Long-Term Effects of Verapamil on Aortic Smooth Muscle Cells Cultured in the Presence of Hypercholesterolemic Serum

Olga Stein, Gideon Halperin, and Yechezkiel Stein

Smooth muscle cells derived from rabbit and bovine aorta were cultured for up to 5 weeks in the presence of d < 1.019 g/ml fraction of hypercholesterolemic rabbit serum. When this fraction was added to serum containing culture medium, there was a significant increase in DNA, protein, and cholesterol ester per dish. Addition of 50 μM verapamil markedly reduced the stimulatory effect of the d < 1.019 g/ml fraction on both DNA and protein content per dish. The effect of verapamil on cholesterol ester content was more complex: there was an increase within the first week, but later the net accumulation of cholesterol ester per dish was lower than in untreated dishes. The recovery of less DNA in verapamil-treated dishes was not due to increased cell loss, as evidenced by retention of a residualizing marker, 3H-cholesterol linoleyl ether. Moreover, verapamil did reduce incorporation of 3H-thymidine into DNA. In verapamil-treated dishes, there was flattening and a cobblestone appearance of the cells. A hypothesis is proposed to explain the inhibitory effect of verapamil on the development of atheroma formation in cholesterol-fed rabbits. Assuming that macrophages play an active role in cholesterol ester removal from atheroma, verapamil, which reduces lysosomal cholesterol ester hydrolysis in macrophages, would permit the lipid-laden macrophage to remove more cholesterol ester per cell from the arterial wall. In addition, the presently reported results support the possibility that verapamil may impede the development of atheroma formation by reduction of smooth muscle cell proliferation. (Arteriosclerosis 7:585–592, November/December 1987)

Atherosclerosis in humans is a multifactorial disease characterized by an insidious rate of development that spans years and decades. These attributes complicate the evaluation of therapeutic interventions in vivo. Simulation of some aspects of the development of atheroma formation in cell culture did help to evaluate certain isolated facets of this process, but most of the studies were limited to relatively short periods of time.4–9 The present experiments were designed to mimic more closely conditions in vivo, and to that end rabbit aortic smooth muscle cells were cultured for a number of weeks in the presence of an atherogenic fraction10–11 isolated from hypercholesterolemic rabbit serum. This experimental system was used to study the effect of verapamil on cholesterol ester accumulation in view of the previously reported12–16 effects of calcium antagonists on the attenuation of atherosclerosis in cholesterol-fed rabbits. The results reported here indicate that in long-term cultures, verapamil reduced the proliferation of smooth muscle cells and, to some extent, the accretion of esterified cholesterol.

This investigation was supported in part by Grant HL28454 from the National Institutes of Health and Grant 3410 from the United States-Israel Binational Science Foundation. Address for reprints: Dr. Yechezkiel Stein, Lipid Research Laboratory, Department of Medicine B, Hadassah University Hospital, P.O. Box 12000, Jerusalem 91120, Israel. Received February 17, 1987; revision accepted May 29, 1987.

Methods

Cell Cultures
Rabbit and bovine smooth muscle cells were grown from explants of aortas and were subcultured in Dulbecco-Vogt medium supplemented with 5% fetal bovine and 5% newborn calf serum.17 For the experiments, 50,000 to 70,000 cells were seeded in 35 mm Falcon Petri dishes and were grown for up to 5 weeks. Human skin fibroblasts were cultured in minimum essential medium supplemented with 10% fetal bovine serum.

Preparation of Lipoproteins
Rabbit lipoproteins were obtained from animals fed 1% cholesterol in their diet for 3 months. The sera were pooled and brought to a density of 1.019 g/ml with NaCl and the lipoproteins were separated by ultracentrifugation for 24 hours in a SW41 rotor at 100,000 g. The top was cut with the help of a tube slicer, and the lipoproteins were resuspended and sterilely filtered through a 0.45 μm Millipore filter. The percent composition by weight of the d < 1.019 g/ml fraction was: 17.9% protein, 42.5% cholesterol, 9.9% triglycerides, and 29.7% phospholipid.

