Progression of coronary atherosclerosis relates to the onset of myocardial infarction in an animal model of spontaneous myocardial intarction (WHHLMI rabbits)

Ito, Takashi

Yamada, Satoshi

Shiomi, Masashi

(Citation)
Experimental Animals,53(4):339-346

(Issue Date)
2004-07

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/90004587

Exp. Anim. 53(4), 339–346, 2004

Progression of Coronary Atherosclerosis Relates to the Onset of Myocardial Infarction in an Animal Model of Spontaneous Myocardial Infarction (WHHLMI Rabbits)

Takashi ITO, Satoshi YAMADA, and Masashi SHIOMI

Institute for Experimental Animals, Kobe University School of Medicine, 7–5–1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Abstract: *Recently, we developed myocardial infarction-prone WHHLMI rabbits from coronary atherosclerosis-prone WHHL rabbits (WHHLCA rabbits) by selective breeding. In this study, we examined the relation of atherosclerotic plaques to the onset of myocardial infarction. We examined myocardial lesions of 378 WHHL rabbits born between 1992 and 2000, and atherosclerosis lesions of 93 WHHLCA and 82 WHHLMI rabbits. The aortic lesions were evaluated as percent surface lesion area. The coronary lesions were evaluated as cross sectional narrowing using sections prepared at 500 or 1,000 μm intervals. Serum lipid levels were assayed with enzymatic methods. The cumulative incidence of fatal myocardial infarction between 11 and 35 months old was 90% in WHHLMI rabbits and 21% in WHHLCA rabbits, respectively. Selective breeding increased the serum cholesterol levels by about 200 mg/dl despite there being no changes in triglyceride levels. Aortic and coronary atherosclerosis progressed markedly in WHHLMI rabbits compared to WHHLCA rabbits. Especially, WHHLMI rabbits over 15 months old showed more than 90% cross sectional narrowing of the left circumflex arteries, main stem of the left coronary artery, and the origin portion of the right coronary artery. In addition, there were no gender differences in atherosclerotic lesions of both aortas and coronary arteries. In conclusion, the present study showed that marked progression of coronary atherosclerosis was probably associated with spontaneous development of myocardial infarction in WHHLMI rabbits.*

Key words: *aortic atherosclerosis, coronary atherosclerosis, myocardial infarction, selective breeding, WHHLMI rabbit*

Introduction

Ischemic coronary heart diseases (myocardial infarction, unstable angina, and sudden cardiac death) are major causes of death in developed countries and studies into them have been carried out [8]. However, the mechanism of the onset of the diseases has not been elucidated and prediction of the patients with a future risk of developing ischemic coronary heart diseases is currently difficult [8]. To promote studies into ischemic

(Received 23 January 2004 / Accepted 22 March 2004)

Address corresponding: M. Shiomi, Institute for Experimental Animals, Kobe University School of Medicine 7–5–1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

coronary heart diseases, the development of a suitable animal model has long been awaited [6, 8]. Recently, several genetically modified mouse models with hypercholesterolemia and atherosclerosis have been reported [2, 4, 7]. In many of them, however, coronary atherosclerosis and subsequent myocardial lesions resembling those of humans have not been documented, possibly due to the difference in lipoprotein metabolism between humans and mice [3, 5]. In addition, the mouse is small in size, which may hamper its use in many surgical manipulations and therapeutic interventions. Therefore, there is a need to develop a relatively large animal model for the study of myocardial infarction. Recently, we first developed a rabbit model for spontaneous myocardial infarction (WHHLMI rabbit) by selective breeding [11]. In 1980, our institute developed Watanabe heritable hyperlipidemic (WHHL) rabbits as an animal model of human familial hypercholesterolemia and atherosclerosis [6, 13]. From this rabbit strain, we further developed coronary atherosclerosis-prone WHHL rabbits (WHHLCA rabbits), which have typical coronary atheromatous plaques similar to those of humans [10]. However, the incidence of myocardial infarction in WHHLCA rabbits was extremely low. Since 1994, we have been attempting to develop an animal model for spontaneous myocardial infarction by serial and selective breeding of the WHHLCA rabbits. After six years of selective breeding, we developed the WHHLMI strain for spontaneous myocardial infarction [11]. At the onset of myocardial infarction, coronary occlusion is important and the coronary atherosclerotic plaques progress markedly in WHHLMI rabbits [11]. WHHLMI rabbits showed findings of fresh myocardial lesions with or without old myocardial lesions [11]. This rabbit model is expected to contribute to studies of ischemic coronary heart diseases and the development of new therapies and/or compounds for the disease [9]. Prior to using WHHLMI rabbits for studies about myocardial infarction, atherosclerosis, and others, it is important to clarify the basic characteristics of this rabbit model. In this study, we examined the degree of atherosclerosis developed in WHHLMI rabbits and studied the relation of coronary atherosclerosis to the onset of myocardial infarction.

