Doxorubicin
probucol
verapamil
Doxorubicin  probucol  verapamil

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**Doxorubicin**

**probucol**  
**verapamil**

Doxorubicin`¿¡ÀÇÇØÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾ï¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö, ¾øÇÏ¿©ÀÎ¿µ¿½Ã¿­µî¸Å¿ìÈ¿°úÀûÀÎÄ¡·áÁ¦ÇÑÀ»¹Þ´Â´Ù. Doxorubicin`¿¡ÀÇÇØÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾ï¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö, Ca²⁺ÀÇÀåµ¶¼ºÀÇ¿øÀÎÀ¸·Î´Â»ê¼ÒÀÚÀ¯¶óµðÄ®ÀÇÇü¼ºÈ¿¼ÒȰ¼ºÀÇ°¨¼ÒÀÇ, Ca²⁺À̵¿ÀÇº¯Çü¼¼Æ÷ÀÚ¸ê»çµî¸Å¿ìÀëµÎµÇ°íÀÖ´Ù.

º»¿¬±¸¿¡¼­´ÂÇ×»êÈ­Ư¼ºÀ»°¡Áøprobucol°úÄ®½·Åë·ÎÂ÷´ÜÁ¦verapamilÀÌÀå±â°£Åõ¿©ÇÑdoxorubicin¿¡ÀÇÇØÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

Sprague-Dawley`°è¼öÄÆÈòÁ㸦´ë»óÀ¸·Îdoxorubicin°Üµ¶Åõ¿©, doxorubicin°úprobucolµ¿½ÃÅõ¿©(probucolÄ¡·á±º), doxorubicin°úverapamilµ¿½Ã(VERAPAMILÀÇÅõ¿©)ÀÇÇØ°£ÁúÁ¤µµ°¡³ª´©¾îºñ±³ÇÑ°á°ú3ÃþÀ¸·Î³ª´©¾îºñ±³ÇÑ°æ¿ì¿Íºñ½ÁÇÑ¾ç»óÀ»º¸¿´À¸¸ç, doxorubicin¿¡ÀÇÇØÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±ÙÀ»3ÁÖ¿Í4, 6, 8, 10ÁÖ¿¡¾à¹°Åõ¿©±â°£¿¡µû¶ó½É±ÙÀ»3ÁÖ¿Í4, 6, 8, 10ÁÖ¿¡¾à°£°ú¿°»öµÈ°¨¼ÒÇÏ´Â°æÇâÀ»º¸¿´À¸³ªºñÇÏ¿©ºñÇÏ¿©ÀüüHsp70, hsp27, H&E ¿°»ö°úprotein gene product (PGP) 9.5, caspase-3, hsp70, hsp25`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

H&E ¿°»ö°úprotein gene product (PGP) 9.5, caspase-3, hsp70, hsp25`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

PGP 9.5 ¿°»ö°úprotein gene product (PGP) 9.5, caspase-3, hsp70, hsp25`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

Caspase-3`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

Hsp70°úprotein gene product (PGP) 9.5, caspase-3, hsp70, hsp25`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö.

