Pergamon

Biochemical Pharmacology, Vol. 49, No. 6, pp. 847–853, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00455-2

DERIVATIZED DEXTRANS MODULATE COLLAGEN SYNTHESIS IN AORTIC SMOOTH MUSCLE CELLS

Y. BENAZZOUG, D. LOGEART,* J. LABAT-ROBERT, L. ROBERT, J. JOZEFONVICZ* and P. KERN†

Laboratoire de Biologie du Tissu Conjonctif, CNRS URA 1460, Université Paris XII, Créteil; *Laboratoire de Recherches sur les Macromolécules, CNRS URA 502, Université Paris-Nord, Villetaneuse, France

(*Received* 29 *July* 1994; *accepted* 26 *September* 1994)

Abstract—The effect of specifically derivatized dextrans, with or without antiproliferative activity on smooth muscle cells (SMC), was investigated on type I and type III collagen biosyntheses and mRNA levels in post-confluent SMC cultures. Our results indicate that dextran derivatives decreased total protein and collagen syntheses independently of their antiproliferative activities. However, the most substituted dextran, the one exhibiting the strongest antiproliferative activity towards SMC, was the most active in modulating type III collagen expression. In addition, only the two dextran derivatives bearing benzylamide groups inhibited collagen excretion.

Key words: dextran; collagen type synthesis; smooth muscle cells; cellular proliferation

Athero-arteriosclerotic processes affect the intima and media of large size arteries and are accompanied by the deposition of lipids and the formation of fibrous plaque [1, 2]. This process shows an age dependence but can be accelerated in pathological conditions such as diabetes mellitus [3, 4].

Vascular tissue is rich in ECM‡, synthesized mainly by SMCs. ECM influences the behaviour of the cells, modulating their shape, migration, proliferation and metabolic functions. SMCs synthesize large quantities of ECM components: collagens (mainly type I and type III), elastin, glycoproteins such as fibronectin, laminin and several proteoglycans [5]. During atherosclerotic plaque formation, proliferation and migration of SMCs from the media into the intima of arteries are considered as an initial event [1, 6, 7]. From a sessile and contractile state SMCs become mobile and synthetic, producing a large number of ECM proteins leading to formation of a fibrous plaque [8–11]. Several studies have shown that heparin and glycosaminoglycans exert antiproliferative effects on vascular SMCs, suggesting that they could play a regulatory role in SMC function in the vessel wall [5, 12–16]. Furthermore heparin can modulate the biosynthesis of collagens and glycoproteins by SMCs [5, 17–19].

We previously demonstrated that low molecular weight heparin fragments possessing the site of antithrombin III but devoid of hemorrhagic properties are capable of: (a) modulating biosynthesis of type III collagen and fibronectin by cultured aortic SMCs [20] and diabetic mouse skin *in vivo* [21]; and (b) significantly decreasing the amount of mRNA coding for type III collagen in cultured aortic SMCs [22].

Specifically derivatized dextrans belong to a category of compounds that mimic some effects of heparin and in particular show an antiproliferative activity on SMCs [23, 24]. Interestingly these dextran derivatives exhibit a weak anticoagulant activity as compared to heparin and therefore minimal hemorrhagic effect.

The aim of the present work was to investigate the effect of several functional dextrans on collagen biosynthesis in cultured aortic SMCs at transcriptional and translational levels. The random distribution of functional groups along the polysaccharide chains renders possible preparation of synthetic compounds with structural requirements for antiproliferative effect and potential activity on collagen biosynthesis.

MATERIALS AND METHODS

Materials. Chemicals for cultures, media and sera were purchased from Boehringer (Mannheim, Germany). All other reagents were obtained from Merck (Darmstadt, Germany).

Soluble dextran derivatives were prepared as described previously [25] from dextran T40 batch 32202 (molecular weight 43,900 g/mol; provided by Pharmacia, France). Briefly, dextran units (D), were successively statistically substituted with carboxymethyl groups (CM), benzylamide groups (B) and sulfonated groups (S). The chemical structures of these synthetic polysaccharides are shown in Fig. 1. In our experiments we used a carboxymethyl dextran (LD5), a carboxymethyl dextran benzylamide (B4) and two carboxymethyl

† Corresponding author: Patrick Kern, 3 Allée du chêne perdu, 91800 Boussy Saint Antoine, France.

