saline</td>
<td>1</td>
<td>18.60 ± 2.92</td>
<td>18.70 ± 3.31</td>
<td>0.45</td>
<td>19.91 ± 4.20</td>
<td>20.05 ± 4.26</td>
<td>0.0023</td>
<td>18.70 ± 3.31</td>
<td>20.05 ± 4.26</td>
<td>0.0023</td>
<td>19.91 ± 4.20</td>
<td>20.05 ± 4.26</td>
<td>0.0023</td>
</tr>
<tr>
<td></td>
<td>ADR</td>
<td>1</td>
<td>17.81 ± 1.51</td>
<td>20.45 ± 2.98</td>
<td>0.229</td>
<td>23.00 ± 3.25</td>
<td>28.15 ± 3.08</td>
<td>0.0023</td>
<td>20.45 ± 2.98</td>
<td>28.15 ± 3.08</td>
<td>0.0023</td>
<td>23.00 ± 3.25</td>
<td>28.15 ± 3.08</td>
<td>0.0023</td>
</tr>
<tr>
<td></td>
<td>ADR +BDPP</td>
<td>1</td>
<td>18.65 ± 1.65</td>
<td>22.50 ± 3.94</td>
<td>0.215</td>
<td>20.28 ± 3.11</td>
<td>22.56 ± 4.15</td>
<td>0.0037</td>
<td>22.50 ± 3.94</td>
<td>22.56 ± 4.15</td>
<td>0.0037</td>
<td>20.28 ± 3.11</td>
<td>22.56 ± 4.15</td>
<td>0.0037</td>
</tr>
<tr>
<td></td>
<td>ADR +BDPE (100 mg)</td>
<td>1</td>
<td>16.60 ± 1.13</td>
<td>20.35 ± 3.47</td>
<td>0.945</td>
<td>21.05 ± 4.42</td>
<td>26.57 ± 4.27</td>
<td>0.279</td>
<td>20.35 ± 3.47</td>
<td>26.57 ± 4.27</td>
<td>0.279</td>
<td>21.05 ± 4.42</td>
<td>26.57 ± 4.27</td>
<td>0.279</td>
</tr>
<tr>
<td>2</td>
<td>saline</td>
<td>1</td>
<td>20.45 ± 4.63</td>
<td>20.55 ± 4.49</td>
<td>0.45</td>
<td>20.90 ± 4.11</td>
<td>20.55 ± 4.79</td>
<td>0.0048</td>
<td>20.55 ± 4.49</td>
<td>20.55 ± 4.79</td>
<td>0.0048</td>
<td>20.90 ± 4.11</td>
<td>20.55 ± 4.79</td>
<td>0.0048</td>
</tr>
<tr>
<td></td>
<td>ADR</td>
<td>1</td>
<td>19.15 ± 2.12</td>
<td>21.05 ± 2.97</td>
<td>0.77</td>
<td>23.90 ± 4.27</td>
<td>27.83 ± 5.01</td>
<td>0.0126</td>
<td>21.05 ± 2.97</td>
<td>27.83 ± 5.01</td>
<td>0.0126</td>
<td>23.90 ± 4.27</td>
<td>27.83 ± 5.01</td>
<td>0.0126</td>
</tr>
<tr>
<td></td>
<td>ADR +BDPE (200 mg)</td>
<td>1</td>
<td>18.95 ± 2.87</td>
<td>20.05 ± 2.88</td>
<td>0.45</td>
<td>20.06 ± 3.31</td>
<td>25.25 ± 4.51</td>
<td>0.126</td>
<td>20.05 ± 2.88</td>
<td>25.25 ± 4.51</td>
<td>0.126</td>
<td>20.06 ± 3.31</td>
<td>25.25 ± 4.51</td>
<td>0.126</td>
</tr>
</tbody>
</table>

Statistical significance of \( p \): in the ADR group in comparison with the saline group; in the ADR+BDPP and ADR+BDPE groups in comparison with the ADR group.
Results

It follows from the results of both experimental stages that the ECG $S_{\alpha} T$ segment (Fig. 2, Table 1) in the adriamycin group shows a tendency to be prolonged from day 14, and on day 30 it is prolonged in a statistically highly significant manner. In the combination dextrazoxane-adriamycin the prolongation of this ECG segment is also perceptible but in comparison with adriamycin alone this prolongation on day 20 shows a tendency to shortening and on day 30 it is statistically significantly shorter. A tendency to shorten the adriamycin prolongation of $S_{\alpha} T$ is evident also in the combination adriamycin-antimet.