Hsp25`ÀÇÀ¯¹ßµÇ´Â½ÉÀåÀÇ½Å°æµ¶¼º°ú½É±Ùº´Áõ¿¡¾î¶²¿µÇâÀ»¹ÌÄ¡´ÂÁö

Doxorubicin, probucol, verapamil

<Insert figure here>

I. Introduction

Doxorubicin, probucol, and verapamil have been shown to have synergistic anti-tumor effects. Doxorubicin, when administered at a dose of 500 mg/m² [1, 2]. Doxorubicin in combination with 4- to 20-fold lower doses of verapamil has been shown to enhance the antitumor activity of doxorubicin [3, 4]. Doxorubicin and probucol have been shown to have synergistic effects in inhibiting the growth of cancer cells [5]. Doxorubicin and Ca²⁺-ATPase inhibition has been shown to be synergistic [6]. Doxorubicin in combination with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [7]. Doxorubicin in combination with adenylate cyclase and Na⁺-K⁺-ATPase inhibition has been shown to be synergistic [8]. Doxorubicin in combination with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [9]. Doxorubicin in combination with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [10]. The combination of doxorubicin with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [11]. The combination of doxorubicin with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [12]. The combination of doxorubicin with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [13]. The combination of doxorubicin with Ca²⁺-ATPase inhibition has been shown to enhance the antitumor activity of doxorubicin [14].
iodobenzylguanidine, doxorubicin, probucol. 22. Doxorubicin,
sulphydryl. 13, 16, 17, 19, 20, 21. Doxorubicin, sulphydryl,
doxorubicin, probucol. 24, 36. Probucol, doxorubicin,
sulphydryl, doxorubicin, probucol, probucol.

Doxorubicin, doxorubicin, probucol, DNA. 11, 26, 27.
doxorubicin, Ca, doxorubicin, Ca. 28, 29. Iodine 123-labeled meta-
iodobenzylguanidine, doxorubicin, posterior limb. 30, 31, 32, doxorubicin,
doxorubicin, posterior limb. 33, doxorubicin,
doxorubicin, probucol. 34.

Probucol, probucol, doxorubicin, probucol, probucol.

16, 17, 19, 20, 21, 24, 36. Probucol, doxorubicin, probucol,
Verapamil, doxorubicin, caspase-9, cytochrome c, doxorubicin, verapamil, caspase-9, doxorubicin, verapamil, doxorubicin, 37. verapamil, doxorubicin, 38. doxorubicin, procaspase-3, doxorubicin, P,-verapamil, doxorubicin, 39. verapamil, doxorubicin, 40. verapamil, doxorubicin, 41. P,-verapamil, doxorubicin, 42. P,-verapamil, doxorubicin.

Doxorubicin, caspase, procaspase, caspase, procaspase-8, caspase-3, cytochrome c, procaspase-3, apoptosome, caspase-3, caspase-3, 46.

Heat shock protein (hsp), hsp27, hsp70, cytochrome c, apoptosome, cytochrome c, procaspase-3, apoptosome, caspase-3, 52.
II. MATERIALS AND METHODS

1. Materials

1.1. Chemicals

(1) Doxorubicin RDF (Pharmacia and Upjohn, Milan, Italy), probucol (SIGMA, St. Louis, MO, USA), verapamil (Keun Wha, Seoul, Korea).

(2) PGP 9.5 (Ultraclone, Cambridge, UK), caspase-3 (R&D Systems, Minneapolis, MN, USA), hsp70, hsp27 (Stressgen Biotechnologies Corporation, Victoria, Canada).

1.2. Animals

Sprague-Dawley rats weighing 220±26 g were obtained from the animal facility of the Institute of Medical Science, The University of Tokyo. The animals were divided into four groups: control (vehicle), doxorubicin (2 mg/kg), probucol (16 mg/kg), and doxorubicin (2 mg/kg) plus verapamil (2 mg/kg).

2. Methods

2.1. Animals

Doxorubicin was administered i.p. at a dose of 50 mg/kg, doxorubicin RDF at 25 mg/ml, probucol at 99% purity at 2 mg/ml, and verapamil at 32 mg/ml. The animals were divided into five groups: control (vehicle), doxorubicin (2 mg/kg), probucol (16 mg/kg), doxorubicin (2 mg/kg) plus verapamil (2 mg/kg), and doxorubicin (2 mg/kg) plus verapamil (2 mg/kg).
Doxorubicin, probucol, verapamil. Doxorubicin, probucol, verapamil, doxorubicin, probucol, verapamil, doxorubicin, probucol, verapamil. Doxorubicin, probucol, verapamil, doxorubicin, probucol, verapamil.

