

ApoE Knockout Mouse에서 원발성 동맥경화부위의 혈관 협착부위가 동맥경화 억제약물의 투여시 미치는 영향-혈관 재구도의 관점에서 본 변화

서홍석 · 이은미 · 안정천 · 황인희 · 황교승 · 송우혁
임도선 · 박창규 · 김영훈 · 심완주 · 오동주 · 노영무

The Effects of Antiproliferative Drugs at Stenotic Area Associated with Primary Atherosclerotic Lesions in ApoE Knockout Mouse-Change of Vascular Remodeling

Hong Seog Seo, MD, Eun Mi Lee, MD, Jeong Cheon Ahn, MD, Soo Mi Kim, MD,
Hwang In Hee, MD, Kyo Seung Hwang, MD, Woo Hyuk Song, MD,
Do Sun Lim, DM, Chang Gyu Park, MD, Young Hoon Kim, MD,
Wan Joo Shim, MD, Dong Joo Oh, MD and Young Moo Ro, MD

Department of Internal Medicine, College of Medicine, Korea University, Seoul, Korea

ABSTRACT

Apolipoprotein (apo) E deficient mouse can produce reproducible fixed stenotic primary atherosclerotic lesion, which reveals failure to remodel of vascular lumen, in the ascending aorta, external carotid, common carotid, iliac, femoral and popliteal arteries. To evaluate the effect of drugs in regarding to both prevention of primary atherosclerotic lesion and vascular remodeling, a systematic analysis of distribution of atherosclerotic lesions was undertaken in chow-fed, 9-month-old apo E deficient mice, which was administrated drugs including aspirin, methotrexate, probucol, sulodexide, diltiazem, cilazapril, trimetazidine, molsidomine, pentoxiphylline and Ginxin for 7 month from 3 month-old. On gross and microscopic examination, formation of primary atherosclerotic lesions could be delayed and/or prevented partially by effects of these drugs. On morphometric examination, failure to remodel forming vascular stenosis could not be seen, though relatively mild atherosclerotic lesion occurred at vascular tree. These data suggest that the stenotic process in advanced atherosclerotic vessels can be delayed and/or prevented by several drugs including methotrexate, probucol, sulodexide, diltiazem, cilazapril, trimetazidine, molsidomine, pentoxiphylline and Ginxin in vivo state. (Korean Circulation J 2000;30(4):517-527)

KEY WORDS : Apolipoprotein E deficient mouse · Atherosclerosis · Vascular remodel · Primary prevention.

서 론

가

가

가 19

: , 152 - 050

80

: (02) 818 - 6634 · : (02) 864 - 3062

E - mail : mdhsseo@unitel.co.kr

가

가

가 , 가

가 3)

가

가

가

가 1996 Seo 4) apoE knockout mouse 가

가 , ad -

ventitia 4) in vitro

가 . 1987 Glagov 1)

가 40%

가

가

가 8 apolipoprotein E(apoE) defi -

ciency mouse

asprin methotrexate

probucol, smooth muscle cell

sulodexide, diltiazem, cilazapril,

trimetazidine, molsidomine, pentoxiphylline Gi -

nexin 7

9

가

연구 방법

2)