Labelling of Lipoproteins
To incorporate 3H-cholesterol linoleyl ether (3H-CLE) into the rabbit lipoproteins, the labelled compound (20 μCi)
was placed in a test tube, the solvent was evaporated, and the dried lipid was sonicated with 2 ml saline containing 100 µl of d > 1.21 g/ml rabbit plasma (10 to 30 mg cholesterol). Sonication was carried out for 2 × 30 seconds using an 11 mm probe and a Braun Sonic 4000 instrument (Melsungen, West Germany). The sonicated labelled lipid, the d < 1.019 g/ml fraction of hypercholesterolemic rabbit plasma (10 to 30 mg cholesterol), and the d > 1.21 g/ml fraction of rabbit plasma serving as a source of cholesteryl ester transfer protein were incubated for 18 hours at 37°C under N₂. The final volume was about 6 ml, and the protein concentration of d > 1.21 g/ml fraction was 30 mg/ml. Thereafter, the lipoproteins were floated either sequentially at d = 1.006 g/ml and 1.019 g/ml or directly at d = 1.019 g/ml at 100,000 g for 24 hours. All labelled lipoproteins were sterilely filtered before use.

Experimental Design

The smooth muscle cells were seeded in 35 mm Petri dishes and grown for 5 to 7 days until confluence. The experimental medium containing the d < 1.019 g/ml fraction, and verapamil where appropriate, was changed twice weekly. To determine the cellular retention of 3H-CLE or 3H-DNA, the d < 1.019 g/ml labelled with 3H-CLE (0.5 to 1.0 × 10⁶ DPM) or 3H-thymidine (0.5 µCi/ml) was added to separate flasks of smooth muscle cells containing 10 ml medium. After 3 days of labelling, the medium was removed, the cell layer was washed, and the cells were trypsinized and seeded in fifteen 35 mm Petri dishes. After 5 days, the zero-time dishes were terminated and the other cells were kept with the experimental media and verapamil as detailed in Table 5. The medium was changed twice weekly. To study the incorporation of 3H-thymidine into DNA, 0.5 µCi was added to 1 ml of the culture medium and incubation was carried out for 24 hours.

At the end of the experiments, the medium was removed, the cell layer was washed three times with phosphate-buffered saline containing 0.2% bovine serum albumin and three times with phosphate-buffered saline. The cells were scraped with 1 ml 50% methanol and 2 × 1 ml 100% methanol using a Teflon policeman. After addition of an equal volume of chloroform, the lipids were extracted and the delipidated residue was separated and used for determination of DNA. Protein was determined on the residue, according to the method of Lowry et al. The incorporation of 3H-thymidine into DNA was determined on 200 µl of the DNA hydrolysate. Alternately, after incubation with 3H-thymidine and exhaustive washing of the cells in the Petri dish with buffered saline, 5% ice-cold trichloroacetic acid and buffered saline, the cells were hydrolyzed in situ with 0.25 N NaOH and aliquots were taken to determine the 3H radioactivity. The lipids were purified according to the method of Folch et al. Total and free cholesterol was determined by gas liquid chromatography after the addition of campesterol as an internal standard. Lipid phosphorus was determined according to the method of Bartlett. Triglycerides and cholesterol were determined using the Technicon AutoAnalyzer II according to the Lipid Research Clinics protocol.

Materials

The culture medium and fetal bovine serum were obtained from Gibco (New York, New York). Horse serum was obtained from Biolog (Jerusalem, Israel). Verapamil was obtained from Ikapharm (Jerusalem, Israel). [7α(n)]-3H-cholesterol and ¹⁴C-cholesterol were obtained from Amersham International, U.K. Bovine serum albumin was obtained from Sigma, St. Louis, Missouri.

Results

In the first experiments, we examined the uptake by rabbit smooth muscle cells of two lipoprotein fractions derived from hypercholesterolemic rabbit plasma and labelled with 3H-CLE, the nonhydrolyzable analogue of cholesteryl ester. The cultured cells were exposed to these fractions for 1 or 2 weeks and at both time intervals a higher uptake of the d = 1.006 to 1.019 g/ml fraction was seen (Table 1). This observation was accompanied by a more prominent accumulation of esterified cholesterol in cells incubated for 2 weeks with the more dense fraction.

