Neointima formation is promoted by surgical preparation and inhibited by cyclic nucleotides in human saphenous vein organ cultures

Intimal thickening is an important cause of late coronary vein graft occlusion, which no variation of surgical technique or pharmacologic intervention has been shown to reduce. We used a recently developed quantitative organ culture of human saphenous vein to investigate whether surgical preparative injury promotes neointima formation. We also investigated the effects on neointima formation of the lipid-soluble cyclic nucleotide analogs, 8-Br-cyclic adenosine monophosphate and 8-Br-cyclic guanosine monophosphate, and the phosphodiesterase inhibitor, isobutylmethylxanthine. These agents are pharmacologic mimetics of endothelium-derived prostacyclin and nitric oxide, which elevate vascular smooth muscle cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations, respectively, and may normally suppress neointima formation. Surgical preparation was found to promote intimal thickening and neointimal smooth muscle cell proliferation by 42% and 48%, respectively. 8-Br-cyclic adenosine monophosphate, 8-Br-cyclic guanosine monophosphate, or isobutylmethylxanthine (which elevated endogenous cyclic adenosine monophosphate concentrations) inhibited intimal thickening by 80%, 40%, and 72%, respectively, at a concentration of 0.1 mmol/L. The results imply that surgical techniques that avoid preparative injury and vasodilator drugs that act by elevating cyclic adenosine monophosphate or cyclic guanosine monophosphate concentrations may reduce neointima formation in vein grafts. (J THORAC CARDIOVASC SURG 1995;109:2-12)

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Intimal thickening is a key contributor to late arteriovenous graft occlusion.[1-3] It is caused by migration and proliferation of vascular smooth muscle cells and laying down of extracellular matrix. Intimal thickening may critically narrow the graft lumen in itself or may promote atheroma formation leading to further stenosis.

Proliferation of vascular smooth muscle cells probably results from the action of several endogenously produced growth factors, the most important of which may be platelet-derived growth factor[4, 5] and basic fibroblast growth factor.[6, 7] The causes of growth factor production in vein grafts is uncertain, although wall stress[8] and injury[9] have

both been implicated. Cell injury appears to be necessary, in particular, for release of basic fibroblast growth factor from cells.[10] A relationship between surgical preparative injury and the extent of subsequent vein graft intimal thickening has been suggested in animal models on the basis of semiquantitative morphologic criteria.[11, 12] Overdistention of vein,[13, 14] used to reverse spasm, and exposure to solutions of low oncotic pressure[15] or low temperature[14, 16, 17] have been implicated as causes of this injury. In this study, the influence of surgical preparation on neointima formation was investigated quantitatively in a recently developed organ culture of human saphenous vein.[9, 18]

Pharmacologic strategies to inhibit neointima formation have included attempts to inhibit mitogen release from platelets,[19] although this appears to limit thrombosis rather than neointima formation.[20] The use of agents that have direct antimitotic activity on vascular smooth muscle cells probably represents the most attractive route. Experiments with heparin[21, 22] have been disappointing, however, probably because of the low potency of this agent. Elevation of intracellular adenosine 3′,5′-cyclic monophosphate (cAMP)[23-29] or guanosine 3′,5′-cyclic monophosphate (cGMP)[29-31] has been shown to inhibit the proliferation of vascular smooth muscle cells. Because endothelium-derived prostaglandins and nitric oxide can elevate cAMP and cGMP concentrations, respectively, in vascular smooth muscle cells,[32] cyclic nucleotides may exert tonic control of their proliferation, which might be potentiated pharmacologically by cyclic nucleotide phosphodiesterase inhibitors. Alternatively, the effect of cAMP and cGMP elevation might be mimicked by membrane-permeable cyclic nucleotide analogs. In this study, the possible inhibitory effects on neointima formation of 8-Br-cAMP, 8-Br-cGMP, and the nonselective cyclic nucleotide phosphodiesterase inhibitor, 3-isobutyl-l-methylaxanthine (IBMX), were also investigated in the organ culture model.