C57/Bl6 mouse apoE

100% apoli -

poprotein E deficiency mouse Jackson La -

boratory

8 6 asprin(50~75

mg/kg/d), methotrexate(0.2 mg/kg), probucol(15

가 mg/kg/d), sulodexide(10 mg/kg), diltiazem(3 mg/kg),

cilazapril(0.04 mg/kg), molsidomine(0.1 mg/kg), tr -

imetazidine(0.6 mg/kg), pentoxiphylline(10 mg/kg),

life cycle Ginexin (4 mg/Kg) Jonde needle

7 . Mouse . apoE deficiency
가 9 ketamine/xylazine(0.3 ml each) mouse . wild
(Table 1),
10% formalin 100~120 mmHg type C57/Bl6 mouse
4~5 apoE deficiency mouse
dissecting microscope 가 (Table 2).
10% formalin immersion
fixation . dissecting microscope 육안적 동맥경화 병소의 비교
Dissecting microscope
paraffin block slide Ha - 가
rris hematoxylineosin , elastine Masson - (severity)
trichrome
morphometric analysis 가
digital image analysis(Optimas 5.2, Optimas Corp., (Table 3) pentoxiphylline
Bothell, WA) probucol sulodexide 가
, internal elastic lamina(IEL) . tree
, IEL , external elastic lamina 가 Gi -
(EEL) , EEL , media , media nexin 가 .
, media smooth muscle cell layer
morphometric parameters 병변의 조직학적 비교
가 가
external carotid artery , common ca -
rotid artery , renal artery
, iliac trifurcation , femoral ar -
tery, popliteal artery

결 과

쥐의 체중비교와 혈중 lipid profile의 비교
ApoE deficiency mouse 7 astrix ,
3 , GNX 1 va - Diltiazem, cilazapril, pentoxiphylline 가

Table 1. Plasma lipid levels of 9-month-old apoE-deficient and wild type mice

Mouse	Cholesterol (mg/dl)	Triglyceride (md/dl)
Wild type (C57/Bl6)	117.2± 26.4	92.0± 57.4
Control apoE (- / -)	550.3± 204.0	173.7± 149.4
ApoE (- / -) + aspirin	903.7± 104.7	186.0± 104.0
ApoE (- / -) + methotrexate	826.3± 274.0	201.8± 111.3
ApoE (- / -) + probucol	827.5± 355.6	113.0± 51.6
ApoE (- / -) + sulodexide	940.7± 201.2	286.0± 155.0
ApoE (- / -) + diltiazem	998.3± 286.2	227.8± 110.8
ApoE (- / -) + cilazapril	964.7± 249.6	189.2± 66.8
ApoE (- / -) + molsidomine	1022.7± 125.2	293.7± 145.3
ApoE (- / -) + trimetazidine	907.4± 122.4	156.2± 58.4
ApoE (- / -) + pentoxiphylline	911.2± 216.0	207.3± 100.6

Table 2. Distribution and severity of peripheral atherosclerotic lesions by dissecting microscope in 9-month-old apoE-deficient mice according to drug administration

ApoE	ECA score	CCA score	AA score	AR score	IT score	FA score	PA score	Sum of score
ApoE (-/-), control								
1	3	3	2	2	3	3	3	19
2	2	1	2	2	1	0	0.5	8.5
3	3	2	2	2	3	3	3	18
4	3	2.5	2	3	1	3	2.5	17
5	3	2	2	3	0	2.5	2.5	15
6	3	2.5	2	3	2.5	2.5	2	17.5
ApoE (-/-) + aspirin								
1	2.5	2.5	2	3	2	1	0	13
2	0.5	0.5	2	0	0	0	0	3
3	2.5	2.5	2	2	0	0	0	9
ApoE (-/-) + methotrexate								
1	0	1.5	2	2	0	0	0	5.5
2	0	1.5	2	2.5	2	1	1	10
3	1.5	2	2	2	2	3	3	14.5
4	0	0	2	3	2	0	0	7
5	0	2	2	2	0	0	0	6
6	0	0	2	3	1	1.5	0	7.5
ApoE (-/-) + probucol								
1	2.5	0	2	3	0	1	1.5	10
2	1	1	2	2	0	1	0	7
3	0.5	0	2	2	0	0	0	4.5
4	0	0	2	2	0	0	0	4
ApoE (-/-) + sulodexide								
1	0	0	2	0	0	0	0	2
2	2	2	2	2	0	1	0	9
3	1.5	2	2	0	0	0	0	5.5
4	1	2	2	3	0	0	0	8
5	1	1	2	3	0	0	0	7
6	3	2.5	2	3	0	0	0	10.5
ApoE (-/-) + diltiazem								
1	0	0	2	2	2	2	0	8
2	0	1	2	2	2	0	0	7
3	0	2	2	2	0	1	0	7
4	2.5	0	2	2	0	1	0	7.5
5	2.5	0	2	2	2	0	0	8.5
6	2	1	2	3	0	0	0	8
ApoE (-/-) + cilazapril								
1	2.5	0	2	2	1	1	0	8.5
2	0.5	0	2	1	0	2	0	5.5
3	1	0	2	2	3	0	0	8
4	1	0	2	3	0	0	0	8
5	0	2	2	2	0	0	0	6
6	1.5	0	2	1	1	1	0	6.5

