

Doxorubicin

probucol verapamil

Doxorubicin

probucol verapamil

2002 12

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가

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	1
I.	3
II.	6
1.	6
가.	6
.	6
2.	6
가.	6
.	7
.	7
(1)	7
(2)	7
(가) PGP 9.5	7
() Caspase-3	8
() Hsp70	8
() Hsp25	8
(3)	8
.	8
.	10
III.	10
1.	10
2. PGP 9.5	11
3. Caspase-3	15
4. Hsp70	17
5. Hsp25	18
IV.	20
V.	22
	23
	29

1.	9
2. 6	11
3. PGP 9.5	13
4.	PGP 9.5	14
5.	PGP 9.5	15
6. Caspase-3	16
7.	caspase-3	17
8. Hsp70	18
9. Hsp25	19

1.	7
2.	10
3.	PGP 9.5	12
4.	PGP 9.5	12
5.	caspase-3	16
6.	hsp70	17
7.	hsp25	19

Doxorubicin

probucol verapamil

Doxorubicin

. Doxorubicin

Ca²⁺

가 probucol

verapamil

doxorubicin

caspase-3

heat shock protein (hsp) 70

hsp27

Sprague-Dawley

doxorubicin

, doxorubicin

probucol (probucol

), doxorubicin verapamil

(verapamil

) 4, 6, 8, 10

. Doxorubicin probucol

, verapamil

2

H&E

protein gene product (PGP) 9.5, caspase-3, hsp70, hsp25

H&E

doxorubicin

가

, verapamil

, probucol

가

PGP 9.5

doxorubicin

verapamil

가

, probucol

가

3

3

, doxorubicin

verapamil

가

. Caspase-3

doxorubicin

verapamil

probucol

. Probucol

Hsp70

4

8

6

10

. Hsp25

doxorubicin

4

6

doxorubicin
 , doxorubicin
 가 doxorubicin
 .
 doxorubicin 2 가
 , hsp70 hsp25 가 , caspase-3
 probucol doxorubicin verapamil
 , hsp70 hsp25
 caspase-3
 . doxorubicin
 가 .

: doxorubicin, probucol, verapamil, , protein gene product 9.5, caspase-3,
 heat shock protein,

¹³, doxorubicin
¹⁶, sulfhydryl
¹⁷,
¹⁸, 가
¹⁹,
^{20, 21} 가
²². Doxorubicin
²³.
²⁴.
²⁵. Doxorubicin
^{26, 27}.
²⁸,
²⁹. Iodine 123-labeled meta-
³⁰.
³¹,
³², doxorubicin
³³.
³⁴.
³⁵. Probucol
^{24, 36}. Probucol
²⁴.
²².

Verapamil
, doxorubicin

verapamil
doxorubicin
³⁷ . verapamil doxorubicin
³⁸ , doxorubicin
doxorubicin 가 ³⁹ ,

verapamil
doxorubicin
⁴⁰ . verapamil doxorubicin 가 ⁴¹ ,
P- doxorubicin 가
⁴² ,

protein gene product (PGP) 9.5
, ⁴³ .

Doxorubicin 가
^{20, 21} , caspase

Caspase procaspase ,
. Caspase
가 ⁴⁴ , cytochrome *c* ⁴⁵ .
caspase-8 caspase-3 , cytochrome *c*

apoptosome
caspase-9 caspase-3 , caspase-3
⁴⁶ .

Heat shock protein (hsp) , hsp
가 ⁴⁷ .
⁴⁸ . Hsp

hsp70
cytochrome *c* apoptosome ^{49, 50} . Hsp27
cytochrome *c* ⁵¹ cytochrome *c* procaspase-3
apoptosome caspase-3 ⁵² .
doxorubicin

doxorubicin ,

가 ,
 probucol verapamil doxorubicin
 ,
 caspase-3 ,
 hsp70 hsp27 .

II.

1.

가.

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

(2) PGP 9.5 (Ultracelone, Cambridge, UK), caspase-3 (R&D Systems, Minneapolis, MN, USA), hsp70, hsp25 (Stressgen Biotechnologies Corporation, Victoria, Canada) .

Sprague-Dawley (220±26 g) 1 22 ,
 50%

2.

가.