Materials and methods

Paired segments of freshly isolated and surplus surgically prepared saphenous vein were obtained from 33 patients (mean age 60.4 years; range 38 to 73 years; 7 women) who were undergoing coronary artery bypass grafting. Ethical permission was obtained from the relevant authority. Freshly isolated segments were obtained immediately after dissection by a "no-touch" technique.[33] Surgically prepared segments had been subjected to side branch ligation, adventitial stripping, and uncontrolled manual distention with the patient's heparinized blood followed by storage in the same medium for 60 to 120 minutes.[9, 14] Segments were obtained after completion of the last proximal anastomosis.

Vein segments were collected in tissue culture medium (RPMI 1640 with HEPES buffer, 20 mmol/L, pH 7.4) containing heparin sodium, 4 IU/ml (CP Pharmaceuticals, Wrexham, United Kingdom), papaverine hydrochloride, 0.2 mg/ml (McCarthy Medical, Essex, United Kingdom), and amphotericin B, 5 μg/ml (Flow Labs, Irvine, Calif.), and transferred to the laboratory at room temperature.

Tissue culture and vein preparation procedures. Vein segments were cleaned free of excess fat and connective tissue under sterile conditions, opened with the luminal surface uppermost, and cut into 1 cm lengths. Vein segments were then incubated in RPMI 1640 medium containing sodium bicarbonate, 2 gm/L, supplemented with 30% fetal bovine serum (Imperial Labs, Hants, United Kingdom), gentamicin, 2.5 μg/ml (David Bull Labs, Warwick, United Kingdom), penicillin:streptomycin, 100 μg/ml (Flow Labs), and glutamine, 2 mmol/L (Flow Labs), and maintained for 14 days at 37° C in 5% carbon dioxide in an air incubator. Tissue culture medium was changed every 48 to 72 hours and was supplemented with [6-^{3}H]thymidine (5 Ci/mmol; Amersham International, Aylesbury, Bucks, United Kingdom) for the last 24 hours in culture. 8-Br-cAMP, 8-Br-cGMP, and IBMX were purchased from Sigma (Poole, Dorset, United Kingdom). 8-Br-cAMP and 8-Br-cGMP were dissolved to 0.1 mmol in tissue culture medium and sterilized by filtration. IBMX was dissolved in dimethylsulfoxide and diluted with tissue culture medium to yield a final concentration 0.1 mmol IBMX and 0.1% dimethylsulfoxide. The culture medium was supplemented with the appropriate pharmacologic agent throughout the culture period. For the experiments with IBMX, control cultures were performed with medium containing 0.1% dimethylsulfoxide.

At the end of the culture period, the vein segments were bisected and one portion was immediately placed into liquid nitrogen while the other was fixed in phosphate buffer, 0.1 mol (pH 7.2), containing 10% formaldehyde. The frozen vein segments were pulverized under liquid nitrogen, extracted with perchloric acid,[34] and the soluble fractions were neutralized by paired ion extraction.[35] Measurements of purine metabolites by high-performance liquid chromatography,[14] deoxyribonucleic acid by fluorimetry,[36] and total [^{3}H]thymidine incorporation by liquid scintillation spectrometry[37] were then carried out as described previously.[18] The concentrations of cAMP and cGMP were measured in neutralized soluble fractions of the same extracts by radioimmunoassay using commercially available kits (Amersham International, RPA 508 and RPA 525, respectively).

The fixed vein segments were embedded in paraffin and transverse sections (5 μm thick) were then cut and stained with Miller's elastic and van Gieson stain.[38] Intimal thickening in culture was measured with a calibrated graticule at 5 to 10 points on two sections from each vein. To count [^{3}H]thymidine-labeled cells, we subjected unstained sections to autoradiography by coating with K2 nuclear emulsion (Ilford Ltd., Cheshire, United Kingdom) as previously described[18] and counterstained with Harris' hematoxylin and eosin.[38] Neointimal and medial cells

