Transcriptional expression of LDL receptor gene in aortic atherosclerotic lesions induced by cholesterol-rich diet

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The present study, aimed at understanding the transcriptional regulation of the LDL receptor (LDL-R) gene in rabbits fed with high-cholesterol diet (atherogenic diet) for different time intervals, revealed that time-dependent increase in serum lipid profile was paralleled by decrease in the transcriptional expression of apoprotein-'B'-specific LDL receptor (receptor 'B') gene at the atherosclerotic lesion sites as compared to normal sites. Based on these and other earlier observations, we propose that cholesterol-dependent transmembrane signalling pathway may be responsible for the initiation of atherogenesis at the cellular level.

THERE exists a general recognition of the fact that the major cell involved in atherogenesis is the arterial smooth muscle cell[1] because of its inherent ability to store lipids, proliferate and synthesize connective tissue elements and undergo necrosis. These cells cultured from atherosclerotic lesions were characterized by high lipid levels and enhanced proliferative activity, and retain the major manifestation of atherosclerosis at the cellular level[2]. Further, it is well known that under hyperlipidemic conditions, uptake of cholesterol by these cells through apoprotein-B-specific LDL receptor (receptor 'B') pathway comprises about 20% of the total cholesterol and the rest is taken up through the LDL-R-independent pathway. A recent study[3] aimed at understanding the interaction of cholesterol with arterial smooth muscle cells *in vitro* revealed that cholesterol uptake through LDL-R-independent pathway had the ability to stimulate all the features of atherogenesis at the cellular level. Consequently, the present study is aimed at understanding the *in vivo* conditions, whether or not transcriptional expression of the LDL-R gene is affected in the atherosclerotic lesions induced by cholesterol.

A group of 18 male rabbits (close-bred New Zealand strain) were acclimatized to the laboratory conditions for a period of one month on stock diet (Hindustan Lever Limited) consisting of 20% crude protein, 3.5% ether extract, 12% crude fibre, 8% ash, 1.2% calcium, 0.6% phosphorus, 47% nitrogen-free extract, stabilized vitamins, all minerals and trace elements. After one month, these animals were divided into three different groups on the basis of basal serum lipid profile and body weight (3–4 kg). Group I ($n = 6$) was fed stock pellet diet. Group II ($n = 6$) was fed atherogenic diet for a period of 2.5 months and group III ($n = 6$) was fed the same diet for a period of 5 months. The atherogenic diet consisted of 70 gm stock pellet diet + 5 gm butter + 100 mg cholesterol[4]. Serum lipid (cholesterol and triglycerides) profile of animals belonging to each group was recorded at day zero as well as before they were sacrificed using standard procedures[4]. The thoracic–abdominal cavity was opened and the aorta of each animal was removed, split open longitudinally and stained for Sudan IV to know the sites of Sudanophilic atherosclerotic lesions. The tissues from the atherosclerotic sites as well as adjacent normal sites were taken for cryostatic sectioning. Transcriptional expression of the LDL-R gene was studied in normal aortic intima/media, atherosclerotic lesions as well as in normal intima/media adjacent to these lesions by employing *in situ* hybridization technology[5]. Cryostatic sections (10 µm) were cut and mounted on gelatin-coated slides and fixed for 1 h in 4% *p*-formaldehyde-buffered saline, washed twice with PBS and incubated with proteinase K for 15 min. Sections were delipidated by passing through graded series of ethanol. Hybridization was carried out at 37°C overnight in a humidified chamber in hybridization buffer (2 × SSC, 10 × Denhardt's solution, 0.1% SDS, 30% formamide and 0.1% Salmon sperm DNA) containing digoxigenin-labelled DNA probe specific for receptor B. After hybridization, the slides were washed with 2 × SSC buffer at room temperature. Digoxigenin-specific antibody conjugated to alkaline phosphatase (Böhringer Mannheim, Germany) was used for the detection of hybridized probe, with NBT and BCIP as substrate and 2 mM levamisol as inhibitor of intrinsic alkaline phosphatase activity. To check for the non-specific hybridization, the sections were hybridized with multiple probes (c-fos and c-myc). Since that data has been communicated somewhere else we are unable to include the data in this paper.