‡ Abbreviations: EMC, extracellular matrix; SMCs, smooth muscle cells; FCS, foetal calf serum; DMEM, Dulbecco's modified Eagle's medium; SSC, standard saline citrate; D, dextran units; CM, carboxymethyl groups; B, benzylamide groups; S, sulfonated groups; LD5, carboxymethyl dextran; B4, carboxymethyl dextran benzylamide; E9 and D1, carboxymethyl dextrans benzylamide sulfonated.

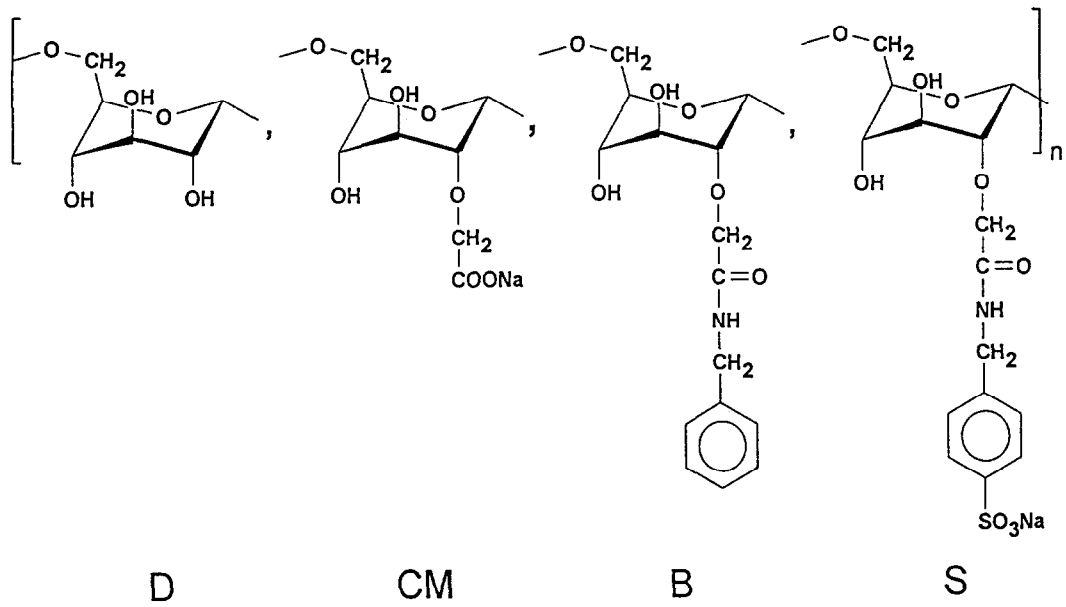

Fig. 1. Structure of water soluble derivatized dextrans. Native dextran contains only D units whereas according to the substitution ratio carboxymethyl dextran bears CM and D units; carboxymethylbenzylamide dextran: B, CM and D units and carboxymethylbenzylamide sulfonated dextran: S, B, CM and D units. Quantitative data on substitution levels are indicated in Table 1.

Table 1. Chemical characterization of dextran derivatives

	Dextran derivatives				
	LD5	B4	E9	D1	Heparin
Unsubstituted dextrans %	20	20	0	62	—
Carboxymethyl content %	80	64	58	30	—
Benzylamide content %	0	16	26	0	—
Sulfonate content %	0	0	16	8	—
Anticoagulant activity (IU.mg)	0	0	2.3	0	173

Their specific anticoagulant activities are expressed in international units per milligram.

dextrans benzylamide sulfonated (E9 and D1). The chemical compositions and specific anticoagulant activities of these compounds are reported in Table 1. In all experiments T40 dextran was used as an internal control to determine the effect of unsubstituted dextran.

Cell culture. SMCs were grown from newborn pig aorta media explants in DMEM supplemented with 20% FCS 100 IU/mL penicillin, 100 μg/mL streptomycin and 2 mM glutamine.

