Effect of hypercholesterolemia on the sequential changes of apoptosis and proliferation after balloon injury to rabbit iliac artery

Jong-Min Song a,c, Hyo-Soo Kim a,c,* , Moo-Young Rhee b, In-Ho Chae a,c, Dae-Won Sohn a,c, Byung-Hee Oh a,c, Myoung-Mook Lee a,c, Young-Bae Park a,c, Yun-Shik Choi a,c, Young-Woo Lee a,c

a Heart Research Institute, Seoul National University, Seoul, South Korea
b Department of Internal Medicine, Dankook University College of Medicine, Cheonan, South Korea
c Department of Internal Medicine, Division of Cardiology, Seoul National University College of Medicine, 28 Yongon-Dong Chomno-Gu, Seoul 110-744, South Korea

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Abstract

To evaluate the effect of hypercholesterolemia on apoptosis and proliferation after vascular injury, iliac arteries of hypercholesterolemic (HC) and normocholesterolemic (NC) rabbits were examined after balloon injury using TUNEL, immunohistochemical staining of PCNA, macrophages, smooth muscle actin and p53. In media, apoptosis occurred massively early after injury and then decreased. HC did not affect this early post-injury apoptosis but significantly increased apoptosis 14 days later (D14). Immediate apoptosis in media was followed by active proliferation. HC sustained a high activity of proliferation until D14. The changes of immunoreactivity to p53 over the same 14 day period parallel that of apoptosis. In intima, where cells were scarce initially, proliferative activity reached a peak at D7 and then decreased. HC significantly enhanced proliferation at D14. In intima proliferation was accompanied by a later low-level apoptosis. HC significantly enhanced this low-level apoptosis at D14. These effects of HC resulted in significantly increased areas of intima and media. The fundamental difference between HC and NC was the infiltration of macrophages in HC. In conclusion, balloon injury induces early massive p53-associated apoptosis followed by proliferation in media, whereas in intima, it induces active proliferation followed by a low-level apoptosis. Hypercholesterolemia does not affect the early post-injury apoptosis but enhances proliferation and low-level apoptosis at a later stage, which in turn results in intimal and medial hyperplasia. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Restenosis after angioplasty is one of the most important problems, which remain to be solved to secure the successful outcome of coronary angioplasty upon patients with ischemic heart disease. One of the main causes of restenosis is known to be neointimal and medial hyperplasia. Various growth factors and cytokines are implicated. Recently, in addition to proliferation, apoptotic activity was found in accelerated atherosclerosis, such as restenosis [1–4]. Foci of apoptosis were more frequently found in restenosis lesions rather than in primary lesions [1]. Neointimal smooth muscle cells were reported to undergo apoptosis after balloon injury [3,5] or stent insertion [6] in experimental animal models. A further study discovered that medial smooth muscle cells underwent massive apoptosis within a half or several hours after denudation balloon injury to rat carotid artery or over-stretch balloon injury to rabbit iliac artery [7]. Recently, a more comprehensive examination demonstrated the sequential changes of apoptosis and proliferation within the me-
dia, neointima and adventitia of balloon-injured porcine coronary artery [8].

Though it is an established fact that apoptosis is an important vascular response to injury, its mechanism has not been elucidated. Amongst the various mechanisms proposed [9], one is a p53-mediated apoptosis that was identified in an in-vitro experiment upon the vascular smooth muscle cells from rat aorta [10] or human atherosclerotic plaques [11]. Actually, p53 expression was also found in in-vivo situations, i.e. in restenotic lesion after coronary angioplasty [12], in atherosclerotic tissue [13] in humans and in the neointimal smooth muscle cells of the rabbit carotid artery (2 weeks after balloon-denudation injury) [14]. However, it has not been demonstrated whether p53 is also implicated in the apoptosis which occurs immediately after injury.