Doxorubicin 50 mg doxorubicin 25
 ml 가 2 mg/ml 100 g
 0.1 ml , probucol 99% 5 ml 160 mg probucol
 32 mg/ml 100 g 0.05 ml , verapamil
 10 ml verapamil 10 mg 1 mg/ml
 100 g 0.1 ml .
 , doxorubicin 2 mg/kg doxorubicin
 , doxorubicin probucol (probucol
) 16 mg/kg probucol 2 mg/kg doxorubicin , doxorubicin
 verapamil (verapamil) 1 mg/kg verapamil 2 mg/kg
 doxorubicin ,

Doxorubicin, probucol, , 4 ,
 6 , 8 , 10 , verapamil
 . Probucol, verapamil,
 doxorubicin 1 .
 2 4
 가 doxorubicin 6, 8, 10 1, 2, 4 가
 , probucol 0, 2, 3 가 , verapamil
 0, 2, 4 가 (1).

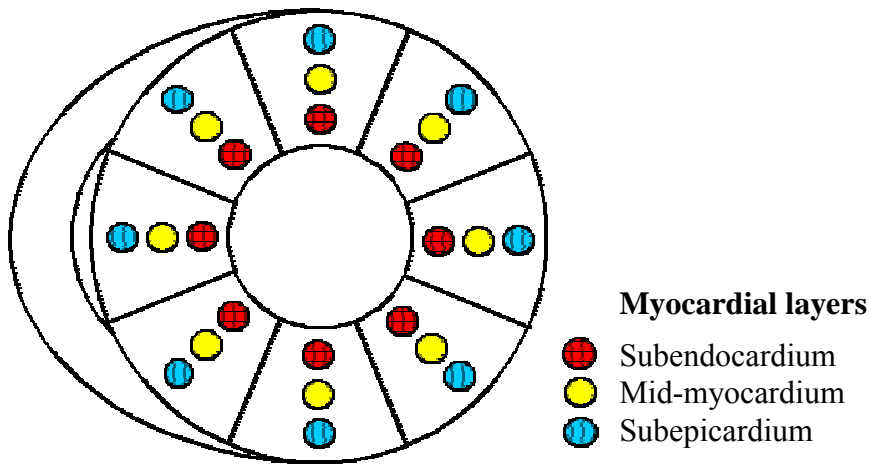
1.

Group	4 weeks	6 weeks	8 weeks	10 weeks
Control	5	5	5	5
DXR	5	5	5	4
DXR + PROB	5	6	5	5
DXR + Ve	5	6	5	4

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

10%
 4 μm
 Hematoxylin & Eosin (H&E)

(1)
 10% 4 μm
 poly-L-lysine 60°C 2
 . Xylene 10 2 가 graded alcohol
 , 3% 10 .
 (2)
 (가) PGP 9.5
 5 1:1200 PGP 9.5 (rabbit,
 polyclonal) 4°C
 Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA)



1. 24 8

PGP 9.5 :
400 10x5 PGP 9.5

Caspase-3 :
200 10x10

Hsp70 hsp25 : (SprintScan 35 Plus,
Polaroid corporation, Cambridge, MA, USA) small computer system interface
(SCSI)

PolaColor Insight 3.5 (Polaroid corporation,
Cambridge, MA, USA) 2700 DPI

1:1 Tagged-Image File Format (TIFF)

Image Pro Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA)
(optical density)

$$\text{Optical density}(x,y) = -\log\left(\frac{\text{Intensity}(x,y) - \text{Black}}{\text{Incident} - \text{Black}}\right)$$

Intensity(x,y) : (x,y)

Black :

Incident :

가 ,
0 가 , 가

(mean)± (standard deviation of the mean, SD)
(one-way ANOVA)
(Scheffe test) , /
- (Mann-Whitney U test)

III.

1.

H&E doxorubicin 가
, verapamil , probucol 가
doxorubicin probucol (2,

2).