Cell proliferation studies. Cells were serially passaged with 10% FCS. In all experiments SMCs were used from passage three to four. Proliferation studies were performed with 1.2×10^4 SMCs plated onto 16 mm multiwell plates in DMEM supplemented with 10% FCS. After 24 hr the cells were growth arrested by placing them in DMEM with 0.1% FCS for 72 hr. Cell proliferation was initiated by incubation in DMEM with 10% FCS followed by addition of dextran derivatives [23]. Cell numbers were measured in quadruplicate samples and counted with a Coulter counter. The degree of proliferation inhibition was determined after 5 days according to the relationship [24]:

$$1\% = \left(1 - \frac{\text{net proliferation with dextran}}{\text{net proliferation in control}}\right) \times 100.$$

Collagen biosynthesis. Cells were serially passaged with 10% FCS up to the third subculture [20], where they were used at over-confluency for all biosynthetic experiments in order to avoid or minimize the inhibitory effect of the investigated compounds (substituted dextrans) on cell proliferation. Cells were incubated at 30° in a 95% air, 5% CO_2 atmosphere. For biosynthetic experiments, culture medium was discarded and cells incubated in the same medium without FCS, in the presence of the

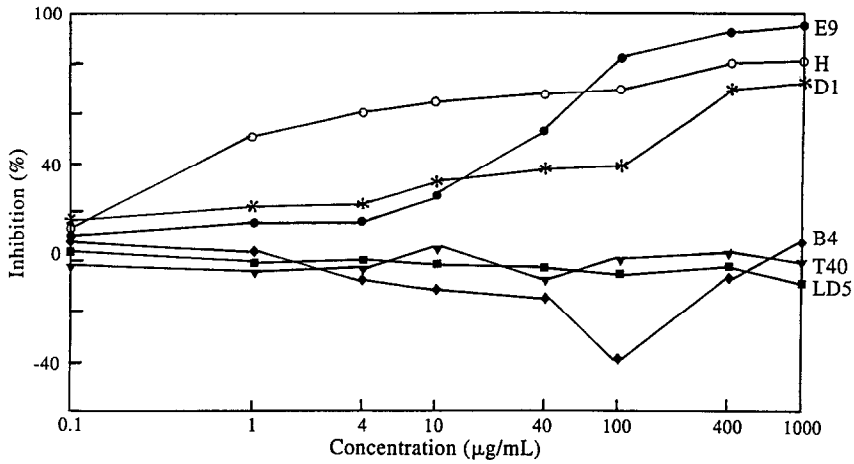

Fig. 2. Effect of dextran derivatives on SMC proliferation. SMCs were cultured in the presence of increasing amounts of each dextran. Inhibition activity of heparin (H) was used as comparative data. Experimental procedure is indicated in Materials and Methods. Each value is the mean of five determinations: SD for all points was less than 5% of the mean value.

above described compounds (400 μg/mL/10^6 cells) for 24 hr.

Collagen and total protein synthesis were determined as previously described [20, 26, 27]. In brief, over-confluent cells were incubated for 24 hr without (control) or with dextran derivatives as indicated above in presence of [2,3,4,5-^{3}H]proline (50 μCi/mL; specific activity 105 Ci/mM; Amersham, France). The medium was supplemented with 50 μg/mL sodium ascorbate. At the end of the incubation, medium and cell layers were extensively dialysed against distilled water. Aliquots were hydrolysed with 6 M HCl, 104° for 24 hr. Radiolabelled hydroxyproline and proline were separated and quantified as described [20, 26, 27]. The rate of collagen biosynthesis relative to total protein synthesis was estimated according to Wiestner *et al.* [28]. Medium and cell dialysate were lyophilised, resuspended in 0.5 M acetic acid and digested with pepsin [20]. Collagen phenotype was determined by SDS–PAGE and the relative proportions of radioactivity recovered in type I and type III collagens were quantified as described previously [20, 26, 27]. Briefly, collagen alpha-chains separated on SDS–PAGE were quantified by excision of the individual collagenous band, hydrolysis in H_2O_2 at 37° for 24 hr and scintillation counting.

RNA analysis. Total ribonucleic acids were isolated from the cells with the guanidine isothiocyanate–phenol–chloroform–extraction procedure of Chromczynski and Sacchi [29]. The purity and integrity of the RNA were checked by the ratio of A_{260}/A_{280} (nm) and by agarose gel electrophoresis.