Table 2. Continued

ApoE	ECA score	CCA score	AA score	AR score	IT score	FA score	PA score	Sum of score
ApoE (- / -) + molsidomine								
1	1	2	2	1	2	3	0	11
2	1.5	0	2	3	0	1	0	7.5
3	3	0	2	3	0	1	0	9
4	3	0	2	3	0	0	0	8
5	0.5	0.5	2	0	0	0	0	3
6	0	3	2	2	3	3	0	14
ApoE (- / -) + trimetazidine								
1	3	2.5	2	2	3	2.5	0	15
2	2.5	0	2	3	2	1	0	10.5
3	3	1	2	2	3	1	0	12
4	0	0	2	0	0	0	0	2
5	0	0	2	2	0	0	0	4
ApoE (- / -) + pentoxiphyline								
1	0	0	2	2	0	0	0	4
2	0	0	2	1	0	0	0	3
3	2	0	2	1.5	0	0.5	0	6
4	2.5	1.5	2	1	0	0	0	7
5	1	0	2	1	0	0	0	4
6	1	2.5	2	1	1	1.5	0	9

Score 0, normal ; 1, fluffy edge not well defined ; 2, edge well defined, cream color, usually oval or round ; 3, hard to touch, pearly looking, concentric. Score represents mean value of left and right side. EC=external carotid artery ; CC=common carotid artery ; AA=aortic arch ; AR=aorta at the level of kidney, IT=iliac trifurcation artery ; FA=femoral artery ; PA=popliteal artery

Table 3. 죽상경맥경화 병변의 조직학적 관찰을 비교하여 본 바 죽상반 형성의 빈도

Mouse	ECA	CCA	AA	AR	IA	FA	PA	Total (%)
Control	12/12	12/12	6/6	3/6	12/12	12/12	12/12	69/72(95.8)
Aspirin	2/ 5	0/ 2	3/3	1/2	0/ 6	0/ 6	1/ 6	7/30(23.3)
Methotrexate	0/ 9	4/10	6/6	3/6	1/11	0/11	0/ 7	14/60(23.3)
Probucol	1/ 7	1/ 8	5/5	4/5	0/ 8	0/ 8	0/ 6	11/47(23.4)
Sulodexide	0/12	2/12	6/6	4/6	0/ 8	0/10	0/ 4	12/58(20.7)
Dilitiazem1	1/12	1/12	5/6	1/6	1/11	0/11	0/10	9/68(13.2)
Cilazapril	0/12	0/ 9	6/6	4/6	1/11	0/10	0/ 7	11/61(18.0)
Molsidomine	6/11	1/10	6/6	4/6	1/11	0/12	0/11	18/67(28.9)
Trimetazidine	3/ 7	2/10	5/5	2/5	1/10	0/12	0/11	12/60(20.0)
Pentoxiphyline	0/12	1/ 6	5/6	2/5	2/10	0/12	0/ 8	10/59(16.9)
GNX	1/ 8	3/ 8	5/5	3/5	0/10	1/ 8	1/ 8	14/52(26.9)

EC=external carotid artery ; CC=common carotid artery ; AA=aortic arch ; AR=aorta at the level of kidney
IT=iliac trifurcation artery ; FA=femoral artery ; PA=popliteal artery

(Table 3). Extracellular cholesterol deposition(cholesterol cleft) , cilazapril, trimetazidine, probucol 가 Smooth muscle cell layer destruction 가 (Table 5). Adventitial inflammatory cell infiltration (Table 4).