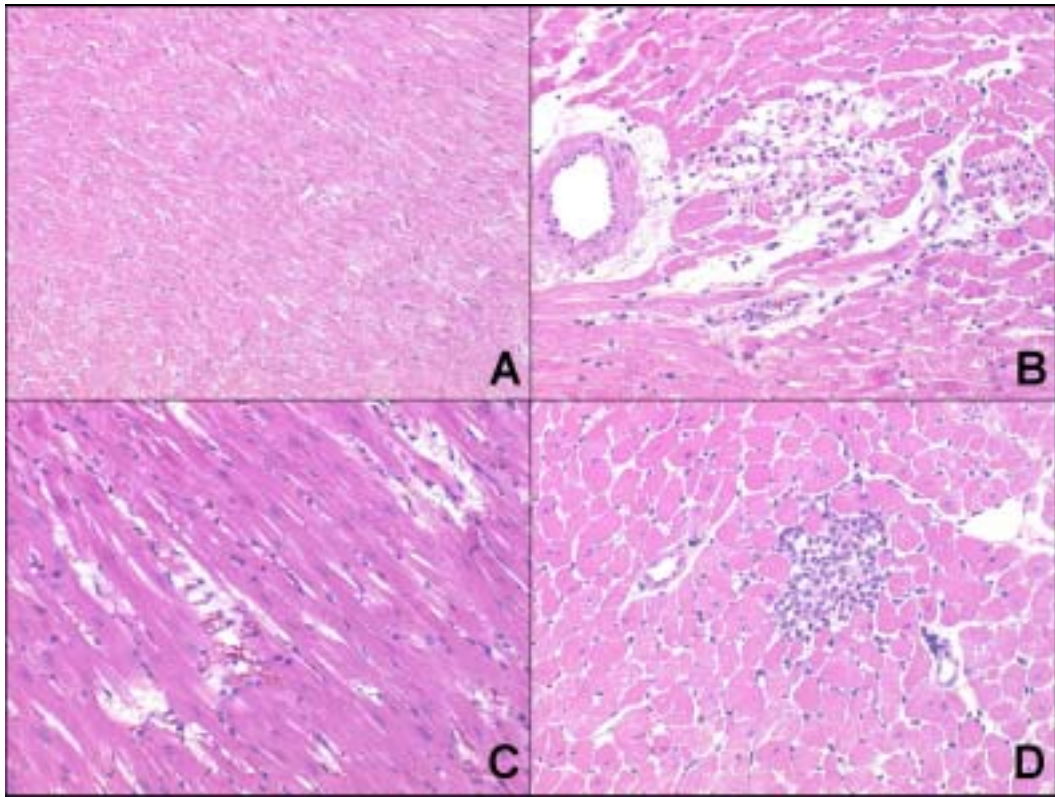
2.

1

Group		4 weeks	6 weeks	8 weeks	10 weeks
Cellularity of inflammatory cells	Control	0	0	0	0
	DXR	0.8	1.2	1.2	0.5
	DXR + PROB	0.1	0.17	0	0.1
	DXR + Ve	0	0.5	0.6	0.13
Vacuolization	Control	0	0	0	0
	DXR	0	0	0.2	0.5
	DXR + PROB	0	0.17	0	0
	DXR + Ve	0	0	0	0

1

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



2. 6 (A) , doxorubicin (B) , doxorubicin probucol (C) , doxorubicin verapamil (D)

2. PGP 9.5

PGP 9.5

. Doxorubicin verapamil 4
 가
 doxorubicin 8 10 4
 , verapamil 8 4 , 10
 4 6 . Probucol 4
 가 , 가
 , (3, 3).
 Doxorubicin . Pearson doxorubicin verapamil

-0.8279 -0.7956 , probucol -0.4598
(p<0.05).