For quantitative assessment, RNA was heat-denatured at 65° for 15 min in a 50° formamide, 6% formaldehyde, 0.2 M 4-morpholinepropasulphonic acid, 0.05 M sodium acetate pH 7.0 and 0.01 M EDTA buffer. Aliquots of total RNA were then transferred to nylon membranes (Hybond N+, Amersham, France) using "Slot Blot" apparatus (Minifold, Schleicher & Schull, Dassel, Germany). RNA was fixed to the membrane by alkali treatment (5 min with 0.05 M NaOH pH 5.0) and then washed with 2 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M sodium citrate pH 7.0).

Prehybridation was carried out at 42° for 24 hr in solution A containing 50% (v/v) formamide, 5 × SSPE (1 × SSPE: 180 mM NaCl, 10 mM Na_2HPO_4, 1 mM EDTA pH 7.4), 0.5% (w/v) SDS, 0.1% each of Ficoll, polyvinylpyrrolidone and BSA and 200 μg/mL of heat-denatured sonicated salmon DNA. Hybridization was carried out in fresh buffer A containing 3–5 × 10^8 cpm/μg of the appropriate ^{32}P-labelled probe for 24 hr at 42°. Labelling was performed using a Nick Translation kit (Boehringer, Mannheim, Germany) with [α-^{32}P]dCTP (111 TBq/mM; Amersham, France). The following cDNA probes were used: pHCAL1U specific for human pro alpha1 (I) chain of type I collagen and pHFS3 specific for human pro alpha1 (III) chain of type III collagen, provided by Sandberg and Vuorio [30]. The membranes were washed at 42° with 2 × SSC, 1 × SSC, 0.1 × SSC (all containing 0.1% SDS). The dried membranes were exposed to X-ray films at −80°.

The autoradiogams were quantified by densitometric analysis (Ultroscan 2202, LKB, Freiburg, Germany). A linear correspondence was obtained between the amounts of RNA applied onto membrane for hybridization and the densitometric reading (data not shown).

Miscellaneous. DNA was measured according to Burton [31]. All statistical analyses were performed using the Student's *t*-test. Viability of the cells in the presence of the compounds studied was tested by determination of lactic dehydrogenase (EC. 1.1.1.27).

RESULTS

Antiproliferative activity of derivatized dextrans

In order to explore the inhibitory capacity of

Table 2. Effect of dextran derivatives on the synthesis of total proteins and collagen by SMCs

Addition to culture medium (400 μg/mL)	Total protein synthesis (cpm.10^{-3} μg DNA)	Total collegen synthesis (% of total protein synthesis)
0	38.2 (±1.9)	16.8 (±0.9)
LD5	15.7 (±0.9)†	6.1 (±0.4)†
B4	20.2 (±1.3)†	7.9 (±0.5)†
E9	15.6 (±0.8)†	11.3 (±0.7)†
D1	42.6 (±2.4)	13.1 (±0.8)*
T40	36.7 (±1.9)	14.9 (±0.9)

Cultures were incubated with [^{3}H]proline and incorporation determined in total protein (as proline) as in collagen (as hydroxyproline) as described in Materials and Methods. Collagen synthesis was calculated according to Wiestner *et al.* [28].

Data are the means of six determinations (±SD).

Statistical analysis as compared to control (no dextran added to the medium): * $P < 0.01$; † $P < 0.001$.

Table 3. Effect of dextran derivatives on type III collagen synthesis by SMCs

Addition to culture medium (400 μg/mL)	% of [^{3}H]proline incorporated in collagen alpha1 (III) chain
0	52.9 (±3.5)
LD5	34.5 (±3.1)*
B4	31.1 (±2.8)†
E9	26.5 (±2.5)†
D1	42.5 (±3.7)
T40	50.7 (±3.6)

SMCs were labelled with [^{3}H]proline. Pepsin resistant radiolabelled collagen types were separated on SDS–PAGE and relative synthesis of total type III collagen (medium + cell layer) was determined as described in Materials and Methods.

Values are means of six determinations (±SD).

Statistical analysis as in Table 2: * $P < 0.01$; † $P < 0.001$.