Materials and Methods

Animals

We examined 931 rabbits for serum lipid levels and 378 rabbits for myocardial lesions. These rabbits were born between 1992 and 2000 in the WHHL rabbit colony of Kobe University. At the time of death, the heart and aorta were excised. We examined aortic and coronary atherosclerosis of 93 WHHLCA rabbits born in 1992 and 1993, and 82 WHHLMI rabbits born in 1999 and 2000. The rabbits were housed individually in metal cages in a room with a constant temperature (22 ± 2°C) and constant lighting cycle (12 h light/dark), and were fed standard rabbit chow (120 g per day; CR-3, Clea Japan, Tokyo, Japan) and water (*ad libitum*). This study was approved by the Institutional Animal Care and Use Committee (Permission number: P-930951, P-961004-R, and P-011010R) and carried out according to the Guidelines of Animal Experimentation of Kobe University.

Selective breeding

To develop a rabbit model for spontaneous myocardial infarction, we [11] carried out selective breeding by selecting descendants of WHHLCA rabbits with the following features; (i) development of myocardial infarction, (ii) macrophage-rich coronary plaque, (iii) coronary lumen stenosis greater than the values of the mean plus the standard error in their parents' generation, and (iv) serum total cholesterol levels greater than 800 mg/dl at 12 months old, and greater than 700 mg/dl at 24 months old. During the selective breeding, the conditions of housing, and feeding were maintained at a constant, and rabbits did not receive any intervention except for selective breeding.

Measurement of serum lipid levels

Blood samples were taken from the marginal ear vein after overnight fasting at the ages of 2, 6, 12, 18, and 24 months old. Serum cholesterol and triglyceride levels were measured by enzymatic methods.

Evaluation of myocardial lesions

Serial heart sections were examined under a light microscope and the degree of myocardial lesions was microscopically evaluated as percent lesion area in the left ventricle wall by two different researchers. We

defined a myocardial lesion area above 10% of the area of the left ventricle wall accompanied by occluded coronary atheromatous plaques at the upper stream as myocardial infarction.

Evaluation of aortic atherosclerosis

After excision, the aortas were immediately cut open longitudinally. The degree of atherosclerosis developed at the aorta was grossly evaluated as percent surface lesion area (surface lesion area/ surface lumen area × 100) by two different researchers.

Evaluation of coronary atherosclerosis

The hearts were fixed with Bouin's fixative or 10% neutral buffered formalin solution and embedded in paraffin [10]. The paraffin blocks were sectioned serially and serial sections prepared at 500 or 1,000 μm intervals were stained with hematoxylin and eosin staining, elastic van Gieson staining, or Azan-Mallory staining. Using sections stained with elastic van Gieson staining, the degree of coronary atherosclerosis was evaluated as percent cross-sectional narrowing (lesion area/area circumscribed by the internal elastic lamina × 100) microscopically by two independent researchers. In our examination of coronary stenosis, we also evaluated percent cross-sectional narrowing of elliptical coronary cross sections. As shown in Fig. 1, theoretically, the areas of cross sections showing the right circle shape (the area is 625 π mm^2 in the large circle, and are 25 π mm^2 in small circles) of a right cylinder (the diameter is 50 mm in the large base circle area, and 10 mm in small base circle areas) are correlated proportionally to those of the elliptical cross sections (the area is 1,250 π mm^2 in the large ellipse, and are 50 π mm^2 in small ellipses) obtained by cutting the same right cylinders obliquely at a 30 degree angle. No difference in the area of the small ellipses located at various positions in a large ellipse indicates that every small circle in the large circle is enlarged to the corresponding ellipse in the large ellipse (Fig. 1) by the same proportion. In addition, the area ratio of the sum of small circles/ large circle (25 π mm^2 × 4 / 625 π mm^2 = 0.16) is equal to that in an elliptical cross section (50 π mm^2 × 4 / 1,250 π mm^2 = 0.16). We confirmed this proportional relation between a circle and the ellipse prepared by cutting obliquely in practice by measuring areas of the right circle shape cross section and the elliptical cross section prepared with one right cylinder with an image analyzer. Therefore, even if arteries were sectioned obliquely, the percent cross-sectional narrowing is not affected by oblique sectioning.