Reactive oxygen or Redox species may well provide us with another important factor, which may determine the course of apoptosis after vascular injury [15,16]. Among many products of oxidative stresses, oxidized low-density lipoprotein (LDL), in association with hypercholesterolemia, is probably the most well known. This induces macrophages to become foam cells which can activate proliferation [17–21] and apoptosis [4,22] of smooth muscle cells, via various growth factors and cytokines. Therefore, hypercholesterolemia may influence both the proliferative and apoptotic activity after vascular injury. However, this effect has not been fully evaluated in vivo.

In this study, we firstly examined the sequential and differential changes of apoptosis in media and neointima compared to proliferative activity after vascular injury and went on to investigate whether apoptosis after balloon-injury is associated with p53. Finally, we investigated the effect of hypercholesterolemia on the p53-associated apoptosis early in media and late in neointima.

2. Methods

2.1. Animal subjects

Male New Zealand white rabbits (weighing 2.5–3.5 kg) were randomly assigned into normocholesterolemic (NC) and hypercholesterolemic (HC) groups. They were fed either a normal diet or a 1% cholesterol diet (Oriental Yeast Co., Japan) for a period of 2 weeks before balloon injury and up to the time of sacrifice. A blood sample was taken from each rabbit to check serum cholesterol level prior to balloon injury.

2.1.1. Balloon injured rabbit iliac artery model

Rabbits were anesthetized with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). Heparin (100 IU/kg) and gentamicin (1 mg/kg) were injected intravenously to prevent thrombus formation and infection, respectively.

Right common carotid artery was exposed and 4F introducer (Check-flo, Cook, USA) was inserted to the descending aorta using a guide wire. Radiocontrast dye was injected through the introducer under fluoroscopic guidance to measure the diameters of both iliac arteries. Balloons, with diameter 1.1–1.6 times that of the iliac arteries, were advanced over a standard 0.014 inch guide wire and placed in both iliac arteries just beyond the bifurcation of the abdominal aorta. They were inflated three times for a period of 30 s, with 30 s reflow after each inflation.

2.2. Tissue collection and preparation

Rabbits were sacrificed after 1 (n = 3, each group), 3 (n = 3, each group), 7 (n = 4, each group), and 14 days (n = 6 in NC group, n = 5 in HC group). Uninjured iliac arteries of NC (n = 2) and HC (n = 2) groups were also obtained. Rabbits were anesthetized with an intramuscular injection of ketamine and xylazine, and then 10% formaldehyde was continuously infused with a pressure of 60 mmHg for 15 min through a catheter inserted into the abdominal aorta for perfusion fixation of both iliac arteries. Both iliac arteries were dissected and immersed in 10% formaldehyde solution for 12–24 h. They were then dehydrated with ethanol and embedded in paraffin. Representative sections from each block were stained with hematoxylin and eosin and with Verhoeff-van-Gieson-elastin to examine the internal and external elastic lamina and measure areas of the media and neointima.

2.3. TdT mediated in-situ nick end labeling (TUNEL)

In order to evaluate apoptotic activity, we used the TUNEL method [23] with apoptosis detection kit (ApopTag, Oncor). Each section was de-paraffinized...
Fig. 2. Sequential changes of p53 expression after over-stretch balloon-injury to the iliac arteries of hypercholesterolemic and normocholesterolemic rabbits. (A, B, C, D) Microphotographs showing p53 immunostaining of sections from iliac arteries of normocholesterolemic rabbits 1, 3, 7 and 14 days after injury. (F, G, H, I) Hypercholesterolemic rabbits after 1, 3, 7 and 14 days. (E) Microphotographs showing p53 immunostaining of sections from uninjured artery. (J) Microphotographs showing p53 immunostaining of sections from human breast cancer tissue. The intima (i), media (m) and internal elastic lamina (arrow) are indicated. P53 positive cells are indicated by arrow heads (dark brown color). Magnification × 400. (K) p53 indices of intima and media of hypercholesterolemic (HC) and normocholesterolemic (NC) rabbits 1, 3, 7 and 14 days after injury. P53 indices both in intima and media were higher in HC than in NC rabbits at 7 days. Results are expressed as mean ± SD. ** indicates P < 0.01 and * indicates P < 0.05 between NC and HC.
with xylene and rehydrated with alcohol. Proteinase K (20 μg/ml) was applied to the section for 15 min, with the intention of producing optimal proteolysis. The nonspecific chromogen reaction, induced by endogenous peroxidase was inhibited with 3% hydrogen peroxide; application period was 5 min. The TdT reaction was performed for 1 h at 37°C, and antidiogenin-peroxidase was applied for 30 min at room temperature. Biochemical controls were made with positive control slides treated with DNase-I (1 mg/ml), instead of proteinase K, and negative control slides were treated with PBS instead of TdT. Diaminobenzidine (DAB) was used as a chromogen and hematoxylin as a counterstain.