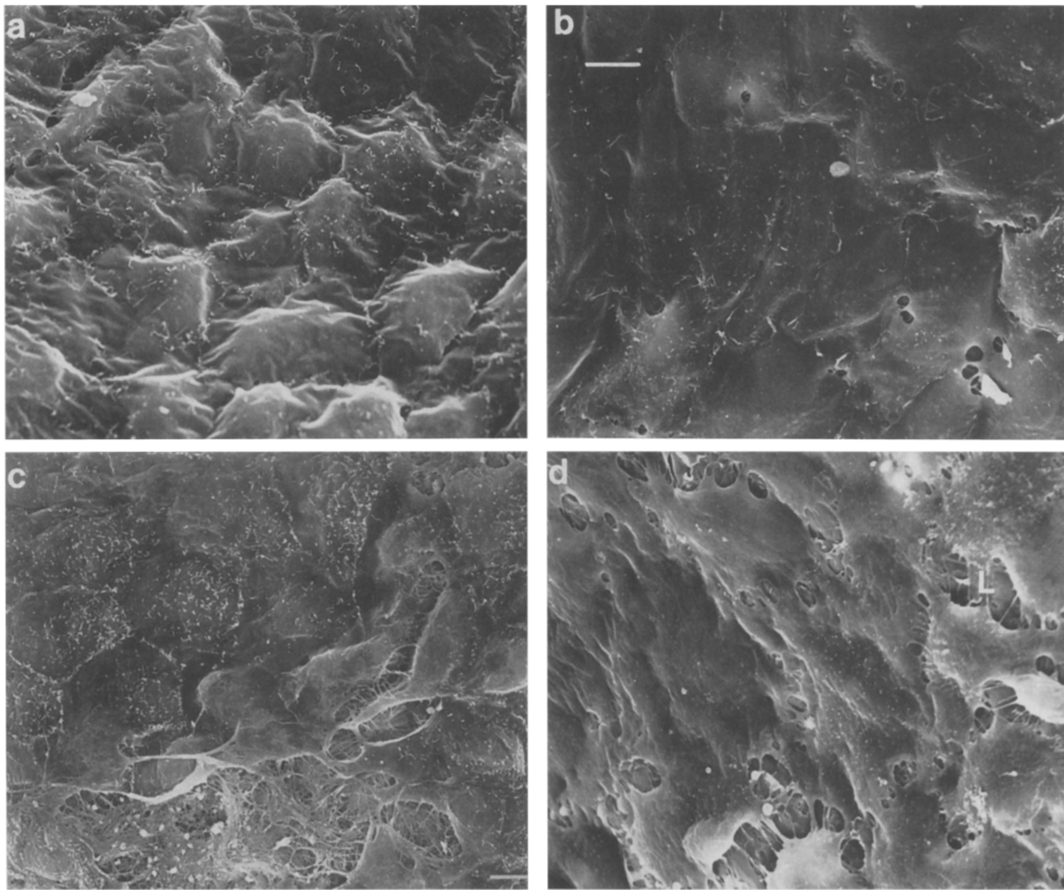

Fig. 1. Intimal surface structure. Scanning electron micrographs of representatives of 20 replicate vein segments examined under each of the following conditions: **a,** Freshly isolated vein after preparation for culture. **b,** Freshly isolated vein after 14 days in culture. **c,** Surgically prepared vein after preparation for culture; note that the endothelium is partly disrupted. **d,** Surgically prepared vein after 14 days in culture; note that the endothelial coverage has been largely restored, although there are lamellipodia *(L)* spanning the gaps between endothelial cells. (The scale bar = 10 μm.)

labeled with more than about 20 silver grains were counted in three sections from each vein and related to the intimal length or intimal length × medial thickness off the section, respectively, which were measured with a calibrated graticule, as described earlier. The total number of nuclei in the neointimal layer was also counted.

Results

Effect of surgical preparation. Freshly isolated veins had a significantly higher adenosine triphosphate (ATP) concentration than surgically prepared veins (164 ± 24 versus 86 ± 15 nmol/mg wet weight, standard error of the mean, $n = 25, p < 0.01$, paired t test). The ratio of adenosine triphosphate/diphosphate (ATP/ADP) concentration was similarly decreased by surgical preparation from 1.28 ± 0.16 to 0.75 ± 0.10 ($p < 0.01$). This confirmed that surgical preparation caused metabolic injury, as previously reported.[9, 14, 39] Scanning electron microscopy confirmed also that freshly isolated veins had an intact endothelium but that surgically prepared veins had partial endothelial injury (Fig. 1, *a* compared to *c*).