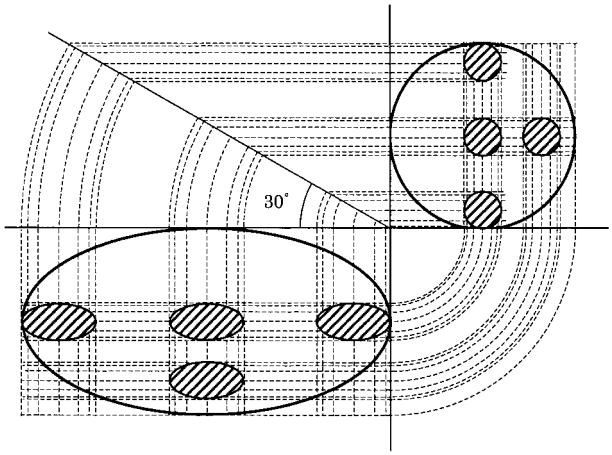

Fig. 1. Relation of the area between the right circle and ellipse of a right cylinder (the diameter is 50 mm). The diameter is 50 mm in a large circle and 10 mm in each small circle. The ellipse shape cross section is obtained by cutting the cylinder at a 30 degree angle obliquely. The short diameter is 50 mm in the larger ellipse and 10 mm in small ellipses, and the long diameter is 100 mm in the larger ellipse and 20 mm in each small ellipse. The area measured with an image analyzer is 625 π mm^2 in the large circle, 25 π mm^2 in each small circle, 1,250 π mm^2 in the large ellipse, and 50 π mm^2 in each small ellipse. The area ratio of the sum of small circles to the large circle is 0.16, and that of the sum of small ellipses to the large ellipse is 0.16.

Statistical analysis

Data are represented as mean ± the SEM. Statistical analyses were carried out with the Mann-Whitney U-test for atherosclerotic lesions and the 2 × 2 or m × n Chi-square test for incidences. Statistical significance was set at $P<0.05$.

Results

Incidence of myocardial infarction

As shown in Fig. 2, the cumulative incidence of fatal myocardial infarction was increased by selective breeding. Although the cumulative incidence between 11 and 30 months old was 12% in WHHLCA rabbits born in 1992 and 1993 (before selective breeding), it in-

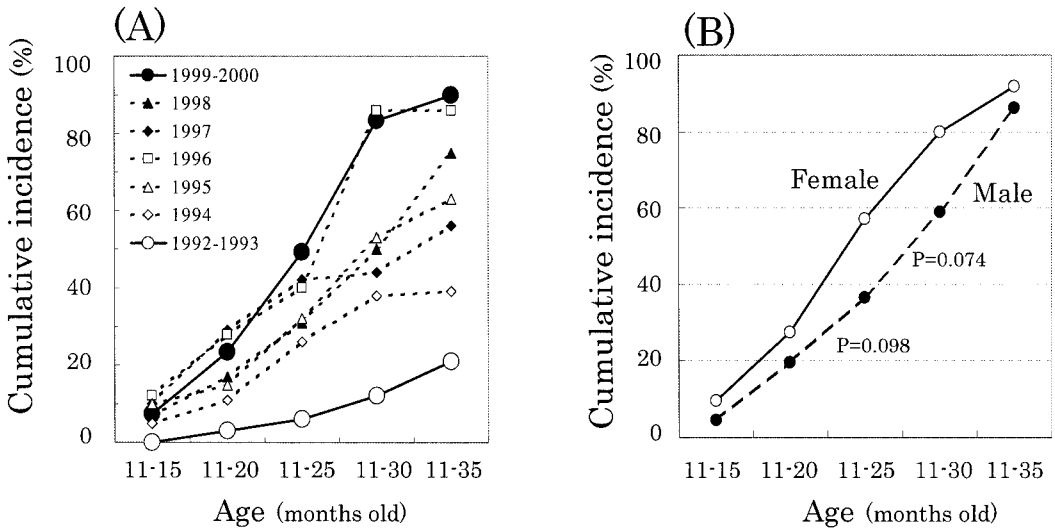

Fig. 2. Cumulative incidence of fatal myocardial infarction in WHHL rabbits. (A) Increase in the cumulative incidence of fatal myocardial infarction in WHHL rabbits during selective breeding. Examined rabbits were 30–82 per group. (B) Cumulative incidence of fatal myocardial infarction was 37–42 in females (○) and 29–46 in males (●) WHHLMI rabbits (born in 1999 and 2000) at each age. Statistical analysis was carried out with the 2 × 2 Chi-square test.