3. PGP 9.5

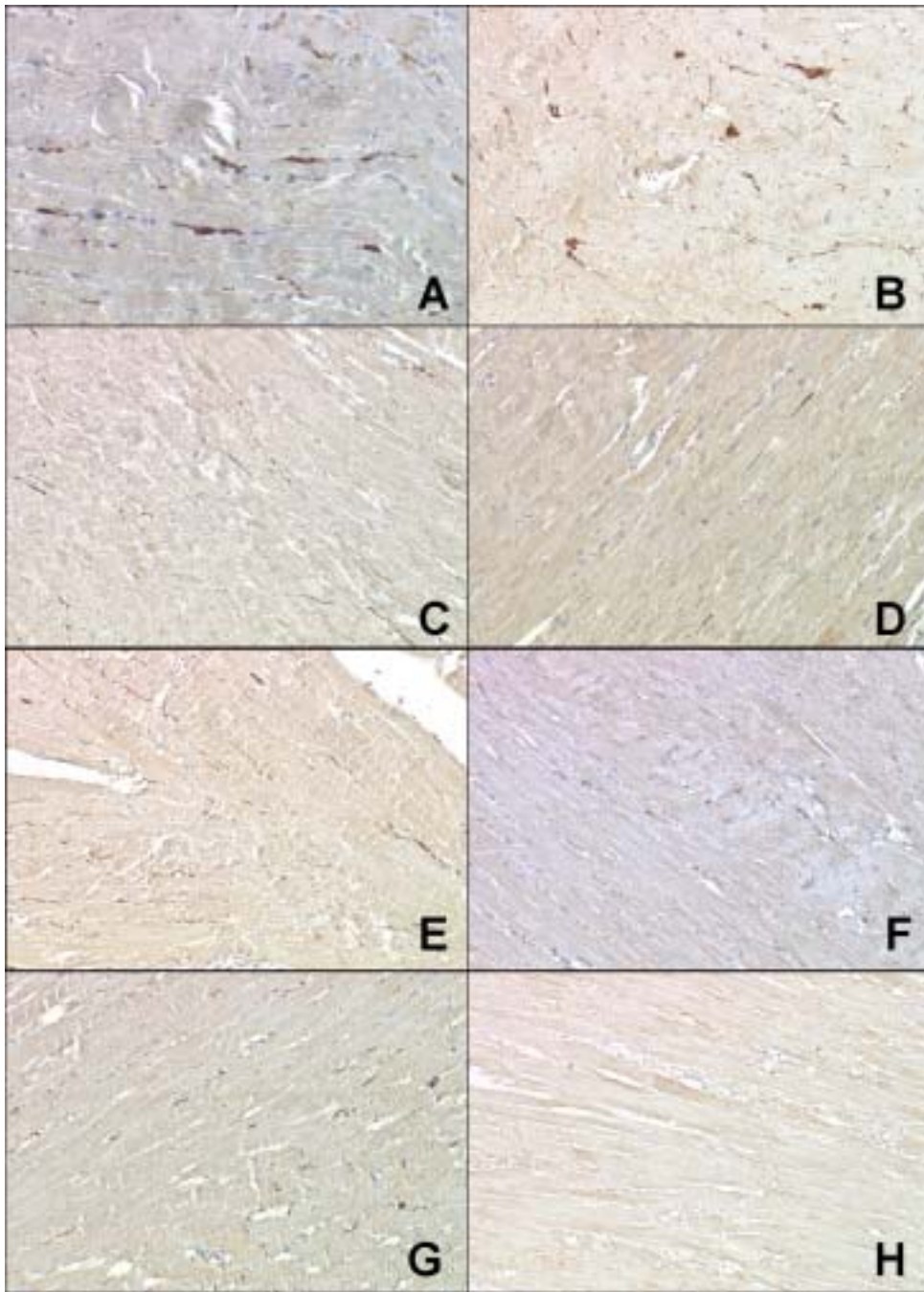
Group	4 weeks	6 weeks	8 weeks	10 weeks
Control	442.00±103.02	448.60±100.74	439.60±94.34	435.80±93.16
DXR	361.40±109.08	242.60±76.46	142.20±41.95*	96.00±23.62*
DXR + PROB	422.00±94.67	389.33±85.03	348.40±64.77	324.20±64.04
DXR + Ve	354.20±110.83	277.67±79.99	147.80±36.02*	115.75±34.75*#

¹ ±
DXR: doxorubicin, PROB: probucol, Ve: verapamil.
* 4 p<0.05, # 6 p< 0.05.

4. PGP 9.5

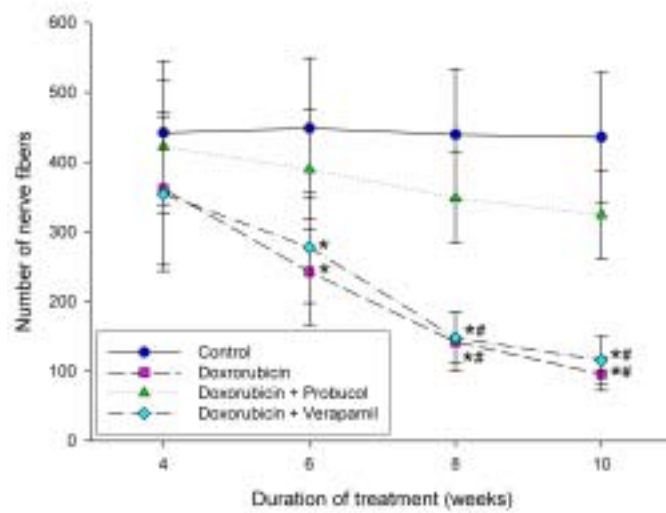
Wks	Group	Subendocardium	Mid-myocardium	Subepicardium	Epi/endo ratio ²
4	Control	67.40±17.50	133.40±43.60	241.20±43.34	3.64±0.34
	DXR	54.80±17.57	100.00±21.04	206.60±73.86	3.71±0.73
	DXR + PROB	60.20±11.80	107.00±41.16	223.00±48.02	3.70±0.39
	DXR + Ve	53.80±17.89	99.60±19.32	200.80±74.61	3.73±0.58
6	Control	65.60±18.12	149.40±47.25	233.60±39.22	3.66±0.59
	DXR	33.80±11.08	78.00±25.95	130.80±41.88	3.95±0.63
	DXR + PROB	55.67±11.38	125.17±37.25	208.50±39.86	3.79±0.50
	DXR + Ve	38.50±13.11	93.67±26.13	145.50±42.53	3.87±0.56
8	Control	68.00±19.20	133.80±36.72	237.80±41.67	3.60±0.52
	DXR	17.20±7.19*	44.80±13.22*	80.20±22.72*	4.90±1.09
	DXR + PROB	51.60±17.78	104.00±18.59	192.80±31.01	3.92±0.71
	DXR + Ve	17.80±6.02*	44.20±15.11*#	85.80±16.75*	5.03±1.02
10	Control	68.60±20.17	125.20±33.97	242.00±39.97	3.64±0.49
	DXR	9.00±2.16*#	31.25±6.40*#	55.75±15.78*	6.24±1.15*#
	DXR + PROB	45.60±14.29	101.20±21.17	177.40±31.79	4.05±0.76
	DXR + Ve	12.25±5.32*#	32.50±8.74*#	71.00±20.94*	6.08±1.05*#