Table 4. Extracellular cholesterol의 deposition (cholesterol cleft)의 빈도

Mouse	ECA	CCA	AA	AR	IA	FA	PA	Total (%)
Control	12/12	12/12	6/6	3/6	12/12	12/12	12/12	69/72(95.8)
Aspirin	1/ 5	1/ 2	1/3	0/2	0/ 6	0/ 6	0/ 6	3/33(9.1)
Methotrexate	0/ 9	2/10	6/6	1/6	0/11	0/11	0/ 7	9/60(15.0)
Probucol	0/ 7	1/ 8	2/5	1/5	0/ 8	0/ 8	0/ 6	4/47(8.5)
Sulodexide	0/12	2/12	6/6	1/6	0/ 8	0/10	0/ 4	7/58(12.1)
Dilifiazem1	1/12	1/12	3/6	0/6	1/11	0/11	0/10	6/68(8.8)
Cilazapril	0/12	0/ 9	2/6	2/6	0/11	0/10	0/ 7	4/61(6.6)
Molsidomine	1/11	0/10	3/6	2/6	0/11	0/12	0/11	6/67(9.0)
Trimetazidine	0/ 7	1/10	2/5	1/5	0/10	0/12	0/11	4/60(6.7)
Pentoxiphyline	0/12	0/ 6	3/6	0/5	0/10	0/12	0/ 8	3/59(5.1)
GNX	1/ 8	2/ 8	4/5	3/5	0/10	0/ 8	0/ 8	10/52(19.2)

EC=external carotid artery ; CC=common carotid artery ; AA=aortic arch ; AR=aorta at the level of kidney
 IT=iliac trifurcation artery ; FA=femoral artery ; PA=popliteal artery

Table 5. Smooth muscle cell layer의 destruction

Mouse	ECA	CCA	AA	AR	IA	FA	PA	Total (%)
Control	12/12	12/12	3/3	3/6	12/12	12/12	12/12	69/72(95.8)
Aspirin	2/ 5	0/ 2	2/3	0/2	0/ 6	0/ 6	0/ 6	4/33(15.2)
Methotrexate	0/ 9	1/10	5/6	4/6	2/11	0/11	0/ 7	12/60(20.0)
Probucol	1/ 7	2/ 8	3/5	1/5	0/ 8	1/ 8	0/ 6	8/47(17.2)
Sulodexide	1/12	2/12	4/6	4/6	0/ 8	0/10	0/ 4	11/58(17.2)
Dilifiazem1	1/12	0/12	1/6	1/6	1/11	0/11	0/10	4/68(5.9)
Cilazapril	0/12	0/ 9	6/6	0/6	0/11	0/10	0/ 7	6/61(11.5)
Molsidomine	3/11	0/10	3/6	2/6	2/11	2/12	0/11	12/67(17.9)
Trimetazidine	1/ 7	2/10	4/5	2/5	1/10	1/12	0/11	11/60(18.3)
Pentoxiphyline	0/12	0/ 6	1/6	1/5	0/10	0/12	0/ 8	2/59(10.2)
GNX	1/ 8	1/ 8	0/5	1/5	0/10	1/ 8	0/ 8	5/52(21.2)

EC=external carotid artery ; CC=common carotid artery ; AA=aortic arch ; AR=aorta at the level of kidney
 IT=iliac trifurcation artery ; FA=femoral artery ; PA=popliteal artery