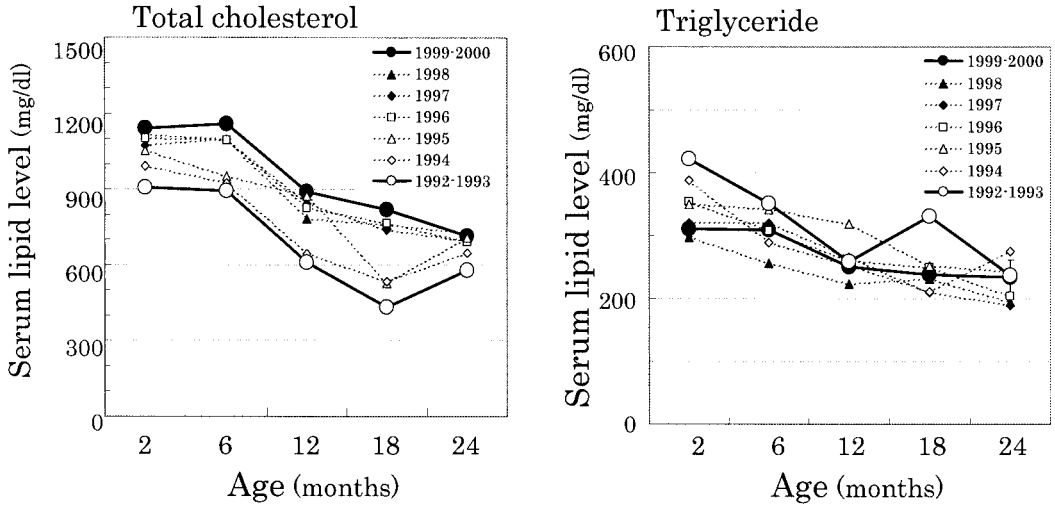

Fig. 3. Changes in serum lipid levels of WHHL rabbits during selective breeding. Serum lipid levels were assayed using 67–288 rabbits in each group. Data are represented as mean ± the standard error of the mean.

creased to 90% in WHHLMI rabbits born in 1999 and 2000 (after selective breeding). In the WHHLMI rabbits, fatal myocardial infarction was observed from 11 months old, and rabbits suffering from fatal myocardial infarction between 11 and 20 months old increased by about 3-fold compared to rabbits aged between 11 and 15 months. Compared to the female rabbits, the cumulative incidence of fatal myocardial infarction was relatively low in males, but this difference was not significant.

Serum lipid levels

Figure 3 shows changes in serum lipid levels of WHHL rabbits during selective breeding. The serum