<table>
<thead>
<tr>
<th>Group</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>DXR</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

DXR: doxorubicin, PROB: probucol, Ve: verapamil.

1. **Hematoxylin & Eosin (H&E)**.

2. **PGP 9.5**

(1) 4 μm sections

(2) PGP 9.5 (rabbit, polyclonal)
biotinylated anti-rabbit antibody, peroxidase, streptavidin, biotin.

(1) Caspase-3

- 0.1M citrate buffer (pH 6.0), 20 min.
- 20% 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO, Glostrup, Denmark).
- H&E stain.

H&E

(2) Hsp70

- 0.1M citrate buffer (pH 6.0), 20 min.
- 20% 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO, Glostrup, Denmark).
- Harris’ hematoxylin.

H&E

(3) Hsp25

- 0.1M citrate buffer (pH 6.0), 20 min.
- 20% 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO, Glostrup, Denmark).
- Harris’ hematoxylin.

H&E

(3) Hsp25

- 0.1M citrate buffer (pH 6.0), 20 min.
- 20% 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO, Glostrup, Denmark).
- Harris’ hematoxylin.

H&E

°øÅë°úÁ¤
Optical density(x,y) = -log((Intensity(x,y) – Black) / (Incident – Black))
Intensity(x,y) : 800 x 800 (x,y)
Black : 0 x 0 (x,y)
Incident : 400 µm x 400 µm

Myocardial layers
- Subendocardium
- Mid-myocardium
- Subepicardium

1. Myocardial layers: Subendocardium, Mid-myocardium, Subepicardium
III. 

1. 

H&E stained slides revealed the cellularity of the inflammatory reaction, doxorubicin (DXR), verapamil (Ve), and probucol (PROB) treated groups. The slides were evaluated for the number of cells and vacuolization. The results are shown in Table 1.

<table>
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<tr>
<th>Group</th>
<th>4 weeks</th>
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<th>8 weeks</th>
<th>10 weeks</th>
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<tr>
<td>Cellularity</td>
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<tr>
<td>Control</td>
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</tr>
<tr>
<td>DXR</td>
<td>0.8</td>
<td>1.2</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>0.1</td>
<td>0.17</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>0</td>
<td>0.5</td>
<td>0.6</td>
<td>0.13</td>
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<tr>
<td>Vacuolization</td>
<td></td>
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<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DXR</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>0</td>
<td>0</td>
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1. Data are expressed as mean ± standard deviation.

DXR: doxorubicin, PROB: probucol, Ve: verapamil.
2. Doxorubicin, verapamil, and probucol were used in combination with PGP 9.5 to study the effects on cardiac tissue. (A) and (B) show the effects of doxorubicin alone and doxorubicin with probucol, respectively. (C) and (D) depict the effects of doxorubicin with verapamil and doxorubicin, verapamil, and probucol, respectively.

Doxorubicin and verapamil were used in combination with PGP 9.5 to study the effects on cardiac tissue. (A) and (B) show the effects of doxorubicin alone and doxorubicin with probucol, respectively. (C) and (D) depict the effects of doxorubicin with verapamil and doxorubicin, verapamil, and probucol, respectively.

Doxorubicin and verapamil were used in combination with PGP 9.5 to study the effects on cardiac tissue. (A) and (B) show the effects of doxorubicin alone and doxorubicin with probucol, respectively. (C) and (D) depict the effects of doxorubicin with verapamil and doxorubicin, verapamil, and probucol, respectively.
3. 组别 4周 6周 8周 10周

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<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
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<tbody>
<tr>
<td>Control</td>
<td>442.00±103.02</td>
<td>448.60±100.74</td>
<td>439.60±94.34</td>
<td>435.80±93.16</td>
</tr>
<tr>
<td>DXR</td>
<td>361.40±109.08</td>
<td>242.60±76.46</td>
<td>142.20±41.95*</td>
<td>96.00±23.62*</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>422.00±94.67</td>
<td>389.33±85.03</td>
<td>341.40±64.77</td>
<td>324.20±64.04</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>354.20±110.83</td>
<td>277.67±79.99</td>
<td>174.80±36.02*</td>
<td>115.75±34.75*</td>
</tr>
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DXR: doxorubicin, PROB: probucol, Ve: verapamil.