Table 6. Adventitial inflammatory cell infiltration의 빈도

Mouse	ECA	CCA	AA	AR	IA	FA	PA	Total (%)
Control	12/12	12/12	3/3	3/6	12/12	12/12	12/12	69/72(95.8)
Aspirin	1/ 5	1/ 2	1/3	2/2	0/ 6	0/ 6	0/ 6	5/33(15.2)
Methotrexate	0/ 9	1/10	5/6	4/6	2/11	0/11	0/ 7	12/60(20.0)
Probucol	1/ 7	2/ 8	3/5	1/5	0/ 8	1/ 8	0/ 6	8/47(17.2)
Sulodexide	1/12	1/12	4/6	3/6	0/ 8	1/10	0/ 4	10/58(17.2)
Dilifiazem1	1/12	0/12	1/6	1/6	1/11	0/11	0/10	4/68(5.9)
Cilazapril	0/12	0/ 9	4/6	1/6	1/11	1/10	0/ 7	7/61(11.5)
Molsidomine	3/11	0/10	3/6	2/6	2/11	2/12	0/11	12/67(17.9)
Trimetazidine	1/ 7	2/10	4/5	2/5	1/10	1/12	0/11	11/60(18.3)
Pentoxiphyline	0/12	0/ 6	3/6	2/5	1/10	0/12	0/ 8	6/59(10.2)
GNX	0/ 8	1/ 8	5/5	3/5	0/10	1/ 8	1/ 8	11/52(21.2)

EC=external carotid artery ; CC=common carotid artery ; AA=aortic arch ; AR=aorta at the level of kidney
 IT=iliac trifurcation artery ; FA=femoral artery ; PA=popliteal artery

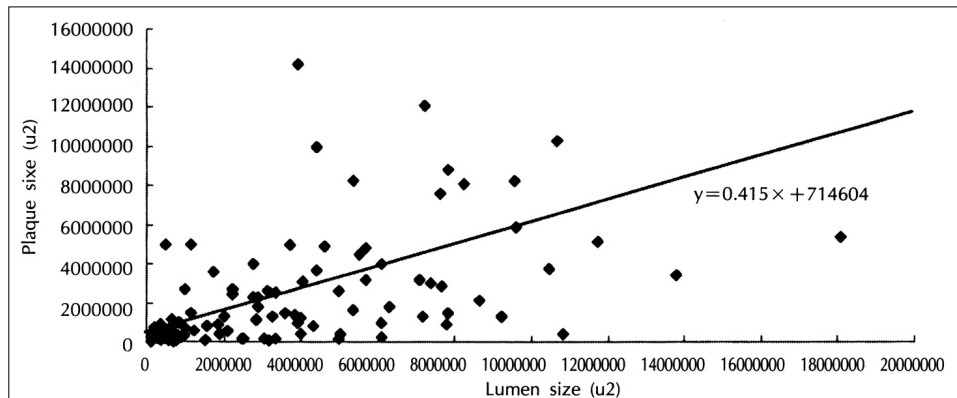


Fig. 1. Relation of plaque size and lumen size in drug-administrated apo E deficient mice from morphometric data shows positive vascular remodeling occurred.