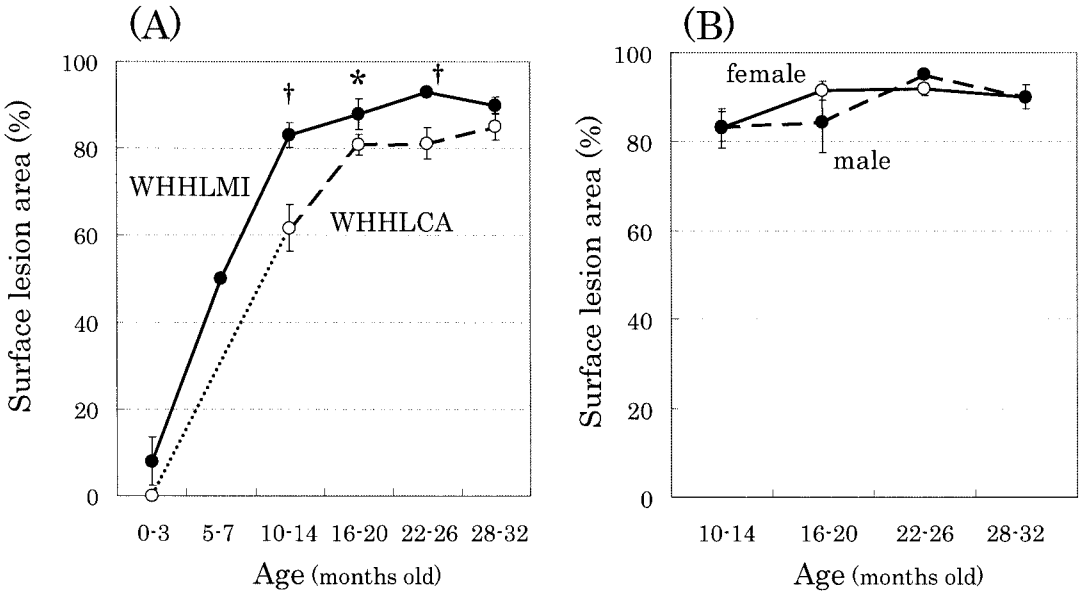

Fig. 4. Changes in percent area of the aortic surface lesion area with aging. (A) 57 WHHLMI rabbits (●) and 41 WHHL rabbits before selective breeding (WHHLCA rabbits, ○). Examined rabbits were 11–13 in WHHLCA and 8–17 in WHHLMI rabbits at ages between 10–26 months, and at other age groups, examined rabbits were less than 5. (B) Percent area of aortic surface lesion area of 5–11 female (○) and 6-7 male (●) WHHLMI rabbits at each age. Data are represented as mean ± the standard error of the mean. Statistical analysis was carried out with the Mann-Whitney U-test. *: $P<0.05$, †: $P<0.01$.

cholesterol levels of WHHLMI rabbits (born in 1999 and 2000) increased by about 200 mg/dl at every assay point compared to the WHHLCA rabbits (born in 1992 and 1993). However, the serum triglyceride levels of WHHLMI rabbits were similar to those of WHHLCA rabbits. Serum lipid levels decreased gradually with aging.

Degree of aortic atherosclerosis

Figure 4 shows that the aortic surface lesion area of WHHLMI rabbits significantly increased compared to the WHHLCA rabbits born before selective breeding. Above 10 months old, the atherosclerotic lesions covered more than 80% of aortic surface lumen in WHHLMI rabbits. In addition, there was no gender difference in the degree of aortic atheromatous lesions in WHHLMI rabbits.

Degree of coronary atherosclerosis

As shown in Fig. 5, percent cross-sectional narrowing of coronary arteries of WHHLMI rabbits significantly increased at each arterial segment at each age compared to WHHLCA rabbits. In the WHHLCA rabbits, the cross-sectional narrowing was mild in left anterior descending, left circumflex, septal, and right coronary arteries despite developing extensive stenosis at the main stem of the left coronary artery and the origin portion of the right coronary artery. In WHHLMI rabbits, the coronary cross-sectional narrowing significantly increased in all arterial segments except the origin portion of the right coronary arteries. Especially, at the left circumflex arteries, which are the main coronary artery in rabbits, the average percent cross-sectional narrowing was greater than 80% above 10 months old and greater than 90% above 15 months old. Figure 6 shows that there was no gender difference in the degree of the atheromatous plaques of the left circumflex arteries of WHHLMI rabbits. Similar findings were observed in the other arterial branches (data not shown).

Relation of coronary atherosclerosis to the onset of myocardial infarction

To examine the relation between coronary atherosclerosis and the onset of myocardial infarction, WHHL rabbits born in 1994, 1995, 1997, and 1998 were analyzed. These rabbits were located between WHHLCA