Therefore, in subsequent experiments, we used higher concentrations of the d < 1.019 g/ml fraction of hypercholesterolemic rabbit serum to study the effect of verapamil on cellular cholesteryl ester accretion. The results of two representative experiments performed with rabbit and bovine aortic smooth muscle cells are shown in Table 2. Addition of the d < 1.019 g/ml fraction of hypercholesterolemic rabbit plasma resulted in a marked increase in cellular cholesteryl ester after 2 days. In the bovine cells, there was a further increase in cholesteryl ester after 7 days. Addition of 50 µM verapamil resulted in a two- to threefold increase in cholesteryl ester in rabbit cells incubated in the presence of d < 1.019 g/ml fraction or control medium. In the bovine cells, the increase was the same and occurred in the presence of both media. The aim of the present study was to learn about the chronic effects of verapamil and therefore the next experiments were carried out for up to 5 weeks.

In addition to cholesterol and protein, we also determined the DNA to relate the cholesterol accretion to the cellular content of the dish. The results of four experiments are shown in Table 3; even though there was good reproducibility within each experiment, there was interexperimental variation in the DNA and cholesteryl ester per dish. When the rabbit aortic smooth muscle cells were cultured in the presence of d < 1.019 g/ml fraction (Table 3) for 5 weeks, there was a 50% to 75% increase in DNA per dish (Table 3). During that time, there was an increase in cellular free cholesterol (18% to 118%) while the esterified cholesterol increased five- to tenfold. When 50 µM verapamil was added to medium supplemented with d < 1.019 g/ml fraction, the DNA per dish was the same as in control dishes, which did not contain the d < 1.019 g/ml fraction. When the cells were cultured in control medium with added verapamil, the DNA per dish was 13% to 30% lower than in the absence of verapamil. Addition of verapamil to medium supplemented with d < 1.019 g/ml fraction resulted in 22% to 37% less accretion of cholesteryl ester than in control dishes cultured with the d < 1.019 g/ml fraction only (p < 0.01). Similar results were also obtained in bovine cells after 3 weeks (Table 3).
Table 1. Enrichment of Rabbit Aortic Smooth Muscle Cells with Cholesteryl Ester Incubated with Fractions of Hypercholesterolemic Rabbit Plasma Labelled with $^3$H-CLE

<table>
<thead>
<tr>
<th>Fraction of rabbit plasma</th>
<th>Protein/dish (μg)</th>
<th>Cellular DPM (3H-CLE (% uptake))</th>
<th>Cellular cholesterol (μg/dish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d&lt;1.006 g/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>180</td>
<td>3447±47</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>230</td>
<td>5863±160</td>
<td>5.6</td>
</tr>
<tr>
<td>d = 1.006 to 1.019 g/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>7863±344</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td>1644±349</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Values are means ± SE of triplicate dishes.

On the day of the experiment, 1 ml of medium containing 1% albumin and the lipoprotein fraction labelled with $^3$H-cholesteryl linoleyl ether ($^3$H-CLE, 60 μg cholesterol and 58,000 DPM) was added. After 24 hours, 1 ml of serum-containing medium was added and left for 48 hours. Then the medium was removed, 1 ml of labelled medium was added for 24 hours, 1 ml serum-containing medium was added, and the mixture was incubated for 72 hours more. The same protocol was used for the second week.

FC = free cholesterol; CE = cholesteryl ester.

Table 2. Effect of Verapamil on Cholesteryl Ester Accumulation In Aortic Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Verapamil (50 μM)</th>
<th>Control medium</th>
<th>Medium + d&lt;1.019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FC CE</td>
<td>FC CE</td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 -</td>
<td>44.0±3.8</td>
<td>4.5±1.5</td>
<td>42±0.7</td>
</tr>
<tr>
<td>2 +</td>
<td>40.0±1.3</td>
<td>6.3±3.6</td>
<td>63.2±1.0</td>
</tr>
<tr>
<td>7 -</td>
<td>40.6±0.1</td>
<td>4.3±1.3</td>
<td>58.4±1.3</td>
</tr>
<tr>
<td>7 +</td>
<td>42.3±0.8</td>
<td>12.0±1.3</td>
<td>68.9±1.7</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 -</td>
<td>37.0±0.7</td>
<td>3.3</td>
<td>45.4±0.8</td>
</tr>
<tr>
<td>2 +</td>
<td>44.6±10.3</td>
<td>11.3±2.2</td>
<td>51.4±1.6</td>
</tr>
<tr>
<td>7 -</td>
<td>37.7±1.2</td>
<td>7.5±2.5</td>
<td>49.7±3.4</td>
</tr>
<tr>
<td>7 +</td>
<td>42.8±1.71</td>
<td>14.3±1.1</td>
<td>57.1±1.5</td>
</tr>
</tbody>
</table>