¹ ±
² Wks: weeks, DXR: doxorubicin, PROB: probucol, Ve: verapamil.
* 4 p<0.05, # 6 p< 0.05.

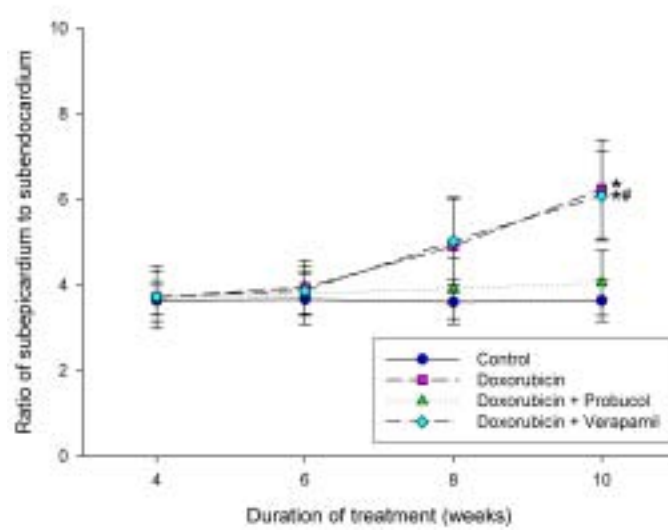


3. PGP 9.5 . PGP 9.5
 4 (A) 10 (B) , doxorubicin 4 (C)
 10 (D) , doxorubicin probucol
 4 (E) 10 (F)
 , doxorubicin verapamil 4 (G) 10 (H)