Feeding of cholesterol-rich atherogenic diet to animals (groups II and III) resulted in significant elevation of serum lipid (cholesterol as well as triglycerides) profile as compared to control group I (Table 1). The aortic atherosclerotic lesions were of either fatty streak or fibro-fatty type. The decrease in receptor 'B' transcriptional expression at the lesion sites was accompanied by increase in the levels of serum lipid profile (Figure 1). These results are in conformity with our earlier results obtained from arterial smooth muscle cells *in vitro* exposed to cholesterol[3] as well as with those reported by other investigators[2,6]. Further, we have recently shown that cholesterol molecule has an inherent capacity to initiate a novel transmembrane signalling

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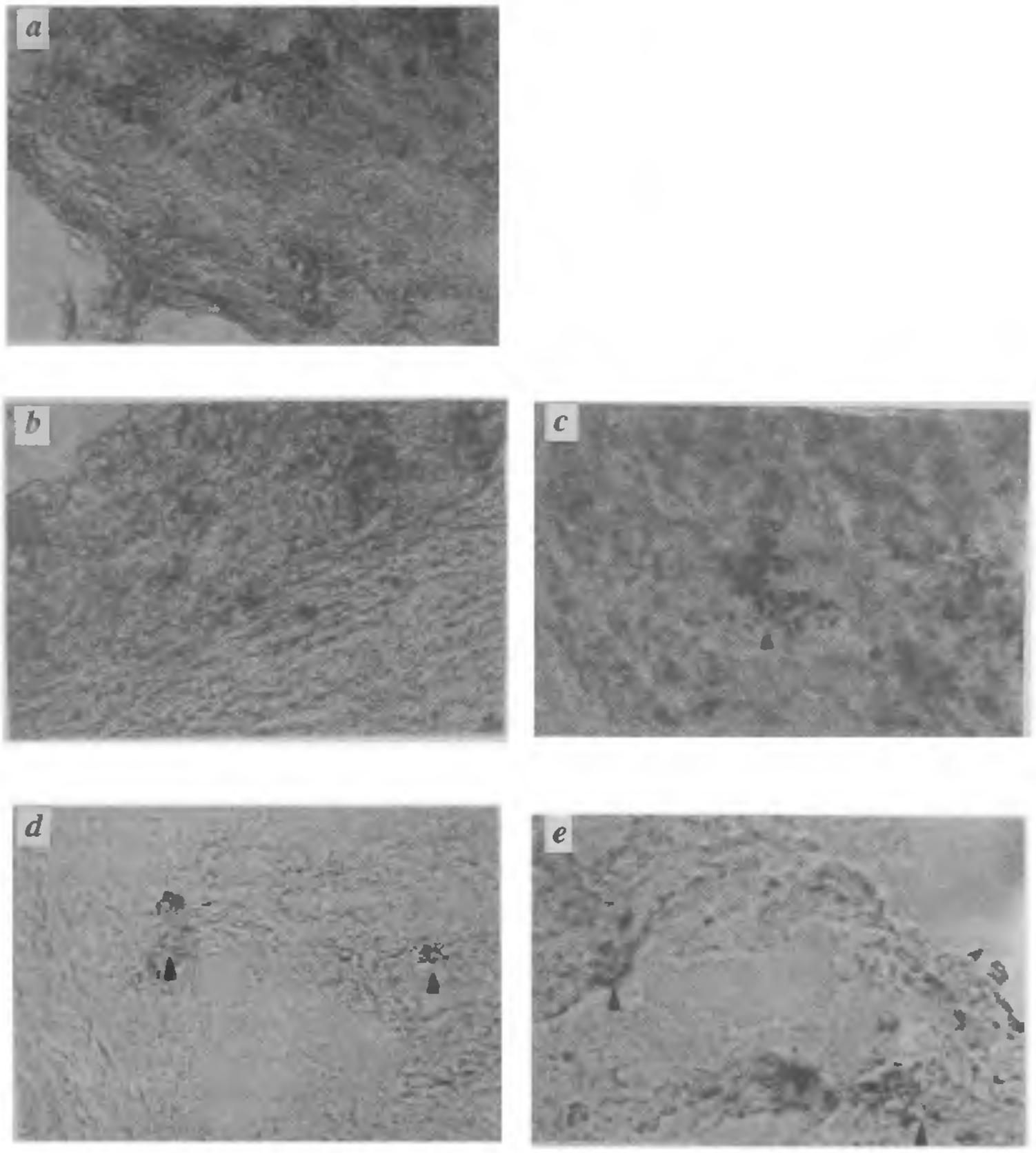