Values are given as μg/mg protein, means ± SE of triplicate dishes.

On the day of the experiment, fresh medium containing the d<1.019 g/ml fraction of hypercholesterolemic rabbit plasma (150 μg cholesterol/ml) was added. Triplicate dishes were terminated after 2 days; in those kept for 7 days, the medium containing appropriate additions was changed on day 3.

FC = free cholesterol; CE = cholesteryl ester.

Table 3. Effect of Long-Term Addition of d<1.019 g/ml Fraction and Verapamil on Cellular Proliferation and Cholesteryl Ester Accumulation in Rabbit and Bovine Aortic Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>d&lt;1.019 added (μg chol/ml)</th>
<th>Verapamil (50 μM)</th>
<th>DNA Weeks</th>
<th>Cellular cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Rabbit</td>
<td>250</td>
<td>-</td>
<td>2.5</td>
<td>8.2±0.4</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>2.5</td>
<td>3.5±0.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>2.5</td>
<td>3.7±0.4</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>2.5</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>5</td>
<td>10.4±0.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>5</td>
<td>6.3±0.1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>5</td>
<td>7.0±1.0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>5</td>
<td>4.9±0.6</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>5</td>
<td>21.2±0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>5</td>
<td>12.1±0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>5</td>
<td>12.2±0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>5</td>
<td>10.6±0.3</td>
</tr>
<tr>
<td>Bovine</td>
<td>250</td>
<td>-</td>
<td>3</td>
<td>12.8±0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>3</td>
<td>8.6±0.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>-</td>
<td>3</td>
<td>8.9±0.5</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>+</td>
<td>3</td>
<td>6.5±0.2</td>
</tr>
</tbody>
</table>

Values (μg/dish) are the means ± SE of triplicate dishes.

At the start of the experiment, the medium was supplemented with d<1.019 g/ml fraction of hypercholesterolemic rabbit plasma and verapamil where appropriate. The medium (containing all additions) was changed twice weekly. a vs. b, c vs. d, e vs. f, p<0.01.
In rabbit cells, the effect of the d < 1.019 g/ml fraction on DNA and cholesteryl ester content was seen also after 2.5 weeks. Addition of verapamil prevented the increase in DNA per dish, while the cholesteryl ester content did not vary from that seen in the presence of d < 1.019 g/ml fraction only. After 5 weeks of culture, the protein content per dish increased markedly in the presence of d < 0.19 g/ml fraction when compared to control medium. Addition of verapamil to either medium resulted in a less pronounced increase in protein content; this effect was less prominent after shorter time intervals in culture (Figure 1).

In several experiments, the cells were cultured in the presence of d < 1.019 g/ml fraction and with lower concentrations of verapamil (0.5 and 5.0 μM). In the verapamil-treated cells, there was a 15% to 20% reduction in protein content per dish when compared to cells incubated with the d < 1.019 g/ml fraction only; no significant differences in cholesteryl ester content per dish were noted. Exposure of cells in control medium to 50 μM verapamil for either 2 or 7 days did not affect 3H-leucine incorporation per milligram of cell protein. The reduction in DNA per dish prompted the investigation of the effect of verapamil on DNA synthesis and the results are shown in Table 4. Exposure of the smooth muscle cells to 50 μM verapamil for 2 days in the presence of the control medium reduced the incorporation of 3H-thymidine into DNA by 90%, and in the presence of the d < 1.019 g/ml fraction, by 85%. After 7 days, synthesis of 3H-DNA was about one-fifth of the initial value, and addition of verapamil reduced the incorporation of 3H-thymidine by two- to threefold. Similar results were also obtained with human skin fibroblasts in which contact inhibition resulted in a more prominent reduction in DNA synthesis after 7 days. Inhibition of incorporation of 3H-thymidine into DNA was also seen when cells were treated with lower concentrations of verapamil for 2 days (Figure 2).