Table 4. Effect of dextran derivatives on the distribution of collagen types between medium and cell layer

Addition to culture medium (400 μg/mL)	Type I collagen in medium (%)	Type III collagen in medium (%)
0	91.2 (±7.1)	82.3 (±5.8)
LD5	93.1 (±7.6)	85.4 (±6.3)
B4	56.0 (±3.9)*	56.4 (±3.8)*
E9	56.9 (±4.0)*	48.2 (±3.5)*
D1	95.1 (±7.8)	93.7 (±7.5)
T40	92.7 (±8.1)	86.2 (±6.2)

The distribution of each collagen type was calculated according to the following formula:

$$\frac{[^3\text{H}]\text{proline incorporated in medium collagen}}{[^3\text{H}]\text{proline incorporated in total collagen (medium + cell)}} \times 100.$$

* $P < 0.001$.

derivatized dextrans, the concentration dependence of functional dextrans on aortic SMC growth was studied. Results are shown in Fig. 2. They are part of systematic studies performed on different functional dextrans. Derivatized dextrans E9 and D1 inhibited SMC proliferation in a dose-dependent manner with an inhibitory capacity equivalent to that of heparin for the highest concentration used. B4 and LD5 as unsubstituted T40 dextran had no inhibitory effect.

Collagen biosynthesis

In order to focus on their potential effect on the biosynthetic capacities of cells only, derivatized dextrans were added to post-confluent cell cultures (400 μg/mL/10^6 cells). During the time course of cell labelling, no significant proliferation was observed in control and derivatized dextran treated cells.

Morphological aspect of over-confluent SMCs in the presence of derivatized dextrans was not altered as compared to control conditions. Lactate dehydrogenase determination showed that there was no deleterious effect on the viability of the cells in the presence of derivatized dextrans (not shown). Total protein synthesis, estimated from the incorporation of total radioactivity per μg DNA, was significantly decreased with all derivatives except for D1 and T40 dextran (Table 2).

Total collagen biosynthesis as related to total protein synthesis was also decreased in the presence of derivatized dextrans with a lesser amplitude with D1 component. T40 had no effect (Table 2).

Pepsin resistant radiolabelled collagen types were separated on SDS–PAGE and used to determine the relative biosynthesis of total type III collagen (cell + medium) to total collagen synthesis (Table 3). Derivatized dextrans had different effects on type III collagen synthesis. The substituted E9 derivative had the most pronounced activity, decreasing type III collagen biosynthesis to approximately 50% of control value. LD5 and B4 components had a similar but less important effect, whereas D1 derivative had no significant activity. Interestingly unsubstituted T40 dextran had no significant effect.

Distribution of type I and type III collagens between the medium and cell layer was estimated by comparing labelled collagen in the medium to total labelled collagen (medium + cell layer) for each type. Only B4 and E9 significantly altered the distribution of both type I and type III collagens (Table 4). It is important to observe that LD5 had no effect on this distribution. Total RNA was extracted from dextran-treated SMCs and analysed by slot blot hybridization. Significant variation occurred for type III collagen mRNA (Fig. 3). Quantitative analysis indicated that LD5, B4 and particularly E9 derivatized dextrans, significantly reduced the steady-state level of type III collagen mRNA (Table 5). However D1 component as unsubstituted T40 dextran had no significant effect

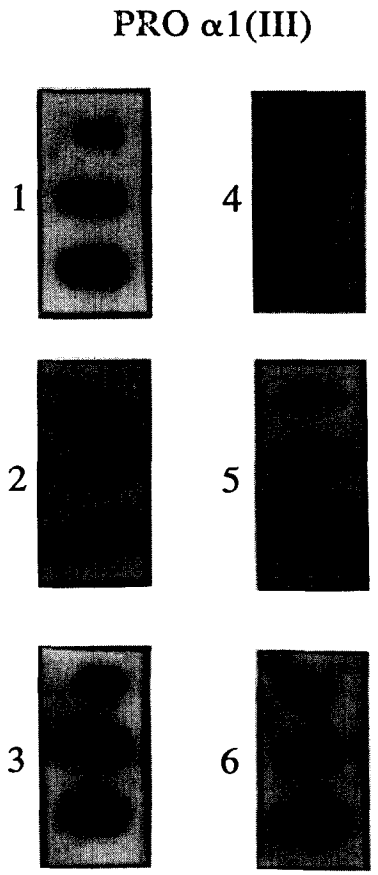

Fig. 3. Slot blot analysis of type III collagen mRNA level. Total RNA was extracted and analysed by slot blot hybridization as described in Materials and Methods. Increased amounts of total RNA (2, 4 and 6 μg) were hybridized with ^{32}P-labeled cDNA probe specific for type III collagen pro alpha1 chain. Quantitative densitometric analysis of the spots is reported in Table 5. 1, control; 2, LD5; 3, B4; 4, E9; 5, D1; and 6, T40.