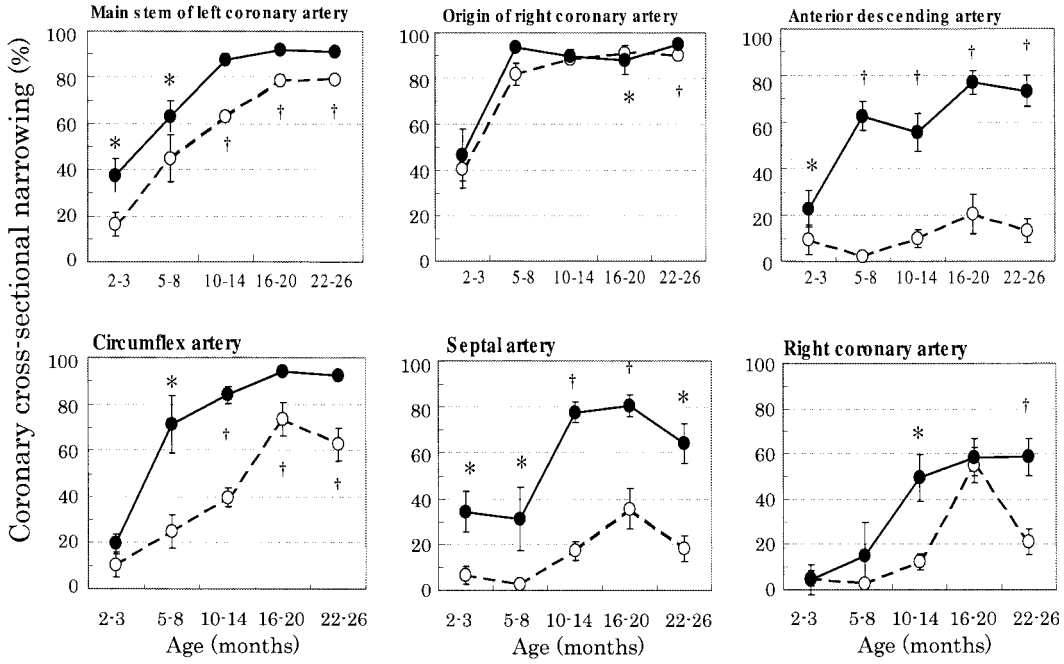

Fig. 5. Coronary atherosclerosis of WHHLMI rabbits (●) and WHHL rabbits before selective breeding (○) at each arterial segment. Examined rabbits were 14–33 for each age and data were presented using the most severe stenosis at each arterial segment. Data are represented as mean ± the standard error of the mean. Statistical analysis was carried out with the Mann-Whitney U-test. *: P<0.05, †: P<0.001.

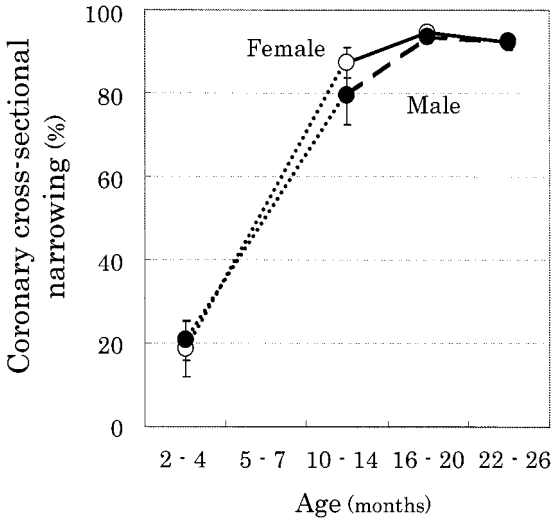

Fig. 6. Progression of coronary stenosis of the left circumflex arteries of female (○) and male (●) WHHLMI rabbits. Examined rabbits were 7–10 males and 7–12 females at each age. Data are represented as mean ± the standard error of the mean. Statistical analysis was carried out with the Mann-Whitney U-test.

and WHHLMI rabbits, and the cumulative incidence of myocardial infarction was 40–80% (Fig. 2-A). Figure 7-A shows that the progression of coronary plaques was significantly related to the onset of myocardial infarction in each age group. Especially, the incidence of myocardial infarction was 100% in rabbits that suffered from coronary plaques with above 90% cross-sectional narrowing. In addition, an increase in serum cholesterol levels was also significantly related to the onset of myocardial infarction (Fig. 7-B).

Discussion

In the WHHLMI rabbits, myocardial infarction was developed from 11 months old and the incidence increased markedly above 15 months old (Fig. 2). The cross-sectional narrowing of the left circumflex arteries was more than 90% above 15 months old (Fig. 5), and the increase of percent cross-sectional narrowing of the left circumflex arteries was closely related to the onset of myocardial infarction at the left ventricle wall of WHHLMI rabbits (Fig. 7-A). In addition, severe coro-