2.4. Immunohistochemistry

To detect proliferative activity immunohistochemically, monoclonal mouse antibody (clone PC10, Dako Corp, 1:50 dilution) to PCNA was used. Monoclonal mouse anti human α-actin (HHF-35, Dako, 1:50 dilution) was used for detection of smooth muscle cell, and monoclonal mouse anti-rabbit macrophage antibody (RAM-11, Dako, 1:50 dilution) for staining macrophages.

To detect p53 overexpression, monoclonal mouse antibody (DO-1, Santa Cruz, 1:100 dilution) was used and the sections were incubated in 6 mol/l urea in distilled water for 30 min at 98°C before application of primary antibody to unmask p53 antigen [13]. Section of human breast cancer was used as a positive control for p53.

Goat biotinylated anti-mouse immunoglobulin (Dako, 1:400 dilution) was used as a secondary antibody. Streptavidine horseradish peroxidase was applied for 30 min and DAB was used as chromogen with hematoxylin counterstain.

Dual staining was performed for TUNEL, PCNA and p53 with RAM-11 to type positively stained cells. Peroxidase was used with chromogen DAB for TUNEL, PCNA and p53 immunostaining and alkaline phosphatase with chromogen New Fuchsin (Dako) was used for RAM-11.

2.5. Quantification and analysis

Media and neointima areas were calculated from sections of Verhoeff-van-Gieson-elastin staining using BMI plus Ver. 1.18 morphometry (BumMi Universe Co., Korea). Sections which were not adequately cross-cut were excluded from area analysis.

Five high power fields (×400) per section were selected randomly and positively stained cells and total cells were counted for TUNEL, PCNA and p53 staining. Two observers analyzed the sections and determined which ones were positively stained cells. Nonspecific cytoplasmic staining without nuclear involvement was considered negative. Sections showing nonspecific cytoplasmic staining for several times of staining, which were probably due to technical reason during fixation, were excluded from index analysis. The total number of positive cells divided by the overall total was defined as an index; TUNEL indices, PCNA indices and p53 indices were calculated for each section.

Values are expressed as mean ± SD. The comparisons of media areas, neointima areas, TUNEL indices, PCNA indices and p53 indices for NC and HC groups were performed by use of the Student’s t-test and a value of P < 0.05 was regarded as significant.

3. Results

Serum total cholesterol level of 14 rabbits of the NC group and 12 rabbits of the HC group were 73.7 ± 27.8 mg/dl and 600.1 ± 188.7 mg/dl, respectively.

3.1. Sequential change of apoptotic activities

TUNEL positive cells with condensed and fragmented nuclei were found both within the media and neointima of the balloon-injured artery (Fig. 1), but were not found in the uninjured artery of either normocholesterolemic or hypercholesterolemic rabbits (data not shown).