가 , 6 doxorubicin , 4 verapamil , 8 doxorubicin (4).
 verapamil probucol



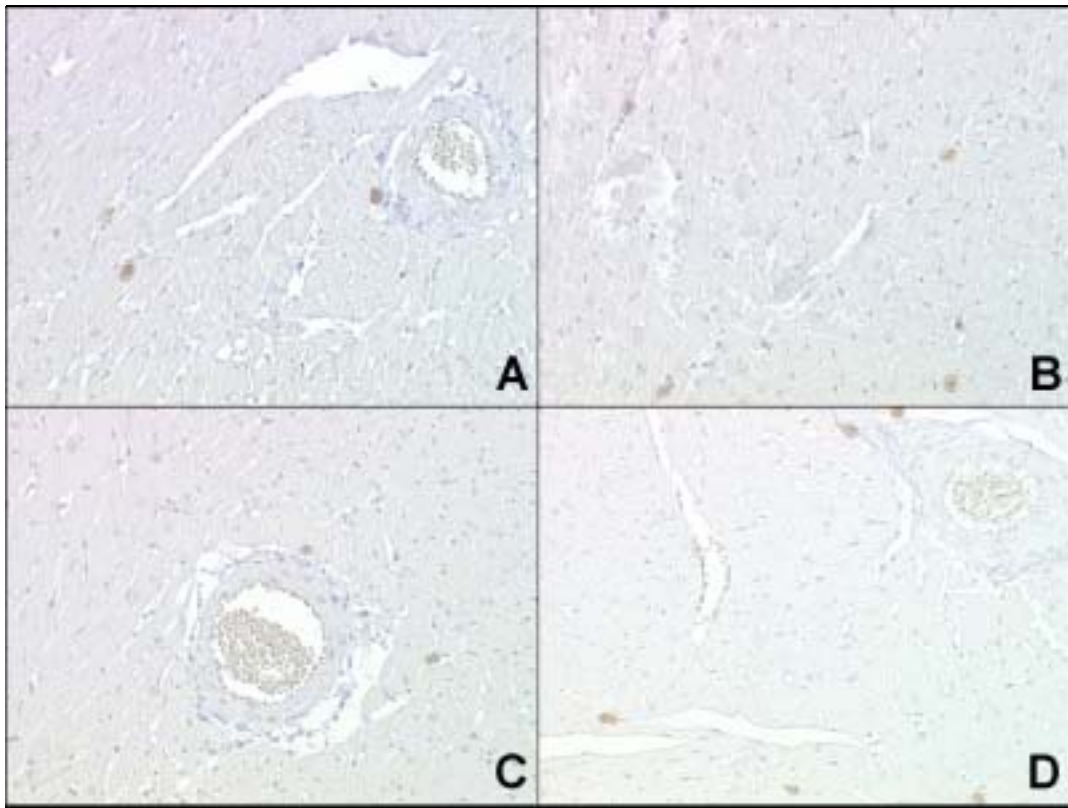
4. PGP 9.5 ±
 * p<0.05, # doxorubicin probucol
 p<0.05.
 3 3
 3
 Mann-Whitney U test doxorubicin
 verapamil 10 4 6
 10 doxorubicin
 verapamil , verapamil
 probucol (4, 5).



5. PGP 9.5
 Doxorubicin doxorubicin verapamil
 가 가 ± *
 p<0.05, # doxorubicin probucol p<0.05.

3. Caspase-3
 Caspase-3

가 , (6).
 Caspase-3 doxorubicin verapamil
 probucol . Probucol
 Doxorubicin verapamil 4 8
 6 10 가 6 doxorubicin
 caspase-3 가 , 10
 , verapamil probucol , 10
 doxorubicin probucol
 (5, 7).



6. Caspase-3

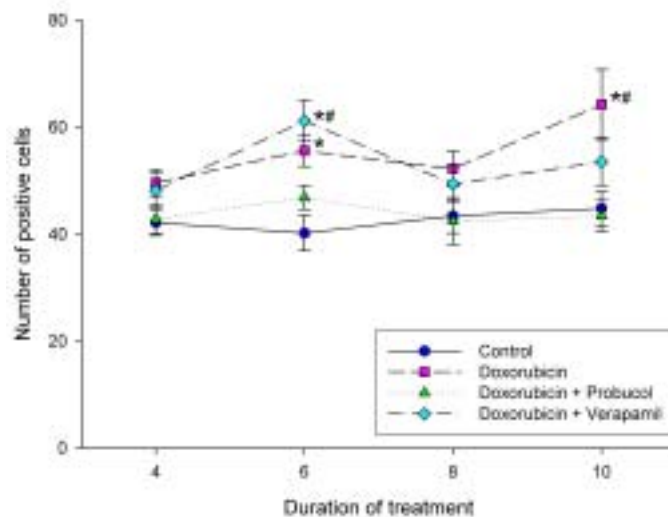
Caspase-3

가 doxorubicin doxorubicin verapamil
 Caspase-3 doxorubicin probucol A: , B:
 doxorubicin , C: doxorubicin probucol , D: doxorubicin verapamil

5. caspase-3

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

¹ ±
 DXR: doxorubicin, PROB: probucol, Ve: verapamil.



7. caspase-3
 Caspase-3 doxorubicin doxorubicin doxorubicin verapamil
 doxorubicin probucol
 * p<0.05, # doxorubicin probucol ±

4. Hsp70

Hsp70

(8).

Hsp70

doxorubicin , probucol , verapamil 4

8 6 10

(6).

6.