Culture in serum for 14 days led to the development of a neointima visible as an elastin-poor layer in transverse histologic sections stained with Miller's elastic–van Gieson stain (Fig. 2, *a* compared to *b*). Proliferating smooth muscle cells were observed in [^{3}H]thymidine autoradiograms in the neointima (Fig. 2, *d* compared to *e*) and less frequently in the media (not shown). The histologic appearance of the neointima was similar in cultured, freshly iso-

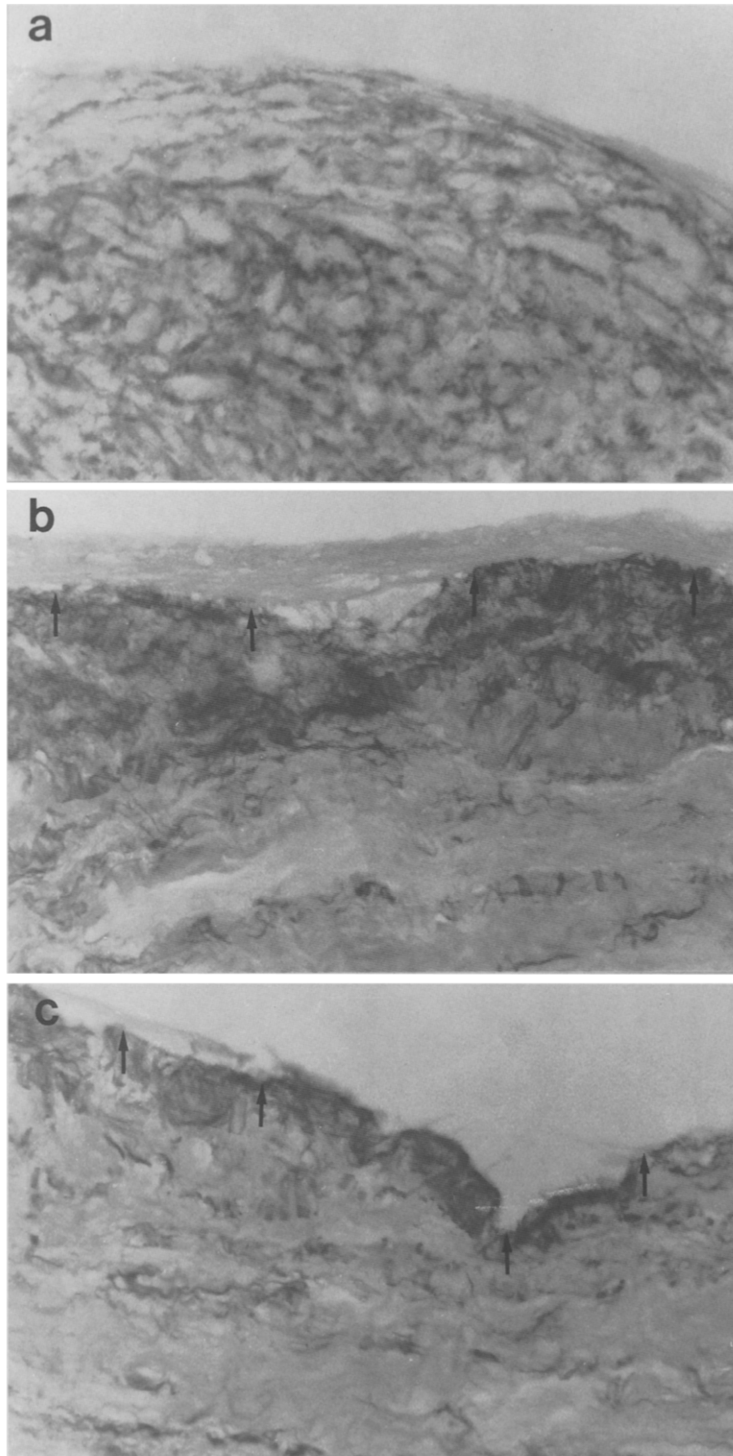

Fig. 2. Transverse sections of veins. **a,** A transverse section (5 μm) of a surgically prepared vein before culturing, stained with Miller's elastic and van Gieson. Note elastic stain extending up to the lumen. **b,** A similar section of a surgically prepared vein after culturing for 14 days in serum alone. Note the lightly stained new intima above the internal elastic layer *(small arrows)*. **c,** A similar section of a surgically prepared vein after culturing for 14 days in serum with 8-Br-cAMP, 0.1 mmol/L. The new intima is greatly attenuated.