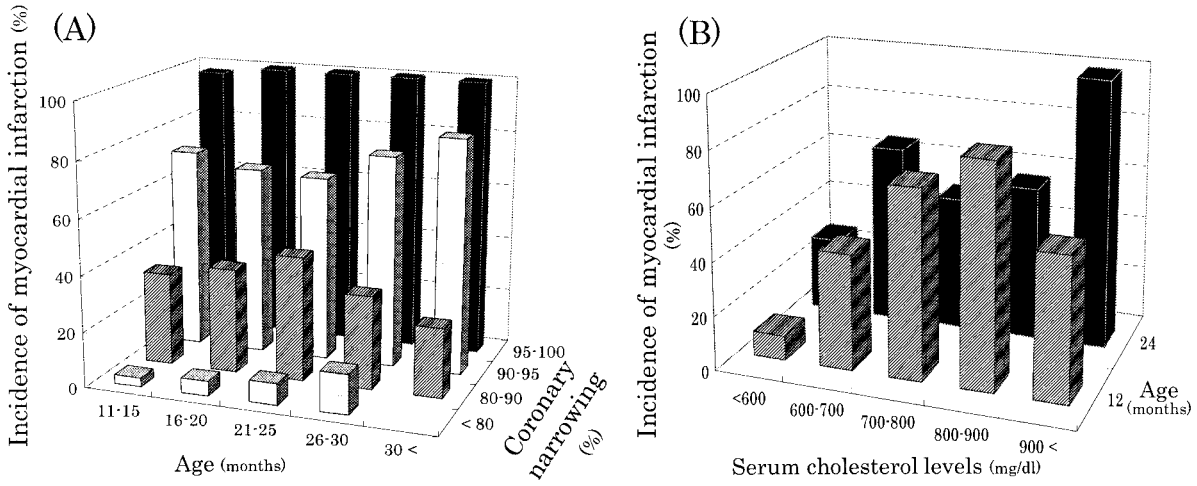

Fig. 7. Relations among incidence of myocardial infarction, degree of coronary atherosclerosis, and serum cholesterol levels in WHHL rabbits born at 1994, 1995, 1997, and 1998. (A) Relation between the incidence of myocardial infarction and degree of coronary cross-sectional narrowing at the left circumflex arteries. Examined rabbits were 6–41 per group with coronary stenosis 95% or above, 14–36 per group with coronary stenosis between 90–95%, 4–30 per group with coronary stenosis between 80–90%, and 0–32 per group with coronary stenosis below 80% at each age group. (B) Relation between the incidence of myocardial infarction and serum cholesterol levels at left circumflex arteries. Examined rabbits were 11–30 in 12 months group, and 7–21 in 24 months group at each serum cholesterol level. Statistical analyses were carried out with the m × n Chi-square test. In Panel-A, differences in incidence of myocardial infarction among each coronary cross-sectional narrowing group were significant (P=0.009 in the above 30 months old group and P<0.001 in other age groups). In Panel-B, differences in incidence of myocardial infarction among each serum cholesterol level were significant in 12 months old group (P=0.001) and 24 months old group (P=0.014).

nary stenosis developed in the main stem of the left coronary artery and the origin portion of the right coronary artery probably relates to circumferential myocardial infarction at the left ventricle wall and ischemic myocardial lesions in the right ventricle wall.

The serum cholesterol levels of WHHLMI rabbits are about 200 mg/dl higher at each age than those of WHHLCA rabbits (Fig. 3). In our recent study [15], the serum lipid levels of male WHHLMI rabbits were lower than those of females. Although it is well known that serum cholesterol levels are closely related to the development of atherosclerosis [1, 6, 10, 14], the present study showed there was no gender difference in the degree of aortic and coronary atherosclerosis of WHHLMI rabbits (Figs. 4 and 6). The onset of myocardial infarction of this model also showed no gender difference (Fig. 2). In addition, some WHHLCA rabbits with equally high serum cholesterol levels did not suffer from myocardial infarction and coronary stenosis was relatively mild. We consider the reason why the degree of atherosclerosis of WHHLMI rabbits did not relate to the serum cholesterol levels to be as follows: (i) risk factors other than serum cholesterol levels probably play an important role in the development / progression of coronary atherosclerosis; and (ii) the sufficiently high serum cholesterol levels of WHHLMI rabbits may have to exceed a threshold to develop or advance atherosclerotic plaques.

In this study, we examined aortic and coronary atherosclerosis of WHHLMI rabbits and showed that atherosclerotic lesions of WHHLMI rabbits progressed markedly compared to the WHHLCA rabbit, which was the progenitor of the WHHLMI rabbit. In the development of WHHLMI rabbits, we selected descendants of WHHLCA rabbits with the following features: (i) development of myocardial infarction; (ii) macrophage rich coronary plaques; (iii) coronary cross-sectional narrowing greater than the values of the mean plus the standard error in their parents; and (iv) serum total cholesterol levels greater than 800 mg/dl at 12 months old and greater than 700 mg/dl at 18 months old [11]. As a result, in the WHHLMI rabbits, the serum cholesterol levels increase by about 200 mg/dl, the coronary atheromatous plaques progress, and myocardial infarction

develops spontaneously [11]. Our present results demonstrate that these parameters set by selective breeding were effective in the progression of atheromatous plaques of coronary arteries as well as aortas and in subsequent myocardial infarction. Since selective breeding markedly increased the frequency of macrophage-rich coronary plaques at ages between 11 and 30 months old (data not shown), the increase of macrophage contents in the plaque is probably related to the progression of atherosclerosis.

In conclusion, the present findings demonstrate that the development of fatal myocardial infarction in WHHLMI rabbits was related to the progression of coronary atherosclerosis.

Acknowledgments

This work was supported in part by unrestricted research grants from Sankyo Co. Ltd. We would like to appreciate Mr. Toshiaki Tamura for preparation of histological sections.