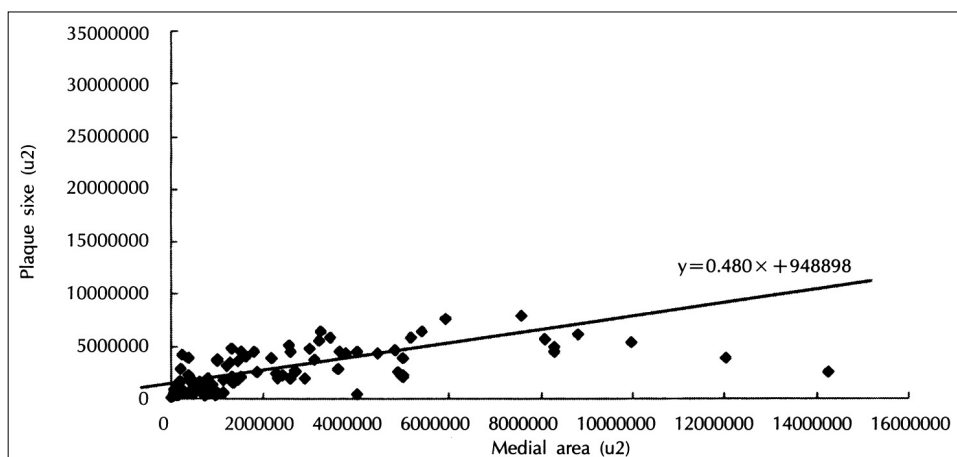


Fig. 2. Relation of plaque size and medial area in drug-administrated apo E deficient mice from morphometric data shows preversed vascular remodeling.

가 , diltiazem, pento -
xiphylline, cilazapril 가 (Table 6). 가 가

Morphometric evaluation의 비교
ApoE deficient mouse

가
(Figs.
1 and 2).
early lesion
fatty streak
고 찰 1 10 15~20

⁵⁾ Glagov ¹⁾ 3~4
가 40% ⁷⁾ .
atheroma가 가 ,
(vascular remodeling)
가 . 1987 Boston Glagov
가 left main coronary artery
가 Clarkson ⁸⁾ . Mintz ²⁾
(rupture)
(erosion) 가 가
⁶⁾ 가 가
가 1996 Seo ⁴⁾
가 apoE knockout mouse가
가 가 가
morphometry 가 가
failure to remodel
가 가
cross - sectional study para - study
meter 가 study 가
population ,
가 가 가
가 가 가
600 mg~1100 mg
. apoE knockout mouse

8 apolipoprotein E(apoE) deficiency mouse

asprin folate cellular humoral immunity cytokine methotrexate,⁹⁾ probucol,¹⁰⁾ sulodexide,¹¹⁾¹²⁾ diltiazem, angiotensin cilazapril, oxygen free radical damage trimetazidine,¹³⁾ endogenous nitric oxide donor molsidomine,¹⁴⁾ tissue factor expression pentoxifylline¹⁵⁾¹⁶⁾ platelet activation factor Ginexin¹⁷⁾ 7 aspirin

, , , angiotensin , platelet activation factor , tissue factor expression , oxygen free radical damage

adventitia in vitro Glagov

■ 본 연구의 한계점 :

life cycle

요 약

연구배경 :

가

가 100%

가

가

방 법 :

8 apo E deficient mouse

6 asprin(50~75 mg/kg/d), methotrexate (0.2 mg/kg), probucol(15 mg/kg/d), sulodexide(10 mg/kg), diltiazem(3 mg/kg), cilazapril(0.04 mg/kg), molsidomine(0.1 mg/kg), trimetazidine(0.6 mg/kg), pentoxifylline(10 mg/kg), Ginexin (mg/Kg)

7

. Mouse 가 9 10% for - malin dissec - ting microscope 10% formalin immersion fixation . Dissec - ting microscope Harris hematoxylineosin , elastine Masson - trichrome

morphometric analysis

가

결 과 :

apoE deficiency mouse

wild type C57/Bl6 mouse

apoE deficiency mouse

가 .

external carotid artery , common carotid artery , renal artery

, iliac trifurcation , femoral artery, po-
pliteal artery dissecting microscope

가

hylline probucol pentoxip -
sulodexide
가 .
tree 가
Ginexin 가 .