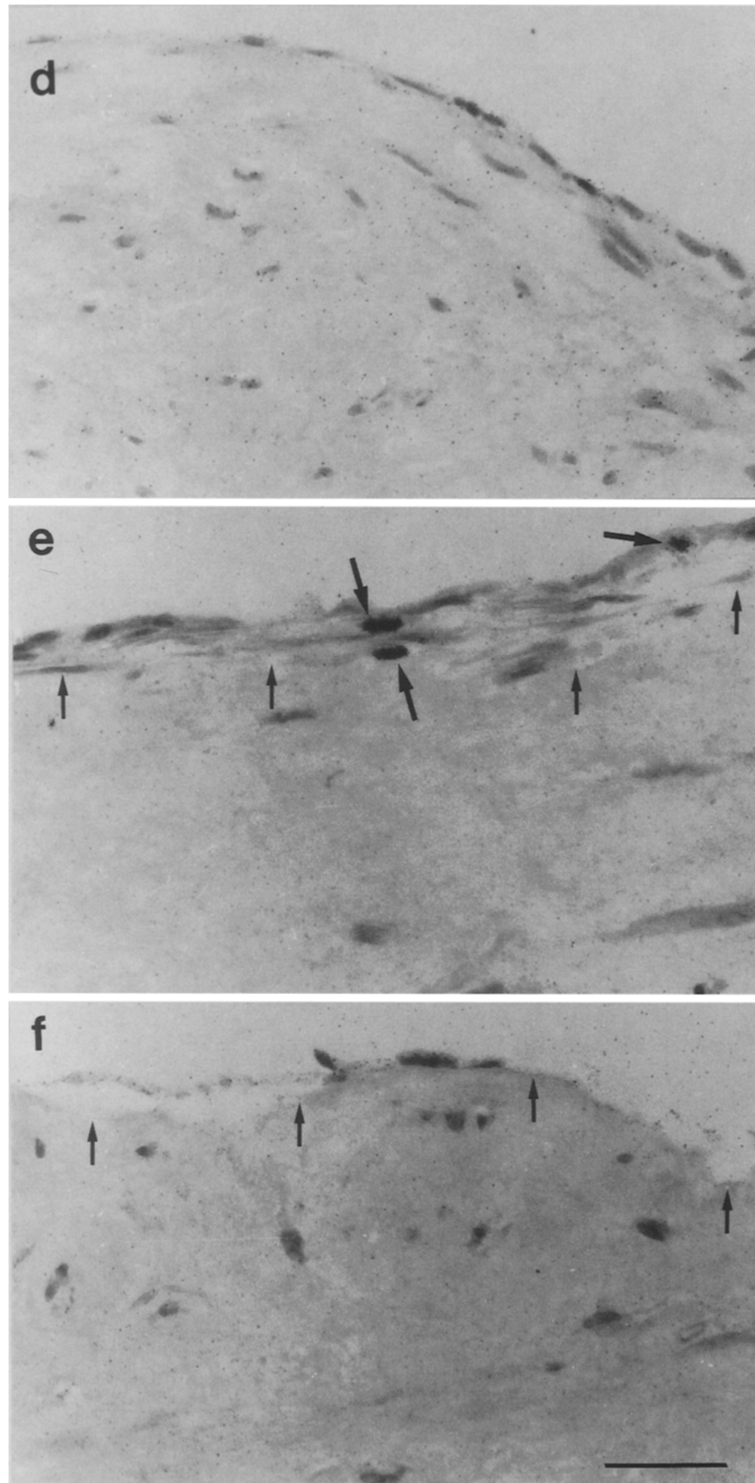

Fig. 2. Cont'd. d, An autoradiogram counterstained with Harris' hematoxylin and eosin of a near-serial section to **a.** There are no silver-stained nuclei. **e,** A similar autoradiogram near-serial to **b.** Labeled cells *(large arrows)* are concentrated in the new intima above the internal elastic layer *(small arrows)*. **f,** A similar autoradiogram near-serial to **c.** There are no silver stained nuclei. The *scale bar* applies to all panels and represents 50 μm.