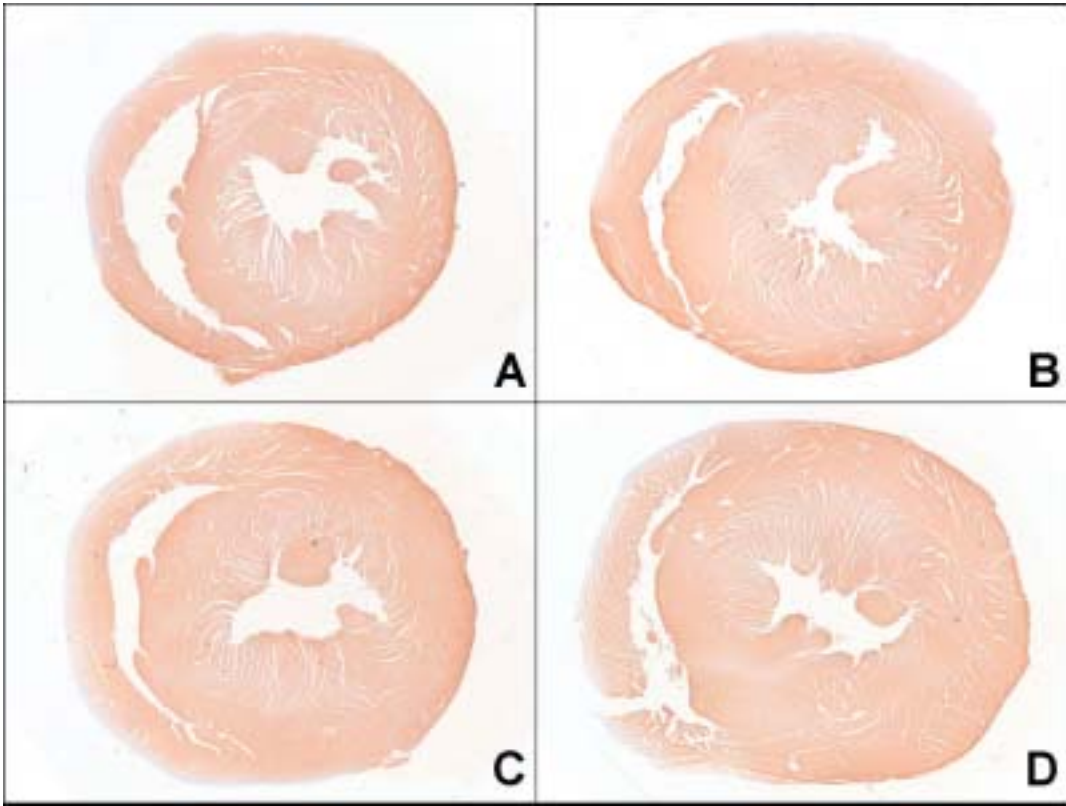
hsp70

1

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

1 ±

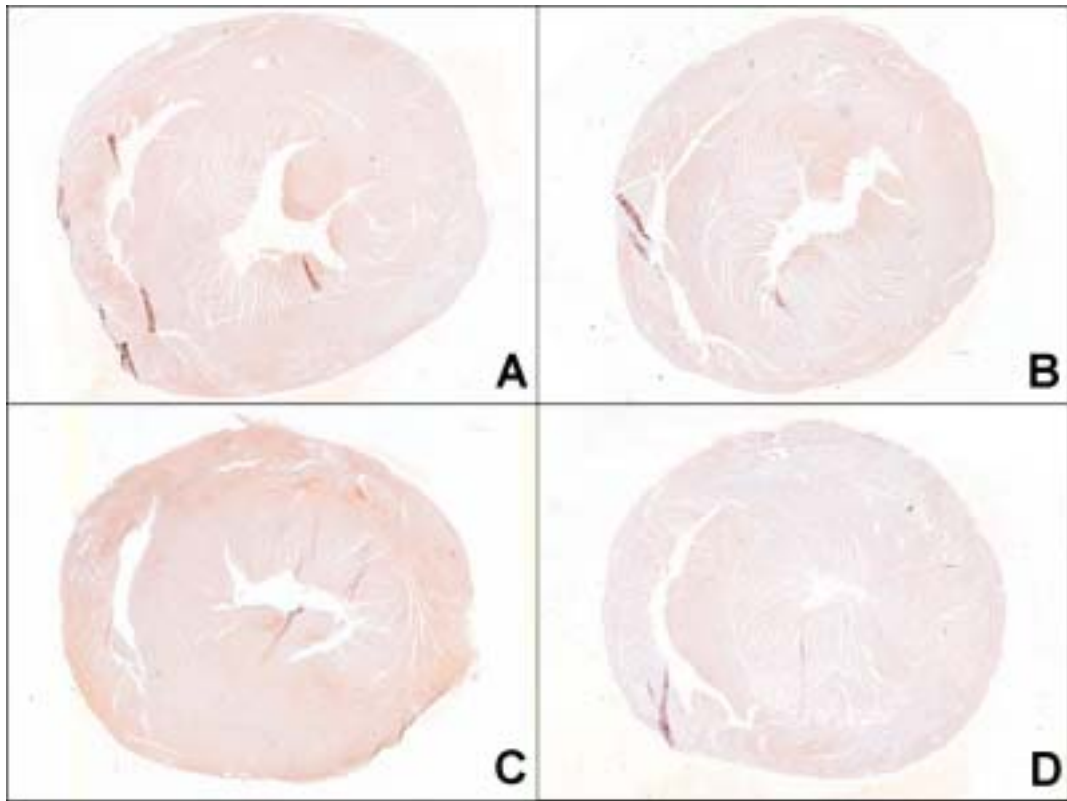
DXR: doxorubicin, PROB: probucol, Ve: verapamil.