TUNEL indices within the media of NC group were 6.3 ± 1.3% (n = 5) at 1 day, 4.5 ± 3.1% (n = 5) at 3
Fig. 3. Sequential changes of proliferation after over-stretch balloon-injury to the iliac arteries of hypercholesterolemic and normocholesterolemic rabbits. (A, B, C) Microphotographs showing PCNA immunostaining of sections from the iliac arteries of normocholesterolemic rabbits 3, 7 and 14 days after injury. (D, E, F) Hypercholesterolemic rabbits after 3, 7 and 14 days. The intima (i), media (m) and internal elastic lamina (arrow) are indicated. PCNA-positive cells are indicated by arrowheads (dark brown color). Magnification × 400. (G) PCNA indices of intima and media of hypercholesterolemic (HC) and normocholesterolemic (NC) rabbits 3, 7 and 14 days after injury. PCNA indices both in intima and media were higher in HC than in NC rabbits at 14 days. Results are expressed as mean ± SD. ** indicates $P < 0.01$ and * indicates $P < 0.05$ between NC and HC.
days, $0.9 \pm 0.8\% (n = 7)$ at 7 days, and $0.9 \pm 0.7\% (n = 6)$ at 14 days, while those of HC group were $6.8 \pm 1.7\% (n = 5)$ at 1 day, $4.7 \pm 2.5\% (n = 5)$ at 3 days, $2.3 \pm 1.5\% (n = 5)$ at 7 days, and $3.3 \pm 1.5\% (n = 6)$ at 14 days. In other words, apoptosis occurred massively in media immediately after the balloon-injury and subsequently decreased. Hypercholesterolemia did not influence the early immediate apoptosis in media on 1 and 3 days but significantly ($P < 0.01$) enhanced the later low-level apoptosis present 14 days after injury (Fig. 11).

TUNEL indices within the neointima of NC group were $1.3 \pm 1.1\% (n = 7)$ at 7 days and $0.3 \pm 0.2\% (n = 6)$ at 14 days, while those of HC group were $1.1 \pm 0.6\%$...

![Fig. 4](image1.png)

**Fig. 4.** Microphotographs showing hematoxylin and eosin staining of the iliac arteries of normocholesterolemic and hypercholesterolemic rabbits 14 days after over-stretch injury. (A) The intima and media of normocholesterolemic rabbit are composed of compact cells. (B) The intima and media of hypercholesterolemic rabbit are composed of foam cells. Intimal and medial hyperplasia are more prominent in hypercholesterolemic than in normocholesterolemic rabbit. The intima (i), media (m) and internal elastic lamina (arrow) are indicated. Magnification $\times 200$. (C) Areas of intima and media of hypercholesterolemic (HC) and normocholesterolemic (NC) rabbits 1, 3, 7 and 14 days after injury. Intimal and medial areas are greater in HC than in NC rabbits at 7 and 14 days. (D) Intima/media ratios of HC and NC rabbits 7 and 14 days after injury. The ratios are larger in HC than in NC rabbits at 7 days. Results are expressed as mean $\pm$ SD. ** indicates $P < 0.01$ and * indicates $P < 0.05$ between NC and HC.

![Fig. 5](image2.png)

**Fig. 5.** Macrophage infiltration into iliac artery after injury. (A, B) Microphotographs showing serial sections of iliac artery from hypercholesterolemic rabbits at day 14. (C, D) Normocholesterolemic rabbits at day 14. A and C have been immunostained with HHF-35 for $\alpha$-actin (dark brown color). B and D have been immunostained with RAM-11 antibody for rabbit monocyte/macrophage (dark brown color). Macrophage infiltration in the outer intima and inner media, adjacent to the internal elastic lamina, only in hypercholesterolemic rabbits. Magnification $\times 100$. 
Fig. 6. Cell types that express p53 or undergo apoptosis or proliferation in iliac artery after injury in hypercholesterolemic rabbits. (A, B) Microphotographs showing macrophage and smooth muscle cells undergoing apoptosis 14 days after injury. This was dual stained with TUNEL (dark brown nucleus: arrowhead) and RAM-11 antibody (red cytoplasm). A and B illustrate macrophages and smooth muscle cells undergoing apoptosis, respectively. Magnification × 1000. (C, D) Microphotographs showing macrophage and smooth muscle cells overexpressing p53 around macrophage infiltration 14 days after injury. This was dual stained with antibody for p53 (dark brown nucleus: arrowhead) and RAM-11 antibody (red cytoplasm). C shows macrophages and D shows smooth muscle cells overexpressing p53. Magnification × 400. (E, F) Microphotographs showing proliferating macrophages and smooth muscle cells around macrophage infiltration 14 days after injury. This was dual stained with antibody for PCNA (dark brown nucleus: arrowhead) and RAM-11 antibody (red cytoplasm). E shows proliferating macrophages and F shows proliferating smooth muscle cells. Magnification × 400.
(n = 5) at 7 days and 0.9 ± 0.4% (n = 6) at 14 days. Hypercholesterolemia, therefore, enhanced the low-level apoptosis in neointima 14 days after injury (P < 0.05).