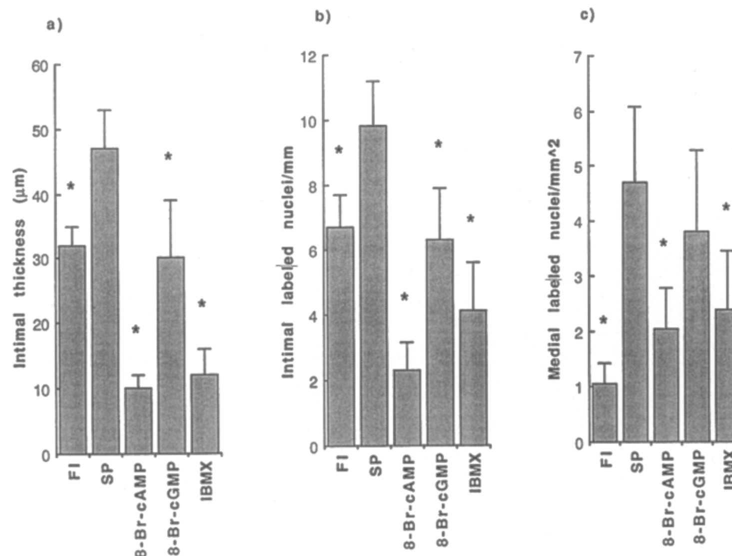

Fig. 3. Unpaired comparison of the effects of surgical preparation, cyclic nucleotides, and IBMX. Freshly isolated *(FI)* ($n = 28$) and surgically prepared *(SP)* veins ($n = 33$) were cultured for 14 days in serum. Further segments of surgically prepared vein were cultured in serum supplemented with 0.1 mmol of 8-Br-cAMP ($n = 16$), 8-Br-cGMP ($n = 12$), or IBMX ($n = 8$) as indicated. The following parameters were measured from transverse sections: **a,** Intimal thickness. **b,** The number of thymidine-labeled cells per millimeter of intimal length. **c,** The number of thymidine-labeled cells per square millimeter of media. $^*p < 0.001$ versus surgically prepared vein cultured in serum alone (unpaired *t* test). For paired comparisons see Tables I to IV.

lated, and surgically prepared veins, both in transverse sections (not shown) and in scanning micrographs (Fig. 1, *b* and *d*).

Surgically prepared veins showed 42% greater intimal thickening during culture, associated with a 48% increase in the number of thymidine-labeled neointimal cells and a 56% increase in the neointimal thymidine index (Fig. 3, Table I). Medial proliferation was also significantly more prevalent in cultured surgically prepared veins (Fig. 3, Table I). ATP concentration, a marker of viable cell numbers, was similar in cultures of surgically prepared veins and freshly isolated veins, despite the lower starting values (Table I). This observation is consistent with there being compensatory cell proliferation in surgically prepared veins, although deoxyribonucleic acid concentrations remained 20% lower than in cultured freshly isolated veins (Table I). The higher ratios of ATP/ADP concentration in cultured surgically prepared veins (Table I) suggests that more of the cells in these cultures had transformed into a synthetic phenotype, because synthetic-state vascular smooth muscle cells have a higher value of this parameter than contractile-state cells.[28]

Table I. *Effect of surgical preparation*

	Freshly isolated veins	*Surgically prepared veins*
Intimal thickness (µm)	33 ± 4	47 ± 6*
Intimal [^{3}H]thymidine cells/mm	6.7 ± 1.1	9.9 ± 1.4*
Intimal [^{3}H]thymidine index (%)	5.3 ± 0.7	8.3 ± 1.0†
Medial [^{3}H]thymidine cells/mm^2	0.8 ± 0.2	5.2 ± 1.5†
ATP (nmol/gm wet weight)	186 ± 19	188 ± 25
ATP/ADP ratio	2.9 ± 0.2	4.54 ± 0.55†
DNA (µg/mg wet weight)	0.53 ± 0.05	0.41 ± 0.04*

Paired segments of freshly isolated and surgically prepared veins from 26 patients were cultured for 14 days with [^{3}H]thymidine present for the last 24 hours. ATP, ADP, and deoxyribonucleic (DNA) concentrations and total [^{3}H]thymidine incorporation were measured in perchloric acid extracts of frozen pulverized tissue. Other parameters were measured on 5 µm thick transverse sections of formalin-fixed vein segments.
$^*p < 0.05$ versus freshly isolated veins (paired Student's *t* test).
$†p < 0.01$ versus freshly isolated veins (paired Student's *t* test).