Even though the reduction in DNA synthesis did account for the lesser amount of DNA per dish in the long-term experiments, the possibility of cell loss induced by prolonged exposure to verapamil was also considered. To investigate this question, we used two residualizing markers. The cells were loaded with d < 1.019 g/ml fraction of rabbit plasma labelled with 3H-cholesteryl linoleyl ether before seeding in Petri dishes. Thereafter, the cells were cultured for 5 days, control dishes were terminated to provide initial radioactivity, and the other cells were cultured with appropriate additions (Table 5). It can be seen that the labelled cholesteryl linoleyl ether was retained during the entire experimental period and that verapamil did not affect this retention. The second probe used was 3H-thymidine incorporated into DNA and again no loss of label was seen (Table 5).

In addition to the biochemical perturbations described so far, prolonged treatment with verapamil also resulted in prominent morphological changes. Representative examples are seen in Figure 3. The cells were cultured for 4 weeks in medium supplemented with d < 1.019 g/ml fraction in the absence and presence of 50 μM verapamil. The control cells grown in the absence of verapamil displayed the characteristic growth pattern of smooth muscle cells and contained refractile lipid droplets. In the presence of verapamil, the cells were larger and flattened and had acquired an almost cobblestone appearance. In many of the cells, prominent lipid inclusions were seen.

Discussion

The development of atherosclerosis is a complex process which involves the entry of plasma lipoproteins and...
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Homologous or heterologous hypercholesterolemic serum or isolated lipoprotein fractions thereof resulted in a variable increase in cellular cholesteryl ester content.4-7 Analogous to our present findings (Table 2), an almost maximal accumulation of cholesteryl ester was seen in monkey aortic smooth muscle cells after 2 days of incubation with hypercholesterolemic monkey serum.6 To evaluate the changes that occurred in the chronic experiments, it is pertinent to compare the ratio of free to esterified cholesterol. Thus, when the cells were cultured in control medium, the cholesteryl ester amounted to 13% to 23% of total cholesterol; the ratio of esterified cholesterol to total cholesterol increased with the concentration of d < 1.019 g/ml fraction in the medium and rose to 60% to 70%.

In addition to the increase in esterified cholesterol in dishes incubated with the d < 1.019 g/ml fraction, there was also a significant increase in DNA per dish representing cellular proliferation. This stimulatory effect of the d < 1.019 g/ml fraction was counteracted by adding verapamil. The effect of verapamil on cholesteryl ester accretion was more complex. Within the first week, more esterified cholesterol was found in dishes exposed to d < 1.019 g/ml fraction and verapamil than with the d < 1.019 g/ml fraction only. After 2.5 to 3 weeks, this difference disappeared, while after 5 weeks there was significantly less cholesteryl ester in dishes cultured with d < 1.019 g/ml fraction and treated with verapamil. It seems plausible that the initial accumulation of cholesteryl ester in the verapamil-treated cells could be related to a partial reduction of cholesteryl ester hydrolysis.5-2 After 3 weeks in culture, the uptake of the d < 1.019 g/ml fraction by the smooth muscle cells decreases (unpublished observations), and thus notwithstanding a slower rate of hydrolysis, a less extensive accumulation of cellular cholesteryl ester would ensue. Since at the same time cellular proliferation, and possibly formation of extracellular material, were attenuated in the presence of verapamil, the net accumulation of cholesteryl ester was indeed less than in controls.

Reduction in proliferation of growth-arrested rat aortic smooth muscle cells by a calcium-blocking agent, nifedipine, was shown to be concentration- and time-depen-

Table 5. Retention of Labelled Markers in Aortic Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Initial radioactivity</th>
<th>Final radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ Verapamil</td>
</tr>
<tr>
<td>Bovine</td>
<td>1242 ± 109</td>
<td>1125 ± 20</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4436 ± 157</td>
<td>4236 ± 330</td>
</tr>
<tr>
<td>Rabbit</td>
<td>197 ± 7</td>
<td>146 ± 36</td>
</tr>
<tr>
<td>Rabbit</td>
<td>568 ± 52</td>
<td>502 ± 41</td>
</tr>
</tbody>
</table>

Values are the means ± SE of 3 to 6 dishes in each experiment.