3.2. Sequential changes of p53 expression which parallel apoptotic activities

The immunoreactivity to p53 was not observed in uninjured rabbit iliac artery, whereas, it was strong in human breast cancer tissue which was used as positive control tissue.

P53 indices within the media of NC group were 52.8 ± 24.8% (n = 6) at 1 day, 49.2 ± 33.2% (n = 6) at 3 days, 4.2 ± 5.9% (n = 5) at 7 days and 24.3 ± 8.7% (n = 8) at 14 days. Corresponding indices of the HC group were: 56.2 ± 8.6% (n = 5) at 1 day, 37.4 ± 17.7% (n = 5) at 3 days, 28.4 ± 15.8% (n = 7) at 7 days and 36.2 ± 18.3% (n = 10) at 14 days. P53 indices, as did the TUNEL indices, increased immediately after vascular injury, then decreased sharply at 7 days and increased again to form a second peak at 14 days. Hypercholesterolemia did not affect the p53 expression during the acute phase immediately after injury, but reduced the transient decrease of p53 at 7 days (P < 0.01) (Fig. 2K).

P53 indices within the neointima of NC group were 5.2 ± 7.5% (n = 5) at 7 days and 13.8 ± 7.8% (n = 8) at 14 days, while those of HC group were 30.3 ± 19.2% (n = 7) at 7 days and 19.7 ± 16.4% (n = 10) at 14 days. P53 indices within the neointima at 7 days of the HC group were significantly higher than those of the NC group (P < 0.05).

3.3. Sequential changes of proliferative activities

PCNA positive cells were rarely found in the uninjured arteries of both NC and HC group (data not shown). However, they were observed within the media 3 days after the early post injury apoptosis and within the neointima after 7 days, accompanying neointimal formation (Fig. 3).

PCNA indices within the media of the NC group were 5.4 ± 1.8% (n = 5) at 3 days, 6.0 ± 3.3% (n = 5) at 7 days, and 3.9 ± 2.8% (n = 6) at 14 days and those of the HC group were 5.9 ± 2.8% (n = 6) at 3 days, 7.5 ± 2.7% (n = 5) at 7 days, and 8.9 ± 2.6% (n = 6) at 14 days. In other words, hypercholesterolemia sustained proliferative activity in the media 14 days after injury (significantly higher than that found in the normocholesterolemic group (P < 0.01)) (Fig. 3G).

PCNA indices within the neointima of the NC group were: 6.4 ± 1.8% (n = 5) at 7 days and 2.5 ± 1.8% (n = 6) at 14 days, while those of the HC group were 7.1 ± 1.9% (n = 5) at 7 days and 5.8 ± 2.2% (n = 6) at 14 days. Hypercholesterolemia also maintained a higher proliferative activity in intima than that found in the NC group, 14 days after injury (P < 0.05).

3.4. The influence of hypercholesterolemia on the medial and neointimal hyperplasia

The medial areas of the NC group were 0.27 ± 0.08 mm² (n = 6) at 1 day, 0.33 ± 0.03 mm² (n = 5) at 3 days, 0.46 ± 0.17 mm² (n = 7) at 7 days and 0.43 ± 0.09 mm² (n = 10) at 14 days, while those of HC group were 0.28 ± 0.04 mm² (n = 6) at 1 day, 0.35 ± 0.07 mm² (n = 6) at 3 days, 0.52 ± 0.12 mm² (n = 7) at 7 days and 0.54 ± 0.07 mm² (n = 12) at 14 days. The medial areas at 14 days of HC group were significantly larger than those of NC group (P < 0.01) (Fig. 4).