Effects of cyclic nucleotides and IBMX. Only surgically prepared veins were used for this study because of their availability in larger amounts than freshly-isolated veins. 8-Br-cAMP decreased intimal thickening by 80%, 8-Br-cGMP decreased it by 40%, and IBMX decreased it by 72% (Fig. 3, *a*, Tables II to IV). 8-Br-cAMP and 8-Br-cGMP also

Table II. *Effect of 8-Br-cAMP*

	Experiment		
	Control: day 0	*Control: day 14*	*8-Br-cAMP: day 14*
Intimal thickness (μm)		50 ± 10	10 ± 2†
Intimal [³H]thymidine cells/mm		9.8 ± 1.8	1.6 ± 0.5†
Intimal [³H]thymidine index (%)		8.5 ± 1.4	3.42 ± 0.8*
Medial [³H]thymidine cells/mm²		8.3 ± 2.8	1.7 ± 0.8*
ATP (nmol/gm wet weight)	80 ± 20	170 ± 20‡	110 ± 10†
ATP/ADP ratio	0.63 ± 0.13	4.6 ± 0.8§	5.7 ± 1.2§
DNA (μg/mg wet weight)	0.4 ± 0.03	0.33 ± 0.03	0.28 ± 0.03*‡

Replicate segments of veins from 15 patients were cultured for 14 days with and without 8-Br-cAMP (0.1 mmol/L) and with [³H]thymidine for the last 24 hours.
*$p < 0.05$ versus control, day 14 (paired Student's t test).
†$p < 0.001$ versus control, day 14 (paired Student's t test).
‡$p < 0.05$ versus control, day 0 (paired Student's t test).
§$p < 0.001$ versus control, day 0 (paired Student's t test).

Table III. *Effect of 8-Br-cGMP*

	Experiment		
	Control: day 0	*Control: day 14*	*8-Br-cGMP: day 14*
Intimal thickness (μm)		53 ± 13	30 ± 10†
Intimal [³H]thymidine cells/mm		7.9 ± 1.8	5.6 ± 1.7*
Intimal [³H]thymidine index (%)		6.1 ± 0.9	5.6 ± 1.1
Medial [³H]thymidine cells/mm²		4.9 ± 1.9	2.4 ± 0.7
ATP (nmol/gm wet weight)	91 ± 27	170 ± 30‡	180 ± 30‡
ATP/ADP ratio	0.74 ± 0.16	4.1 ± 0.70§	4.8 ± 0.9§
DNA (μg/mg wet weight)	0.35 ± 0.03	0.29 ± 0.03	0.25 ± 0.03‡

Replicate segments of veins from 11 patients were cultured for 14 days with and without 8-Br-cGMP (0.1 mmol/L) and with [³H]thymidine for the last 24 hours.
*$p < 0.05$ versus control, day 14 (paired Student's t test).
†$p < 0.001$ versus control, day 14 (paired Student's t test).
‡$p < 0.05$ versus control, day 0 (paired Student's t test).
§$p < 0.001$ versus control, day 0 (paired Student's t test).

decreased the number of [³H]thymidine-labeled cells in the neointima (Fig. 3, *b*) and 8-Br-cAMP the neointimal thymidine index (Table II), although neither effect was seen with IBMX (Fig. 3, *b,* Table IV). Medial proliferation was inhibited (Fig. 3, *c*) significantly by 8-Br-cAMP (Table II) and IBMX

Table IV. *Effect of IBMX*

	Experiment		
	Control: day 0	*Control: day 14*	*IBMX: day 14*
Intimal thickness (μm)		40 ± 6	12 ± 4†
Intimal [³H]thymidine cells/mm		7.4 ± 2.7	4.2 ± 1.5
Intimal [³H]thymidine index (%)		8.0 ± 1.8	9.3 ± 2.9
Medial [³H]thymidine cells/mm²		9.4 ± 2.9	2.4 ± 1.1*
ATP (nmol/gm wet weight)	80 ± 30	200 ± 25‡	125 ± 30*
ATP/ADP ratio	0.68 ± 0.2	6.2 ± 0.85§	6.0 ± 1.3§
DNA (μg/mg wet weight)	0.33 ± 0.05	0.38 ± 0.05	0.27 ± 0.05*

Replicate segments of veins from 8 patients were cultured for 14 days with and without IBMX (0.1 mmol/L) and with [³H]thymidine for the last 24 hours.
*$p < 0.05$ versus control, day 14 (paired Student's t test).
†$p < 0.001$ versus control, day 14 (paired Student's t test).
‡$p < 0.05$ versus control, day 0 (paired Student's t test).
§$p < 0.001$ versus control, day 0 (paired Student's t test).