8. Hsp70
 A: verapamil, B: doxorubicin, C: doxorubicin + probucol, D: doxorubicin

5. Hsp25
 Hsp25, hsp70 (9).

Hsp25, doxorubicin 4 6 (7).



9. Hsp25, hsp70, Hsp25, A: , B: doxorubicin, C: doxorubicin + probucol, D: doxorubicin + verapamil.

7. hsp25

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

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

IV.

Doxorubicin , doxorubicin
 3. doxorubicin
 가,
 6, doxorubicin
 doxorubicin doxorubicin
 가 , 21, 24, 36, 37 ,
 doxorubicin
 가 , 4 6 8
 Jeon 33 . Probucol verapamil
 doxorubicin 가
 , probucol
 pro-inflammatory cytokine interleukin (IL)-1 β IL-6
 53, 54, probucol
 55. Verapamil pro-inflammatory gene signaling
 56, superoxide가 Ca²⁺
 가 superoxide - α
 verapamil 57. probucol
 verapamil doxorubicin 가
 PGP 9.5 4
 . Doxorubicin verapamil 8 4
 33, 34. Probucol
 4 가
 가 . probucol
 , probucol doxorubicin
 24 ,
 가

가
 doxorubicin ()
 , doxorubicin verapamil
 , probucol
 doxorubicin
 33
 4
 가 6 가 doxorubicin verapamil
 , Jeon 33
 Probucol 가 , 8
 doxorubicin verapamil probucol
 가 - 가
 가 58. Doxorubicin
 doxorubicin
 33, doxorubicin 21
 가 가
 Purkinje 가 21. doxorubicin
 가 가
 / 가 , probucol
 가 가 probucol doxorubicin
 doxorubicin probucol 21.
 Doxorubicin
 5,
 doxorubicin
 , doxorubicin 가
 가 21.
 doxorubicin verapamil probucol
 caspase-3 가 , 6 10
 doxorubicin
 4 21 , probucol
 21 doxorubicin

²¹ .
 Bax caspase-3 ^{59, 60} ,
 doxorubicin 가가 Bax 가
 ,
 caspase-3 4
 10 16 ²¹ . bovine aortic endothelial cell (BAEC)
 doxorubicin caspase-3가
 4 가 ⁶¹ . caspase-3 가
 , caspase-3
 14 caspase-3
 가 가 .
 hsp27 hsp70 hsp27 hsp70 가
 hsp 가 가 ⁶² ,
 hsp 가
 doxorubicin shock protein ⁶³ ,
 hsp70
⁶⁴ . doxorubicin 가
 hsp25 doxorubicin 가
 , 8
 가 ⁶⁵ . hsp27 가
 , 가 hsp25 rabbit polyclonal
 . Hsp70 25
 ,
 hsp 가 .

V.

Doxorubicin probucol verapamil
 doxorubicin , doxorubicin probucol ,
 doxorubicin verapamil 4, 6, 8, 10 ,
 2 H&E PGP 9.5, caspase-3, hsp70, hsp25

1. H&E
가 , verapamil , probucol doxorubicin 가

2. PGP 9.5
가 doxorubicin verapamil
, probucol
가
3 3
, doxorubicin
verapamil 가

3. Caspase-3
doxorubicin verapamil
probucol . Probucol

4. Hsp70
4 8 6 10
, hsp25 doxorubicin
4 6
doxorubicin doxorubicin
, doxorubicin doxorubicin
가 doxorubicin 가
2 가 verapamil
doxorubicin 가 , caspase-3
, hsp70 hsp25 가
probucol doxorubicin
, hsp70 hsp25
caspase-3
doxorubicin
가

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Abstract

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

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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 administered intraperitoneally once a week and verapamil was administered 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 cardial 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