Diltiazem, cilazapril, pentoxiphylline 가
Extracellular cholesterol deposition
(cholesterol cleft)
cilazapril, tri -
metazidine, probucol 가 . Smooth
muscle cell layer destruction

가
. Adventitial inflammatory cell infiltration

가 , diltiazem, pen -
toxiphylline, cilazapril 가 (Table
6). morphometric evaluation

결 론 :

600 mg~1100 mg

8 apolipoprotein E(apoE) deficiency
mouse
asprin folate cellular

humoral immunity cytokine
methotrexate,⁹⁾
probucol,¹⁰⁾
sulodexide,¹¹⁾¹²⁾
diltiazem, angiotensin
cilazapril, oxygen free radical damage
trimetazidine,¹³⁾ endogenous nitric oxide
doner molsidomine,¹⁴⁾ tissue factor ex -
pression pentoxiphylline¹⁵⁾¹⁶⁾ platelet
activation factor Ginexin¹⁷⁾ 7
aspirin

, angiotensin , platelet
activation factor , tissue factor expression
, , oxygen free radical damage

중심 단어 : Apolipoprotein E deficient mouse .

감사문

1997

()가 ,

REFERENCES

- 1) Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. *Compensatory enlargement of human atherosclerotic coronary arteries. N Eng J Med* 1987;316:1371.
- 2) Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong C, et al. *Arterial remodeling after coronary angioplasty: A serial intravascular ultrasound study. Circulation* 1996;94:35-43.
- 3) Rapacz J, Hasler-Rapacz J, Taylor KM, Checovich WJ, Attie AD. *Lipoprotein mutations in pigs are associated with elevated plasma cholesterol and atherosclerosis. Science* 1986;234:1573.
- 4) Seo HS, Lombard DM, Polinsky P, Powell-Braxton L, Bunting S, Schwartz SM, et al. *Peripheral vascular stenosis in Apolipoprotein E-deficient mice. Potential roles of lipid deposition, medial atrophy, and adventitial inflammation* 1997;17:3595-601.
- 5) Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Rosenfeld ME, et al. *A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. Circulation*

- 1994;89:2462-78.
- 6) Cheng GC, Loree HM, Kamm RD, Fishben MC, Lee RT. *Distribution of circumferential stress in ruptured and stable atherosclerotic lesions. A structural analysis with histopathological correlation.* *Circulation* 1993;87:1179-87.
 - 7) Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. *ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree.* *Arterioscler Thromb* 1994;14:133.
 - 8) Clarkson TB, Prichard RW, Morgan TM, Petrick GS, Klein KP. *Remodeling of coronary arteries in human and nonhuman primates.* *JAMA* 1994;271:289-94.
 - 9) Cronstein BN. *Molecular therapeutics. Methotrexate and its mechanism of action.* *Arthritis & Rheumatism* 1996;39:1951-60.
 - 10) Steinberg D. *Studies in the mechanism of action of probucol.* *Am J Cardiol* 1986;57:16H-21H.
 - 11) Teien AN, Abildgaard U, Hook M. *The anticoagulant effect of heparan sulfate and dermatan sulfate.* *Thromb Res* 1976;8:856-67.
 - 12) Tiozzo R, Cingi MR, Pietrangeli A, Albertazzi L, Candalina S, Milana MR. *Effect of heparin-like compounds on the in vitro proliferation and protein synthesis of various cell types.* *Arzneim-Forsch/Drug Res* 1989;39:15-20.
 - 13) Guarneri C, Muscari C. *Effect of trimetazidine on mitochondrial function and oxidative damage during reperfusion of ischemic hypertrophied rat myocardium.* *Pharmacology* 1993;46:324-1.
 - 14) Kukovetz WR. *Mechanism of vasodilation by molsidomine.* *Am Heart J* 1985;109:637-40.
 - 15) Ollivier V, Houssaye S, Ternisien C. *Endotoxin-induced tissue factor mRNA in human monocytes is negatively regulated by cyclic AMP-dependent mechanism.* *Blood* 1993;81:973-9.
 - 16) Levi M, ten Cate H, Bauer KA, van der Poll T, Edgington TS, Buller HR, et al. *Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees.* *J Clin Invest* 1994;93:114-20.
 - 17) Smith PF, MacLennan K, Darlington CL. *The neuroprotective properties of the Ginkgo biloba leaf: A review of the possible relationship to platelet-activating factor (PAF).* *J Ethnopharmacol* 1990;50:131-9.