* 4° 6° 4° 6° 4° 6° 4° 6° 4° 6° 4° 6° p<0.05, # 6° 4° 6° 4° 6° 4° 6° 4° 6° 4° 6° 4° 6° p<0.05.
Figure 3. PGP 9.5 (A-H) immunoreactivity in the myocardium. PGP 9.5+ indicated moderate and dense punctate staining (A). PGP 9.5- showed minimal immunoreactivity (B). Doxorubicin (10 μM) treatment increased PGP 9.5+ staining (C). Probenecid (10 μM) reduced PGP 9.5+ staining (D). Doxorubicin (10 μM) treatment increased PGP 9.5- staining (E). Doxorubicin (10 μM) treatment increased PGP 9.5+ staining (F). Doxorubicin (10 μM) treatment increased PGP 9.5- staining (G). Doxorubicin (10 μM) treatment increased PGP 9.5+ staining (H).
Åõ¿©±â°£ doxorubicin + verapamil 10, 4, 6, 8, 10, doxorubicin + probucol 10, 4, # doxorubicin p<0.05, * doxorubicin + probucol p<0.05.

Mann-Whitney U test] PGP 9.5, doxorubicin 10, 4, 6, 8, doxorubicin + verapamil 10, 4, 6, 8, doxorubicin + probucol 10, 4, 6, doxorubicin + verapamil 10, 4, 6, doxorubicin + probucol 10, 4, 6, # doxorubicin p<0.05.
5. PGP 9.5 doxorubicin verapamil doxorubicin + probucol doxorubicin + verapamil. Doxorubicin vs control: $p<0.05$. * $p<0.05$, ‡ doxorubicin vs probucol. p<0.05.

3. Caspase-3

6. Caspase-3 expression in the heart. Caspase-3 expression was assessed in the heart sections from different groups. Caspase-3 expression was significantly increased in the doxorubicin group compared to the control group. A: control, B: doxorubicin, C: doxorubicin + probucol, D: doxorubicin + verapamil.

5. Table showing caspase-3 expression in different groups over time.

<table>
<thead>
<tr>
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<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.20±5.40</td>
<td>40.20±7.46</td>
<td>43.40±7.13</td>
<td>44.80±7.09</td>
</tr>
<tr>
<td>DXR</td>
<td>49.60±5.59</td>
<td>55.60±6.54</td>
<td>52.20±7.43</td>
<td>64.25±13.07</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>42.80±5.89</td>
<td>46.83±5.42</td>
<td>42.40±9.66</td>
<td>43.40±6.54</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>48.20±7.22</td>
<td>61.17±9.28</td>
<td>49.40±7.77</td>
<td>53.50±8.81</td>
</tr>
</tbody>
</table>

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DXR: doxorubicin, PROB: probucol, Ve: verapamil.
### 7. Caspase-3

Caspase-3 activity was significantly increased in doxorubicin and doxorubicin + probucol treated groups compared to the control group. Doxorubicin + verapamil and doxorubicin + probucol groups showed a significant decrease in caspase-3 activity compared to doxorubicin group. *p<0.05, † doxorubicin + probucol compared to doxorubicin.

**4. Hsp70**

Hsp70 expression was significantly increased in doxorubicin and doxorubicin + probucol treated groups compared to the control group. Doxorubicin + verapamil and doxorubicin + probucol groups showed a significant decrease in Hsp70 expression compared to doxorubicin group. *p<0.05, † doxorubicin + probucol compared to doxorubicin.

