The Influences of In Vivo Cholesterol Feeding on the Growth Properties of Enzyme-dispersed Rabbit Aortic Smooth Muscle Cells in Primary Culture*


Introduction

Experimental atherosclerosis of cholesterol-fed rabbits is one of the most popular animal models of arteriosclerosis. However, it is possible that the atherosclerotic lesions caused by the extremely high cholesterol levels are somewhat different from those of human beings.

In the present study we examined the influences of short-term cholesterol feeding on the proliferative activity of enzyme-dispersed rabbit aortic smooth muscle cells (SMCs) in primary culture.

Materials and Methods

Male Japanese White rabbits weighing about 3 kg (n=3) were fed rabbit chow containing 1% cholesterol for 2 weeks. Control rabbits (n=3) were fed the chow without added cholesterol.

Thoracic aortas were isolated aseptically and the intima and media were dissected from the adventitia. And the intima-medias were cut into small pieces and dispersed into single cells by collagenase and elastase1). No attempt was made to remove endothelium so as not to make a biased cell population. Isolated aortic cells were seeded in tissue culture dishes (Falcon 3001, ø35 mm) in Eagle's minimum essential medium (MEM) containing 10% of fetal bovine serum. The cultures were incubated in 5% CO₂−95% air at 37°C. The medium was changed every three days. For growth curves cell counts were made in triplicate for each dish, using a hemocytometer. Part of the aortic tissue was prepared for pathological examination.

Cells from control rabbits were passaged when they reached confluence and were used as subcultured SMCs. The influences of normolipidemic serum from control rabbits and hyperlipidemic serum from cholesterol-fed rabbits on the growth curves of the cells were then studied.

Results

Serum biochemical data at autopsy were as follows: total cholesterol (TC) 31.0±5.3 mg/dl, phospholipid (PL) 62.9±6.5 mg/dl, triglyceride (TG) 56.8±16.6 mg/dl in control rabbits, and TC 991.3±320.0 mg/dl, PL 457.7±160.0 mg/dl, TG 194.8±90.6 mg/dl in cholesterol-fed rabbits.

Pathologically no abnormal findings were observed in control aortas. In cholesterol-fed rabbits, marked subendothelial edema, so-called standing-up phenomenon of medial SMCs and occasionally a small number of intimal foam cells were observed.

SMCs in primary culture grew to confluence, showing so-called hills-and-valleys pattern. The enzyme-dispersed SMCs from control rabbits had a lag phase of 4–5 days before they started to proliferate. On the other hand, cells from cholesterol-fed rabbits started to divide 2–3 days after seeding (Fig. 1) and also tended to grow to higher densities than control (saturation density; 12.9±2.35×10⁵ cells/dish in cholesterol-fed rabbits vs. 9.0±0.75×10⁵ cells/dish in controls, doubling time at the logarithmic growth phase; 34.8±12.9 hrs vs. 35.8±8.4 hrs).

Hyperlipidemic serum (HLS) from cholesterol-fed rabbits slightly enhanced the proliferation of subcultured SMCs compared to normolipidemic serum (NLS) when the serum was added at the concentration of 5% in the medium, however rather suppressed it when added at 10 or 20% concentration (Fig. 2).

* This paper was presented at the winter meeting of the Japan Atherosclerosis Society in Japan on January 1985
** Department of Geriatrics, Nagoya University School of Medicine, Nagoya 466, Japan
Discussion

The explant method of Ross has been used to culture vascular SMCs. However, the cells which migrated from the explant and proliferated have already undergone 'modulation' and show synthetic phenotype\(^2\). Chamley-Campbell et al.\(^1\,\,^2\) reported the enzyme-dispersed primary culture of SMCs, and by using the method we can obtain isolated SMCs in a morphologically well-differentiated and contractile state\(^2\). Enzymatically dissociated SMCs from adult pig aortic media are in the contractile state and do not divide in the first few days. After 6–8 days the cells spontaneously undergo a change in phenotype and start to divide\(^3\). However, we already reported that SMCs from bovine fetuses do not have such a long lag phase and start to grow within 2 days after seeding\(^4\).

In the present study, the lag phase of enzyme-dispersed rabbit aortic SMCs in primary culture was shortened after cholesterol feeding for only 2 weeks. It is suggested that such short-term cholesterol feeding can affect the tendencies of rabbit aortic SMCs to proliferate. Very recently, Kallioniemi et al.\(^5\) reported that enzyme-isolated SMCs from normal aortas of NZW rabbits did not start to grow and incorporate radioactive thymidine until 5–6 days after seeding, whereas those from atherosclerotic aortas of the rabbits fed cholesterol for 4 months did so within 2 days. The present study shows that the change of proliferative activity of aortic SMCs might occur sooner after cholesterol feeding. And it was also reported that mitotic counts in SMCs in the trifurcation region, which is a site of predilection for atherosclerosis, of the aortas of cholesterol-fed swine for 3 days were significantly greater than in controls\(^6\).

It is reported that hyperlipidemic serum or the LDL stimulates the proliferative activity of SMCs and is rather cytotoxic in high concentrations. And our results support this. However, our results also show that the enzyme-dispersed SMCs in primary culture from cholesterol-fed rabbits have greater proliferative activity independent of the presence of hyperlipidemic serum and that the cells themselves have acquired increased proliferative characteristics.

References

4) Naito, M., et al.: The effect of Ca\(^{2+}\)-antagonists on...
Enzyme-dispersed Rabbit Aortic Smooth Muscle Cells in Primary Culture


Summary

We studied the influences of short-term (2-week) cholesterol feeding on the proliferative activity of enzyme-dispersed rabbit aortic smooth muscle cells in primary culture. The cells were cultured in Eagle's minimum essential medium containing 10% fetal bovine serum. Marked subendothelial edema was observed in the aortas of cholesterol-fed rabbits by pathological examination. The enzyme-dispersed smooth muscle cells from control rabbits had a lag phase of 4–5 days before they started to proliferate. On the other hand, cells from cholesterol-fed rabbits started to divide 2–3 days after seeding and tended to grow to higher densities than controls.

Hyperlipidemic serum from cholesterol-fed rabbits slightly enhanced the proliferation of subcultured smooth muscle cells compared to normal lipidemic serum when the serum was added at the concentration of 5% in the medium, but rather suppressed it when added at 10 or 20% concentration.

Our results indicate that the enzyme-dispersed smooth muscle cells in primary culture from cholesterol-fed rabbits have greater proliferative activity independent on the presence of hyperlipidemic serum and that the cells themselves have acquired increased proliferative abilities.

Key words: hypercholesterolemia, smooth muscle cells, growth curves, enzyme-dispersion, primary culture.