References

1. Anitchkow, N. and Chalatow, S.S. 1913. Uber experimenteller Cholesterinsteatose und ihre Bedeutung fur die Entstehung einiger pathologischer Prozesse. *Zentralbl. Allg. Pathol. Anat.* 24: 1–9.
2. Braun, A., Trigatti, B.L., Post, M.J., Sato, K., Simons, M., Edelberg, J.M., Rosenberg, R.D., Schrenzel, M., and Krieger, M. 2002. Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Cir. Res.* 90: 270–276.
3. Brousseau, M.E. and Hoeg, J.M. 1999. Transgenic rabbits as models for atherosclerosis research. *J. Lipid Res.* 40: 365–375.
4. Carligiuri, G., Levy, B., Pernow, J., Thoren, P., and Hansson, G.K. 1999. Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. *Proc. Natl. Acad. Sci. USA* 96: 6920–6924.
5. Fan, J., Challah, M., and Watanabe, T. 1999. Transgenic rabbit models for biomedical research: current status, basic methods, and future perspectives. *Pathol. Int.* 49: 583–594.
6. Goldstein, J.L., Kita, T., and Brown, M.S. 1983. Defective lipoprotein receptors and atherosclerosis. Lessons from an animal counterpart of familial hypercholesterolemia. *N. Engl. J. Med.* 309: 288–296.
7. Kuhlencordt, P.J., Gyurko, R., Han, F., Scherrer-Crosbie, M., Aretz, T.H., Hajjar, R., Picard, M.H., and Huang, P.L. 2001. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. *Circulation* 104: 448–454.
8. Naghavi, M., Libby, P., Falk, E., Casscells, S.W., Litovsky, S., Rumberger, J., Badimon, J.J., Stefanadis, C., Moreno, P., Pasterkam, G., Fayad, Z., Stone, P.H., Waxman, S., Raggi, P., Madjid, M., Zarrabi, A., Burke, A., Yuan, C., Fitzgerald, P.J., Siscovick, D.S., de Korte, C.L., Aikawa, M., Airaksinen, K.E.J., Assmann, G., Becker, C.R., Chesebro, J.H., Farb, A., Galis, Z.S., Jackson, C., Jang, I., Koenig, W., Lodder, R.A., March, K., Demirovic, J., Navab, M., Priori, S.G., Rekhter, M.D., Bahr, R., Grundy, S.M., Mehran, R., Colombo, A., Boerwinkle, E., Ballantyne, C., Insull, W. Jr., Schwartz, R.S., Vogel, R., Serruys, P.W., Hansson, G.K., Faxon, D.P., Kau, L.S., Drexler, H., Greenland, P., Muller, J.E., Virmani, R., Ridker, P.M., Zipes, D.P., Shah, P.K., and Willerson, J.T. 2003. From vulnerable plaque to vulnerable patient, a call for new definitions and risk assessment strategies: Part I. *Circulation* 108: 1664–1672.
9. Shiomi, M., Fan, J., and Kawashima, S. September 5, 2003. Relation of vulnerable plaque to development of myocardial infarction/acute coronary syndromes—Lessons from an animal model of spontaneous myocardial infarction. Commentary to the International Atherosclerosis Society, IAS Website (http://www.athero.org/comm-index.asp).
10. Shiomi, M., Ito, T., Shiraishi, M., and Watanabe, Y. 1992. Inheritability of atherosclerosis and the role of lipoproteins as risk factors in the development of atherosclerosis in WHHL rabbits: Risk factors related to coronary atherosclerosis are different from those related to aortic atherosclerosis. *Atherosclerosis* 96: 43–52.
11. Shiomi, M., Ito, T., Yamada, S., Kawashima, S., and Fan, J. 2003. Development of an animal model for spontaneous myocardial infarction (WHHLMI rabbits). *Arterioscler. Thromb. Vasc. Biol.* 23: 1239–1244.
12. Stamler, J., Wentworth, D., and Neaton, J.D. 1986. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screeners of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 256: 2823–2828.
13. Watanabe, Y. 1980. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). *Atherosclerosis* 36: 261–268.
14. Working Committee on JAS Guideline for Diagnosis and Treatment of Hyperlipidemia. 2002 Report of the Japan Atherosclerosis Society (JAS) guideline for diagnosis and treatment of hyperlipidemia in Japanese adults. *J. Atheroscler. Thromb.* 9: 1–27.
15. Yamada, S., Ito, T., and Shiomi, M. 2004. Age-related changes in serum/plasma biochemical parameters of WHHLMI rabbits. *Exp. Anim.* 53: 159–163.