The labelled compounds were added to smooth muscle cells 3 days before subculture. Thereafter, the cells were seeded in 35-


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Figure 3. A, B. Rabbit aortic smooth muscle cells were cultured for 4 weeks in medium containing only $d < 1.019$ g/ml fraction of hypercholesterolemic rabbit plasma (250 $\mu$g cholesterol/ml). C, D. These cells were exposed to medium containing $d < 1.019$ g/ml fraction and 50 $\mu$M verapamil. Note the characteristic growth pattern of the smooth muscle cells in the control dishes (A and B). The refractile material apparently represents droplets of cholesteryl ester. In the presence of 50 $\mu$M verapamil, the cells acquired an almost cobblestone appearance (C). At a higher magnification (D), prominent cellular lipid inclusions are seen in certain regions. Bar = 100 $\mu$m.
in that study, verapamil was shown also to reduce the number of labelled nuclei in growth-arrested cells stimulated by PDGF. 29

More recently, 5 × 10−5 M verapamil was shown to reduce 3H-thymidine incorporation into DNA of human aortic smooth muscle cells in culture. 30 The mechanism of the verapamil-induced reduction in cellular proliferation could be related to lowering of intracellular calcium. In bovine aortic smooth muscle cells, reduction of DNA synthesis was noted when calcium was absent from the medium. 31 In our long-term experiments the amount of protein in the verapamil-treated dishes was markedly reduced. Smooth muscle cells grown for a number of weeks produce abundant extracellular matrix, in which collagen and elastin are prominent components. 32, 33 Thus it appears that the reduction of protein in the verapamil-treated dishes could represent, in part, reduction in collagen formation because verapamil has been shown to inhibit collagen synthesis in fetal rat bone. 34 The use of 3H-cholesteryl linoleyl ether, which was shown to be completely retained for a number of weeks after injection of various lipoproteins into rats and mice, 35, 36, 37 proved a convenient marker for the studies in culture. Its retention throughout the experimental period support the conclusion that the reduction in DNA per dish after verapamil treatment is due to decreased cell proliferation and not to cell loss. It seems of interest that the morphologic changes in the verapamil-treated rabbit aortic smooth muscle cells, namely flattening and cobblestone appearance, markedly resembled those seen in bovine aortic smooth muscle cells exposed to 10−5 M lanthanum for 14 days. In that study, the decrease in cell number per dish became evident only after a week of treatment.

The present and previously reported results 29 provide some new insights as to the possible inhibitory effect of verapamil on the development of atheroma in cholesterol-fed rabbits. In the in vitro studies, both cellular components of the atheroma (i.e., macrophages and smooth muscle cells) responded to verapamil. The putative salutary effect of verapamil on macrophage-derived foam cells could be related to the lysosomal retention of the ingested lipoprotein cholesteryl ester. Assuming that the lipid-laden macrophages do leave the arterial wall, 39 then in cholesterol-fed rabbits treated with verapamil each cell could remove more cholesteryl ester from the aorta. Verapamil treatment of aortic smooth muscle cells, the other cellular component of atheroma, could counteract the stimulatory effect of hypercholesterolemic serum on cellular proliferation and elaboration of extracellular matrix and thereby impede accumulation of esterified cholesterol and development of atheroma.

Acknowledgments
The excellent assistance of Mazal Ben-Nalm, Yedida Dabach, and Gideon Hollander is gratefully acknowledged.

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Index Terms: atherosclerosis • smooth muscle cells • verapamil • cell proliferation • cholesteryl ester

Long-term effects of verapamil on aortic smooth muscle cells cultured in the presence of hypercholesterolemic serum.
O Stein, G Halperin and Y Stein

Arterioscler Thromb Vasc Biol. 1987;7:585-592
doi: 10.1161/01.ATV.7.6.585

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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