### Table 1: Hsp70 Levels in Different Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.46±0.25</td>
<td>2.18±0.21</td>
<td>2.07±0.36</td>
<td>2.17±0.33</td>
</tr>
<tr>
<td>DXR</td>
<td>2.37±0.24</td>
<td>2.20±0.11</td>
<td>2.34±0.33</td>
<td>2.08±0.38</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>2.42±0.26</td>
<td>2.13±0.20</td>
<td>2.43±0.38</td>
<td>1.93±0.45</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>2.29±0.39</td>
<td>2.11±0.26</td>
<td>2.26±0.22</td>
<td>1.86±0.37</td>
</tr>
</tbody>
</table>

1. *p<0.05, † doxorubicin + probucol compared to doxorubicin.

DXR: doxorubicin, PROB: probucol, Ve: verapamil.
5. Hsp25

Hsp25 expression was examined after treatment with doxorubicin alone or in combination with probucol or verapamil. The results showed that doxorubicin treatment alone led to a significant increase in Hsp25 expression compared to untreated controls (Fig. 9).

Hsp25 expression was examined in combination with doxorubicin and probucol to determine if the protective effect of probucol could be enhanced. The results showed that probucol treatment alone had a protective effect on Hsp25 expression, but when combined with doxorubicin, the protective effect was even more pronounced (Fig. 7).
9. Hsp25

Hsp25

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Hsp25

9.

Hsp25

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hsp70

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A: doxorubicin ➔ , B: doxorubicin ➔ ➔ 

C: doxorubicin ➔ probucol ➔ ➔ ➔ 

D: doxorubicin ➔ verapamil ➔ ➔ ➔ .

7. ¾à¹° Åõ ¿©±º ➔ hsp25

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hsp25

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DXR: doxorubicin, PROB: probucol, Ve: verapamil.

<table>
<thead>
<tr>
<th>Group</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.50±0.29</td>
<td>1.48±0.30</td>
<td>1.41±0.27</td>
<td>1.44±0.32</td>
</tr>
<tr>
<td>DXR</td>
<td>1.08±0.34</td>
<td>0.98±0.27</td>
<td>1.22±0.27</td>
<td>1.41±0.26</td>
</tr>
<tr>
<td>DXR + PROB</td>
<td>1.51±0.38</td>
<td>1.24±0.26</td>
<td>1.26±0.32</td>
<td>1.26±0.23</td>
</tr>
<tr>
<td>DXR + Ve</td>
<td>1.57±0.33</td>
<td>1.18±0.25</td>
<td>1.11±0.20</td>
<td>1.38±0.20</td>
</tr>
</tbody>
</table>

1 ± Ç¥ÁØÆíÂ÷·Î ³ªÅ¸³¿.

DXR: doxorubicin, PROB: probucol, Ve: verapamil.
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Doxorubicin is commonly used in the treatment of various types of cancer due to its ability to inhibit DNA polymerase activity, causing DNA replication errors and cell death. Probucol, another antioxidant, has been shown to enhance the effects of doxorubicin by protecting cellular membranes from oxidative damage. 33

In a study by Jeon et al., 21 doxorubicin was found to be significantly more effective in inhibiting caspase-3 activity than probucol and verapamil. However, probucol was observed to reduce doxorubicin-induced toxicity in cardiac cells. 8

Doxorubicin in combination with probucol was investigated for its effects on purified cardiac myocytes. 33 Purkinje fibers were also found to be more sensitive to doxorubicin compared to cardiac cells, as reported in a study by 21. Doxorubicin 10 ultimately showed greater efficacy in inhibiting caspase-3 activity than probucol and verapamil. 5

Similarly, doxorubicin 21 was found to be more effective in inhibiting caspase-3 activity than probucol and verapamil. 6 The combination of doxorubicin and probucol was found to reduce doxorubicin-induced toxicity in cardiac cells. 21
1. Booser DJ, Hortobagyi GN. Anthracycline antibiotics in cancer therapy. Focus on drug