The neointima could be observed from 7 days after injury in both NC and HC groups. Neointimal areas of the NC group were: 0.10 ± 0.07 mm² (n = 5) at 7 days and 0.23 ± 0.08 mm² (n = 8) at 14 days, while those of the HC group measured 0.20 ± 0.08 mm² (n = 7) at 7 days and 0.42 ± 0.15 mm² (n = 10) at 14 days. The neointimal areas of the HC group were significantly larger than those of the NC group (P < 0.01) at 7 and 14 days. Intima/media ratios in the NC group were 0.21 ± 0.13 at 7 days and 0.56 ± 0.15 at 14 days, while those in the HC group were 0.44 ± 0.16 at 7 days and 0.78 ± 0.33 at 14 days. The ratios in the HC group were significantly larger than those in the NC group (P < 0.05) at 7 days (Fig. 4D).

3.5. Hypercholesterolemia induces macrophage infiltration

Macrophage infiltration was observed from 7 days after injury in both the outer neointima and the inner media of the HC group but there was no or little infiltration into the arteries of NC group and no infiltration into the uninjured artery. Adjacent sections showed that the space not occupied by smooth muscle cell was filled with macrophages (Fig. 5).

3.6. Cell types that undergo apoptosis or proliferation

In dual staining, we found out that both smooth muscle cells and macrophages were TUNEL-positive (Fig. 6A,B), immunoreactive to p53 (Fig. 6C,D) and exhibited proliferative activity (Fig. 6E,F).

4. Discussion

In this study, we have demonstrated that over-stretch balloon injury induced an early massive apoptosis and proliferation which was accompanied by a low-level apoptosis at a later phase in the media of rabbit iliac artery. In intima the proliferative activity reached its peak a little later than in media, again accompanied by
a second low-level apoptosis. The sequential changes of p53 expression were similar to those of apoptotic activities, showing an early large peak and at a later stage a smaller secondary peak. Hypercholesterolemia did not affect the early post injury apoptosis in media but enhanced the later proliferation and low-level apoptosis. This resulted in medial and neointimal hyperplasia because the effect of HC was more prominent on proliferation rather than apoptosis, which was largely mediated by the macrophage infiltration in outer intima and inner media.

4.1. Apoptotic activity

We found that the apoptotic activity after vascular injury was comprised of two components, early massive apoptosis associated with the injury and the later low-level apoptosis associated with proliferation.

We observed the highest apoptotic activity within the media at day 1. This early massive apoptosis after vascular injury, has been reported in previous studies, which showed that about 70% of medial cells underwent apoptosis as early as 30 min after denudation balloon injury to rat carotid artery [7], and that about 9% of medial cells were TUNEL-positive at 18 h after over-stretch balloon injury to porcine coronary artery [8].

In addition to this early medial apoptosis, we observed a low-level of apoptosis at a later stage, which accompanied proliferation in both the media and the intima. This proliferation-associated apoptosis in either intima or media was observed from 7 until 14 days after injury, which is consistent with previous reports [3,8]. One study demonstrated that about 40% of neointimal cells were TUNEL-positive 9 days after injury to rat carotid arteries, 20% at 2 weeks, and 10% at 4 weeks [3]. Another reported that TUNEL indices in intima reached a peak, 2%, 7 days after porcine coronary angioplasty [8].

The timing of the stages involved in apoptosis after vascular injury, i.e. the early medial apoptosis within 24 h and the late proliferation-associated apoptosis at 1–2 weeks after injury, are consistent with those of previous studies, however, the absolute value of TUNEL indices differ. Variations of reported TUNEL indices seem to originate from the species of animal used, the severity and duration of balloon injury and the sensitivity of TUNEL staining.

We find it interesting that hypercholesterolemia did not influence the early medial apoptosis but enhanced the proliferation, and the later apoptosis which occurred between the 7th and 14th day and was associated with proliferation. These findings suggest that the mechanism of the early medial apoptosis might differ from that of the later proliferation-associated apoptosis. In other words, it appears that the early massive apoptosis after balloon injury is primarily caused by the mechanical injury and its resulting disruption of the integrin–matrix protein interactions. This has been proposed previously [7]. In contrast, the later low-grade apoptosis that was associated with proliferation may be caused by biochemical factors, related to hypercholesterolemia, such as oxidized LDL that has dual effect, induction of proliferation and apoptosis [24]. One of the important factors that enhanced the late apoptosis in HC rabbits in this study seems to be the infiltration of macrophages. These can secrete proapoptotic factors such as reactive oxygen species and nitric oxide, etc. [25–28].