Table 5. Effect of dextran derivatives on the relative amount of collagen mRNAs expressed by smooth muscle cells in culture

Addition to culture medium (400 μg/mL)	alpha1 (I) mRNA (% of control)	alpha1 (III) mRNA (% of control)
0	100.0 (±7.8)	100.0 (±6.3)
LD5	109.1 (±9.7)	66.7 (±4.3)†
B4	98.0 (±7.3)	73.9 (±4.1)*
E9	93.8 (±8.2)	61.1 (±3.8)‡
D1	110.2 (±9.9)	88.9 (±6.6)
T40	97.3 (±8.8)	95.1 (±7.4)

Relative levels of collagen mRNA were determined by densitometric analysis of slot blot hybridized with specific cDNA probes (see Fig. 3) as described in Materials and Methods. Results are expressed as a percentage of control value (with no dextran in the medium). Control value was set to 100% (±SD based on cross-comparisons between six determinations under control conditions).

Each value is the mean of six determinations (±SD), * $P < 0.02$; † $P < 0.01$; ‡ $P < 0.001$.

on this parameter. In similar conditions the level of pro alpha1 (I) collagen mRNA was not significantly modified by any dextran derivatives.

DISCUSSION

The proliferation of vascular SMC and excessive secretion of ECM macromolecules, particularly collagens, are postulated to be early key steps in the pathogenesis of atherosclerosis. Highly antiproliferative molecules, devoid of significant anticoagulant activity, could be efficient in the control of SMC phenotype. As collagen metabolism is disturbed in atherosclerosis, the activity of these compounds on collagen biosynthesis was also studied.

We previously showed that low molecular weight heparin fragments (CY222) with antiproliferative properties towards SMC and low anticoagulant activity modulated the collagen gene expression of SMC *in vitro* [20]. This modulation was studied in post-confluent cells and appeared unrelated to their antiproliferative activity.

To extend this investigation to structure–activity relationship, we studied new heparin-like molecules, dextrans grafted with function groups [25]. We previously showed that heparin-like biomaterials exhibiting no or very low anticoagulant activity had the capacity to inhibit aortic SMC proliferation [23, 24]. Native dextran and carboxymethyl dextrans were totally inactive. In order to study the charge effect of these polymers, we compared carboxymethyl dextrans bearing varying amounts of carboxyl group. No antiproliferative effect was observed. It also appeared that benzylamide groups are required to increase inhibitory capacity. It was also observed in SMCs that the E9 derivative was very efficient in inhibiting cell proliferation. The D1 derivative exhibited less inhibitory potency than the E9 derivative.

Our studies were focused on collagen phenotype expression of SMCs. Experiments were carried out on post-confluent cultures in the absence of serum to distinguish the effects on collagen gene expression from general antiproliferative activity. During our biosynthetic experiments no detectable effect of dextran derivatives on proliferation of post-confluent SMC cultures were observed. However, three of the dextran derivatives decreased total protein synthesis and, in an even more efficient fashion, collagen synthesis.

As compared with proliferative SMC, decreases in protein and collagen biosynthesis have been described for quiescent cultures, growth arrested by cell density and/or mitogen deprivation [9, 10]. Experiments carried out on quiescent SMC culture indicate that dextran derivatives were able to decrease total protein synthesis and more specifically collagen biosynthesis by a mechanism distinct from their antiproliferative activity.

Furthermore, the D1 derivative exhibited no effect on total protein synthesis, type III collagen synthesis or collagen excretion but did inhibit SMC proliferation. On the contrary, LD5 and B4 derivatives, which had no inhibitory effect on SMC proliferation, did decrease type III collagen synthesis.