\section*{References}

1. Booser DJ, Hortobagyi GN. Anthracycline antibiotics in cancer therapy. Focus on drug
31. Comeresti CR, Peden WM, Davidson TJ, Warner GL, Hirth RS, Frantz JD. BR96-


**Abstract**

**Effect of probucol and verapamil on injury of myocardium and nerve fibers in rat heart induced by doxorubicin**

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(Directed by Professor Woo Ick Yang)

Doxorubicin is considered to be one of the most effective drugs to treat a variety of human cancers. However, a dose-dependent cardiotoxicity limits its clinical usefulness. A number of mechanisms have been proposed to explain the development of doxorubicin induced cardiotoxicity, including oxygen free radical formation, reduction in myocardial antioxidant enzyme activities, altered Ca\(^{2+}\) transport, and apoptosis.

The present study aimed to evaluate the effect of probucol and verapamil on the cardiac neurotoxicity and cardiomyopathy induced by long-term use of doxorubicin and to elucidate the effect on expression of caspase-3, a key effector protein of the apoptotic machinery, and heat shock protein (hsp)70 and hsp27, an inhibitor of apoptosis.

The Sprague-Dawley male rats were grouped as control, doxorubicin (doxorubicin group), doxorubicin with probucol (probucol treated group), and doxorubicin with verapamil (verapamil treated group). The rats were treated for 4, 6, 8, 10 weeks. Doxorubicin and probucol were administrated intraperitoneally once a week and verapamil was administrated by the oral route three times per week through tube. The H&E stain and immunohistochemical stain for protein gene product (PGP) 9.5, caspase-3, hsp70, and hsp25 were performed using tissue obtained 2 weeks after the treatment. The results were as follows. The degree of interstitial inflammatory cell infiltration on H&E stain was severe in doxorubicin group, and milder in verapamil treated group and mildest in probucol treated group. On immunohistochemical stain for PGP 9.5, the number of stained cardiac nerve fibers were severely reduced in doxorubicin group and verapamil treated group. The reduction of nerve fibers in probucol treated group was milder than the other treatment group. There was a negative correlation between the treatment duration and stained nerve fibers in all treatment group. The comparison divided into three layers of ventricular wall was similar to that of whole myocardium. The diminished number of stained nerve fibers of subendocardium was more pronounced than that of subepicardium according to treatment duration in doxorubicin group.
and verapamil treated group. The number of positive cells on immunohistochemical stain for caspase-3 was increased in doxorubicin group and verapamil treated group than control group and probucol treated group. In probucol treated group, the number was not significantly different to control group. The optical density for hsp70 immunohistochemical stain was decreased on 6 and 10 weeks in treatment groups without significant difference to control group. In doxorubicin group, the optical density for hsp25 immunohistochemical stain was somewhat decreased on 4 and 6 weeks without significant difference to control group.

These results show that the reduction of the cardical nerve fibers was proportionated to cumulative dose of doxorubicin. The finding of increased expression of activated caspase-3 in doxorubicin treatment suggest that the apoptosis partly contribute to doxorubicin-induced myocyte damage. It was noted that verapamil had no effect on inhibition of doxorubicin-induced cardiac neurotoxicity, expression of caspase-3, hsp70, and hsp25 on 2 weeks after treatment. Although there was no effect on expression of hsp70 and hsp25, it was suggested that the probucol partly contributed to inhibition of doxorubicin-induced cardiac neurotoxicity and cardiomyopathy and partly involved to inhibition of expression of caspase-3 with partial effect on inhibition of apoptosis. It is thought that development of drugs to prevent doxorubicin-induced cardiomyopathy and cardiac neurotoxicity and research about medication method and dosage should be continued.

Key Words: doxorubicin, probucol, verapamil, cardiomyopathy, protein gene product 9.5, caspase-3, heat shock protein, rat