4.2. P53 over-expression

P53 was reported to be in atherosclerotic lesions [9,10] and to be an important mediator of apoptosis of smooth muscle cells from human atherosclerotic plaque [11]. We demonstrated that p53 expression was remarkably induced in media immediately after balloon-injury and that it decreased afterwards allowing vascular cells to proliferate. In intima p53 expression, which accompanies cell proliferation, was observed from 7 to 14 days after injury. This is in accord with the findings of a previous publication [14].

Hypercholesterolemia did not change the early remarkable induction of p53 expression in media, whereas it increased the later low-level p53 expression in media and neointima. These sequential changes of p53 expression and the effect of hypercholesterolemia are very similar to the pattern of apoptosis after injury, which suggests that these two phenomena are linked. However, p53 indices were generally much higher than TUNEL indices, which suggested that among the many cells in which DNA was damaged, only a fraction had irreversibly damaged DNA and it was these which were finally consigned to apoptosis.

The mechanism of the hypercholesterolemia-independent p53 expression in the earlier phase might involve DNA damage, due to oxidative stresses, which are induced immediately after the balloon-injury [16]. The later hypercholesterolemia-dependent p53 expression might be due to superoxide anions, which are generated by infiltrating macrophages.

4.3. Proliferative activity

As a result of the over-stretch balloon injury to rabbit iliac arteries in this study, proliferation peaked after 3 days in media and 7 days in intima and subsequently decreased. These sequenced changes of PCNA indices in media and intima, and its peak values (5%), were comparable to those of a previous study [8]. Hypercholesterolemia did not affect the initial surge of proliferation in media, whereas it increased prolifera-
tion in media or maintained proliferative activity in intima during the later phase. These results suggest that the stimuli producing proliferation during the initial and the later stages may differ.

The initial surge of proliferation follows the apoptosis of media immediately after the injury. This suggests that the release of cell-to-cell contact inhibition may initiate the proliferation or that release of growth factors, such as the basic fibroblast growth factor from cells undergoing apoptosis, may stimulate adjacent surviving cells to proliferate. The proliferation at this initial phase would be expected to be independent of hypercholesterolemia, because its main triggering factor, early massive apoptosis, is not influenced by hypercholesterolemia.

In contrast, the proliferation during the later phase was enhanced by hypercholesterolemia, which suggests that the proliferation at this phase may be mediated by the growth factors released from the infiltrating macrophages [29,30].

4.4. Intimal and medial area

Hypercholesterolemia increased areas of the neointima and media and enhanced proliferation and apoptosis accompanying proliferation. PCNA indices in the HC group were higher than those in the NC group by 5% in the media and 4% in the neointima, but TUNEL indices were only slightly increased (2 and 0.5%, respectively). In other words, hypercholesterolemia enhanced proliferation more than the accompanying apoptosis, in turn this resulted in an increase in the areas of neointima and media in the HC group. Another explanation is that infiltration of macrophages itself in the HC group may contribute to the increase in areas. We observed that at 7 and 14 days, 30 and 50%, respectively of total proliferating cells were surviving cells to proliferate. The proliferation at this initial phase would be expected to be independent of hypercholesterolemia, because its main triggering factor, early massive apoptosis, is not influenced by hypercholesterolemia.

In conclusion, balloon injury induces early massive, p53-associated, apoptosis followed by a surge of proliferation in media. Conversely in intima, it induces active proliferation followed by a low-level apoptosis. The early post injury apoptosis and the surge of proliferation which follows, were not modulated by hypercholesterolemia. At a later stage, hypercholesterolemia induces macrophage infiltration and enhances proliferation to a greater extent than it enhances the low-level apoptosis which accompanies proliferation, which results in neointimal and medial hyperplasia.

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References