Differences in the ratio of collagen type appear

to reflect differences in steady-state procollagen mRNA levels, implicating primary control by transcription or mRNA stabilization [32–34]. Our data indicate that changes in proportion of type III collagen synthesis are accompanied by comparable modulations in type III procollagen mRNA levels. In particular, E9, the most substituted dextran, appears to be the most active component in modulating type III collagen expression. E9 decreased type III collagen synthesis to 50.1% of control value and similarly decreased type III procollagen mRNA to 61.1% of control level. Among the dextran derivatives used in this study the E9 derivative exhibited the strongest antiproliferative activity towards SMC.

No correlation could be demonstrated between the absence of effect of LD5 and B4 on SMC proliferation and the ability of these compounds to decrease total collagen biosynthesis and type III collagen proportion. In addition only the two derivatized dextrans bearing benzylamide groups (B4 and E9) altered collagen distribution between the medium and cell layer. It was demonstrated that high molecular weight dextran sulphate (M_r 500,000) increased processing of collagen in cell culture, leading to an alteration of collagen distribution between cell and medium [35]. However in our study unsubstituted native dextran (M_r 43,900) had no such effect on this parameter. These data suggest that benzylamide groups could play a role in the decrease of collagen proportion in medium of SMCs exposed to B4 and E9 derivatives.

In conclusion it appears that derivatized dextrans exhibit different bioactivities depending on their respective quality and degrees of substitution and could involve different mechanisms in SMC proliferation and collagen biosynthesis. E9, the most substituted dextran in the present study, was the most efficient in decreasing both SMC proliferation and SMC collagen gene expression.

REFERENCES

1. Ross R, The pathogenesis of atherosclerosis: an update. *N Eng J Med* **314**: 488–500, 1986.
2. Schwartz CJ, Valente AJ and Sprague EA, A modern view of atherogenesis. *Am J Cardiol* **71**: 98–148, 1993.
3. Robert L, Vascular aging and arteriosclerosis; the role of the elastin receptor. *Sang Thrombose Vaisseaux* **3**: 273–281, 1991.
4. Koschinsky T, Bünting CE, Rütter R and Griess FA, Vascular growth factors and the development of macrovascular disease in diabetes mellitus. *Diabète et Métabolisme* **13**: 318–325, 1987.
5. Snow AD, Bollender RP, Wight TN and Clowes AW, Heparin modulates the composition of the extracellular matrix domain surrounding arterial smooth muscle cells. *Am J Pathol* **137**: 313–330, 1990.
6. Clowes AW, Reidy MA and Clowes MM, Kinetics of cellular proliferation of arterial injury. (I) Smooth muscle growth in the absence of endothelium. *Lab Invest* **49**: 327–333, 1983.
7. Campbell GR and Campbell JH, Phenotypic modulation of smooth muscle cells in primary culture. In: *Vascular Smooth Muscle in Culture* (Eds. Campbell JH and Campbell GR), pp. 39–55. CRC Press, Boca Raton, 1987.
8. Barnes MJ, Collagens in atherosclerosis. *Coll Rel Res* **5**: 65–97, 1985.
9. Ang AK, Tachas G, Campbell JH, Bateman JF and Campbell GR, Collagen synthesis by rabbit cultured smooth muscle cells. Alteration with phenotype. *Biochem J* **265**: 461–469, 1990.
10. Majors AK and Ehrart LA, Cell density and proliferation modulate collagen synthesis and procollagen mRNA levels in arterial smooth muscle cells. *Exp Cell Res* **200**: 168–174, 1992.
11. Asundi V, Cowan K, Matzura D, Wagner W and Dreher KL, Characterization of extracellular proteoglycan transcripts expressed by vascular smooth muscle cells. *Eur J Cell Biol* **52**: 98–104, 1990.
12. Clowes AW and Karnovsky MJ, Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature* **265**: 625–626, 1977.
13. Guyton JR, Rosenberg RD, Clowes AW and Karnovsky MJ, Inhibition of rat arterial smooth muscle cell proliferation by heparin. *In vivo* studies with anticoagulant and non-anticoagulant heparin. *Circ Res* **46**: 625–634, 1980.
14. Clowes AW and Clowes MM, Kinetics of cellular proliferation after arterial injury. (II) Inhibition of smooth muscle growth by heparin. *Lab Invest* **52**: 611–616, 1985.
15. Clowes AW and Clowes MM, Kinetics of cellular proliferation after arterial injury. (IV) Heparin inhibits rat smooth muscle mitogenesis and migration. *Circ Res* **58**: 839–845, 1986.
16. Scott-Burden T and Buhler FR, Regulation of smooth muscle proliferative phenotype by heparinoid-matrix interactions. *Trends Pharmacol Sci* **9**: 94–98, 1988.
17. Majack RA and Bornstein P, Heparin regulates the collagen phenotype of vascular smooth muscle cells; induced synthesis of an M_r 60,000 collagen. *J Cell Biol* **100**: 613–619, 1985.
18. Tan EML, Levine E, Sorger P, Unger GA, Hacobian N, Planck B and Iozzo RV, Heparin and endothelial cell growth factor modulate collagen and proteoglycan production in human smooth muscle cells. *Biochem Biophys Res Commun* **63**: 84–92, 1989.
19. Gavriel P and Kagan HM, Inhibition by heparin of the oxidation of lysine in collagen by lysyl oxidase. *Biochemistry* **27**: 2811–2815, 1988.
20. Asselot-Chapel C, Kern P and Labat-Robert J, Biosyntheses of interstitial collagens and fibronectin by porcine aorta smooth muscle cells. Modulation by low molecular weight heparin fragments. *Biochim Biophys Acta* **993**: 240–244, 1989.
21. Asselot-Chapel C, Labat-Robert J and Kern P, Heparin fragments regulate collagen phenotype and fibronectin synthesis in the skin of genetically diabetic mice. *Biochem Pharmacol* **38**: 895–899, 1989.
22. Asselot-Chapel C, Combacau L, Labat-Robert J and Kern P, Expression of fibronectin and interstitial collagen genes in smooth muscle cells. Modulation by low molecular weight heparin fragments. *Biochem Pharmacol* **49**: 653–659.
23. Avramoglou T and Jozefonvicz J, Derivatized dextran inhibition of smooth muscle cells proliferation. *J Biomater Sci Polymer Edn* **3**: 149–154, 1991.
24. Le Tourneur D, Logeart D, Avramoglou T and Jozefonvicz J, Antiproliferative capacity of synthetic dextrans on smooth muscle cell growth: the model of derivatized dextrans as heparin-like polymers. *J Biomater Sci Polymer Edn* **4**: 1–14, 1993.
25. Mauzac M and Jozefonvicz J, Anticoagulant activity of dextran derivatives: I. Synthesis and characterization. *Biomaterial* **5**: 301–304, 1984.
26. El Nabout R, Martin M, Remy J, Robert L and Lafuma C, Heparin fragments modulate the cellular phenotype