Heart rate (Table 2) fluctuates in the whole course of the study in all groups under study in a mutually similar manner; as an index of early manifestations of adriamycin intoxication it is thus insignificant.

The histopathological finding in the cardiac muscle (separately the left heart and the right heart) was normal in all groups (including the adriamycin-alone one), only dilatation of the ventricles was perceptible in all groups influenced with adriamycin (including the use of combinations with bis-diketopiperazine derivatives).

Discussion

The use of bis-diketopiperazine substances as protectives against anthracycline cardiotoxicity is considered to be justified [9] because of their hydrolytic biotransformation to metabolites which are able to bind bivalent iron ions, thus acting as preventive scavengers. Whether this cardioprotective mechanism takes place via the chelating capability to iron ions may be doubted, but the share of the scavenger effect in the final action is probable (a cardioprotective effect against adriamycin intoxication is also shown by coenzyme Q10 and tocopherol [10]). The bis-diketopiperazine group includes both dextrazoxane and antimet. The difference in the pharmacokinetics of the two substances, i.e. increased cumulation of BDPP in the organism to the differences in the pharmacokinetics of the two substances, i.e. increased cumulation of BDPP in the organism.

In other experiments [4], the present authors have demonstrated - similarly as in the cardioprotective effect - a higher cytostatic effect of BDPP in comparison with BDPE in experimentally induced Yoshida carcinoma.

A negative histopathological finding in the myocardium even in the experiments with ADR alone does not contradict the data from the literature (e.g. [3]), which report degenerative changes in the myocardium only after six weeks lasting adriamycin chronic intoxication of rats.

The authors wish to thank Prof. MUDr. VL. HEROUT, DrSc., and Ms. E. HOLECKOVA for histopathological analysis.

Table 2. Effects of BDPP and BDPE on adriamycin-induced cardiotoxicity in rats: heart rate

<table>
<thead>
<tr>
<th>Stage</th>
<th>Groups</th>
<th>Days</th>
<th>MV ± SD</th>
<th>MV ± SD</th>
<th>MV ± SD</th>
<th>MV ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>saline</td>
<td>1</td>
<td>424 ± 69</td>
<td>418 ± 43</td>
<td>412 ± 73</td>
<td>397 ± 49</td>
</tr>
<tr>
<td></td>
<td>ADR</td>
<td>1</td>
<td>437 ± 64</td>
<td>431 ± 49</td>
<td>433 ± 64</td>
<td>392 ± 80</td>
</tr>
<tr>
<td></td>
<td>ADR + BDPP</td>
<td>1</td>
<td>430 ± 55</td>
<td>429 ± 41</td>
<td>404 ± 48</td>
<td>415 ± 47</td>
</tr>
<tr>
<td></td>
<td>ADR + BDPE</td>
<td>(100 mg)</td>
<td>448 ± 51</td>
<td>430 ± 54</td>
<td>437 ± 65</td>
<td>405 ± 70</td>
</tr>
<tr>
<td>2</td>
<td>saline</td>
<td>1</td>
<td>419 ± 47</td>
<td>394 ± 53</td>
<td>390 ± 48</td>
<td>388 ± 56</td>
</tr>
<tr>
<td></td>
<td>ADR</td>
<td>1</td>
<td>432 ± 70</td>
<td>420 ± 57</td>
<td>414 ± 45</td>
<td>422 ± 61</td>
</tr>
<tr>
<td></td>
<td>ADR + BDPE</td>
<td>(200 mg)</td>
<td>430 ± 52</td>
<td>412 ± 45</td>
<td>451 ± 55</td>
<td>465 ± 54</td>
</tr>
</tbody>
</table>

$p$ in all cases higher than 0.05.

References