Figure 1. Transcriptional expression of LDL-R gene in normal atherosclerotic aortic sections *a*, representative pictures from group I animals or normal aortic portions adjacent to lesion site; *b, c*, representative picture showing LDL-R expression at lesion sites of group II animals; *d, e*, depicting LDL-R expression at lesion sites in group III animals *a, b, d*, ×550; *c, e*, ×1375. Positive staining is shown by arrows.

Table 1. Serum lipid profile of animals belonging to groups I, II and III

Group type	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
Group I (control)	49 60 ± 6 24	8 20 ± 4 32
Group II	590.70 ± 14 60*	293.22 ± 12.56*
Group III	1209 80 ± 25 40*	696 16 ± 26 86*

Values represent mean ± SEM.
*Denotes $p < 0.001$ as compared to control group (basal values).

pathway through its specific receptor C[7–10] and also the cholesterol-dependent transmembrane signalling pathway is responsible for the regulation of LDL-R gene transcription[11]. Further, we have shown earlier that cholesterol-dependent down-regulation of LDL-R expression in arterial smooth muscle cells was reversed in the presence of calcium antagonist trifluoperazine[4]. The recent finding that second messengers, especially cAMP and calcium, play an important role in the transcriptional regulation of the LDL-R gene in various cell lines[12] is in conformity with this proposition. Based on these studies, we propose that cholesterol-dependent transmembrane signalling pathway may play a crucial role not only in the LDL gene transcription but also in the initiation of atherosclerotic process.

1. Geer, J. C., McGill, B. C. and Strong, J. P., *Am. J. Pathol*, 1961, 38, 263–287.
2. Orekhov, A. N., Tertov, V. V., Kudryashov, S. A, Khashimov, Kh A. and Smirnov, V. N., *Atherosclerosis*, 1986, 60, 101–110.
3. Mehta, Uma and Kaul, D., *Exp. Mol. Pathol*, 1991, 55, 13–24.
4. Kaul, D and Kukreja, R S., *Atherosclerosis*, 1987, 64, 211–214
5. Harper, M E., Marselle, L. M., Gallo, R. C. and Wong-Staal, F., *Proc Natl. Acad. Sci. USA*, 1986, 83, 772–776.
6 Chen, R M, Getz, G. S, Fischer Dzoga, K and Wissler, R. W., *Exp. Mol Pathol.*, 1977, 26, 359–374.
7. Kaul, D, *Curr Sci*, 1993, 65, 883–886
8. Kaul, D and Singh, J, *Cellul. Signal*, 1994, 6, 141–145
9 Kochhar, N. and Kaul, D, *FEBS Lett*, 1992, 314, 17 19

10 Kochhar, N and Kaul, D, *FEBS Lett*, 1992, **299**, 19–22
11 Goel, R and Kaul, D, *Atherosclerosis*, 1994, **109**, 109
12 Auwerx, J H, Chait, A, *et al*, *Mol Cell Biol*, 1989, **9**, 2298–2302.

Received 7 November 1994, revised accepted 24 March 1995

A polyurethane–polyvinylpyrrolidone interpenetrating polymer network for mammalian cell encapsulation

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A biocompatible and noncytotoxic interpenetrating polymer network (IPN) membrane was developed for medical applications. The biostable membranes developed could also support the growth and adhesion of mice L929 fibroblast cells. This membrane could, therefore, serve as a candidate material for mammalian cell encapsulation.