of fibroblasts from radiation-induced subcutaneous fibrosis. *Exp Mol Pathol* **51**: 111–122, 1989.
27. El Nabout R, Martin M, Remy J, Kern P and Lafuma C, Collagen synthesis and deposition in cultured fibroblasts from subcutaneous radiation-induced fibrosis. Modification as a function of cell aging. *Matrix* **9**: 411–420, 1989.
28. Wiestner M, Rohde H, Helle O, Krief T, Timpl R and Muller PK, Low rate of procollagen conversion in dermatosparatic sheep fibroblasts is parallel by increased synthesis of type I and type III collagen. *EMBO J* **1**: 513–516, 1982.
29. Chromczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
30. Sandberg M and Vuorio E, Localization of types I, II, III collagen mRNAs in developing human skeletal tissues by *in situ* hybridization. *J Cell Biol* **104**: 1077–1084, 1987.
31. Burton K, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* **62**: 315–323, 1956.
32. Stepp MA, Kindy MS, Franzblau K and Sonenshein GE, Complex regulation of collagen gene expression in cultured bovine aortic smooth muscle cells. *J Biol Chem* **261**: 6542–6548, 1986.
33. Kindy MS, Chang CJ and Sonenshein GE, Serum deprivation of vascular smooth muscle cells enhances collagen gene expression. *J Biol Chem* **263**: 11426–11430, 1988.
34. Chang CJ and Sonenshein GE, Increased collagen gene expression in vascular smooth muscle cells cultured in serum or isoleucine deprived medium. *Matrix* **11**: 242–252, 1991.
35. Bateman JF and Golub SB, Assessment of procollagen processing by fibroblasts cultured in the presence of dextran sulphate. *Biochem J* **267**: 573–577, 1990.