THE presently available therapy of type 1 diabetes mellitus mainly involves therapy with insulin injections. However, this form of therapy cannot prevent the nonphysiological fluctuations in blood glucose levels and secondary complications may develop over the years leading to blindness, kidney disease, etc. Success of an alternate therapy such as transplantation of normal insulin-producing cells or islets of Langerhans is limited by immunorejection[1]. Immunorejection may be overcome either by transplanting the islets under heavy doses of toxic immunosuppressive drugs or by immunoisolation[2]. The success of the more desirable immunoisolation therapy would depend on the availability of extremely biocompatible, semipermeable membranes which would prevent the cells of the immunological system from migrating to the encapsulated islet cells and at the same time allow the diffusion of glucose and insulin through the membranes.

Microcapsules of alginate and polylysine have been utilized for encapsulating islets[3]. Limited stability, biocompatibility and permselectivity[4] have prevented their extensive use in humans. Hollow fibres of acrylates, cellulose acetate, etc., have been used with limited success[5] to encapsulate the islets. The main drawbacks limiting their use appear to be fragility and biocompatibility. The most significant development in this area was made by Calafiore *et al.*[6], who encapsulated islet cells in alginate-polylysine membranes and further entrapped the encapsulated cells within the walls of a PTFE vascular prosthesis. Permeability control, biocompatibility and optimum mechanical properties can be better achieved by implanting the cells in polyurethane membranes.

Polyurethanes are extensively used in biomedical applications but suffer from degradative processes in long-term applications. Techniques of interpenetrating polymer network synthesis have been utilized to achieve better resistant polyurethanes in long-term applications. We have carried out the interpenetration of polyurethane networks with both hydrophilic networks such as polyacrylamide[7], polyvinylpyrrolidone[8], polyhydroxyethylmethacrylate[9] and hydrophobic network such as polymethylmethacrylate[10] to obtain better resistant polyurethanes. Extensive *in vitro*[11] and *in vivo*[12] studies carried out for these IPNs demonstrated the biocompatible and biostable nature for many of the compositions. This study highlights the application of a polyurethane–polyvinylpyrrolidone membrane for cell encapsulation.

Synthesis and characterization of the polyurethane (PU) and the polyurethane–polyvinylpyrrolidone interpenetrating polymer network (PU–PVP IPN) have been reported elsewhere[7,8]. Characterization studies of the IPNs involving studies on chemical resistance, mechanical behaviour, spectroscopy, dynamic mechanical behaviour, surface hydrophilicity and morphology characteristics by scanning electron microscopy were all carried out using standard procedures. Biostability of the PU and IPN membranes was assessed by monitoring the changes in the mechanical properties of the membranes subsequent to implantation in rats. Cytotoxicity of the synthesized membranes was evaluated using an indirect contact test based on a tetrazolium dye (MTT) assay[13].

The membranes were assessed for their cell growth support characteristics by fixing them on tissue culture Petri dishes using a medical-grade silastic adhesive (Dow corning). The growth of L929 fibroblast cells in contact with the membranes was assessed by comparing the cell shape, appearance and spread relative to that of control grown in similar Petri dishes, by visual observance through a microscope.

The biocompatibility aspects of the membranes were also assessed by implanting them intramuscularly in black-hooded Liverpool strain Lister rats for periods of one month and three months. After explanation, the tissues around the implants were stained by an immunostaining method[14] that used monoclonal antibodies for specifically staining the ED2 macrophages. Specific stains were also employed to stain B cells, T cells and neutrophils. The cell number and distribution were quantified using a computer-aided image analysis system.

Characterization results are summarized in (Table 1). Mechanical properties of the IPN are consistent with the behaviour of a typical viscoelastic material. Shift of the loss ($\tan\delta$) peak or glass transition temperature of the IPN to a higher temperature than that obtained for the homopolymer polyurethane is indicative of