(Table IV), whereas the inhibitory effects of 8-Br-cGMP (Table III) just failed to reach significance ($p = 0.1$).

None of 8-Br-cAMP (Table II), 8-Br-cGMP (Table III), or IBMX (Table IV) caused a decline in ATP concentration to values below those observed on day 0, which implies that they did have a cytotoxic action. 8-Br-cAMP and IBMX, however, prevented the increase in ATP concentration that occurred during culture and significantly depressed the concentration of deoxyribonucleic acid (8-Br-cGMP showed an inhibitory trend, $p = 0.06$). This information provides further evidence that 8-Br-cAMP and IBMX inhibited proliferation, thereby decreasing the final number of viable cells. None of the agents affected the ratio of ATP/ADP concentration, which implies that they did not inhibit phenotypic modulation.

To clarify the basis for the inhibitory effects of IBMX, we measured cyclic nucleotide concentrations. The concentration of cAMP was not increased by culturing in the presence of serum with or without 0.1% dimethylsulfoxide (vehicle for IBMX) (Table V). By contrast, cGMP concentration was significantly elevated by culturing for 1 day in serum with or without dimethylsulfoxide. Culturing tended to increase cGMP after 14 days also. This may be due to stimulation of endothelial nitric oxide production by agents such as serotonin, which are

Table V. *Effect of IBMX on intracellular cyclic nucleotide concentrations*

Nucleotide	*cAMP (pmol/μg DNA)*			*cGMP (fmol/μg DNA)*		
	Day 0	*Day 1*	*Day 14*	*Day 0*	*Day 1*	*Day 14*
Control	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	32 ± 4	260 ± 80†	70 ± 40
DMSO (0.1%)		1.1 ± 0.2	1.0 ± 0.1		150 ± 50	40 ± 8
IBMX (0.1 mmol/L)		1.8 ± 0.2*	0.9 ± 0.1		150 ± 50	60 ± 20

Replicate segments of veins from 10 patients were cultured for 1 or 14 days with and without IBMX (0.1 mmol/L). Cyclic nucleotide concentrations were measured by radioimmunoassay in perchloric acid extracts of frozen pulverized tissue.
*$p < 0.05$ versus control on the same day (paired Student's t test).
†$p < 0.05$ versus control on day 0 (paired Student's t test).

known to be present in fetal calf serum.[40] IBMX significantly increased cAMP concentration after 1 day, but not after 14 days (Table V), and did not affect cGMP concentration at either time (Table V). The effects of IBMX are more likely therefore to have resulted from cAMP than cGMP elevation.

Discussion

To our knowledge, these results provide the first direct quantitative evidence that surgical preparative injury promotes neointimal thickening and neointimal proliferation of vascular smooth muscle cells. A previous qualitative morphologic study in dogs reached the same conclusion,[11] but a quantitative study in pigs found no difference.[41] The study in pigs,[41] however, considered only patent grafts. Because surgical preparation was significantly correlated with early graft occlusion,[42] these patent grafts were probably those least injured among the "surgically prepared" group. The effect of surgical preparation may have been thereby obscured. In a previous organ culture study,[9] we found that surgical preparation increased medial proliferation, as confirmed here, but not neointimal proliferation. In that study,[9] labeling with [^{3}H]thymidine was carried out continuously rather than for the last 24 hours only. As a result, the final neointimal thickening was less (30 μm[9] versus 47 μm [present data] in surgically prepared veins), probably owing to the known antiproliferative effect of [^{3}H]thymidine.[43] This inhibition may have counteracted the stimulatory effect of surgical preparation. In addition, the differences observed in this study were based on comparison of a larger group (n = 26).

The data presented here further demonstrate that analogs of cAMP and cGMP inhibit neointimal and medial proliferation of vascular smooth muscle cells in human saphenous vein at concentrations equal to or less than those previously shown to inhibit other preparations of vascular smooth muscle cells.[29, 44] IBMX, a cyclic nucleotide phosphodiesterase inhibitor that increased endogenous cAMP concentrations by only twofold after 1 day, was almost as effective as 8-Br-cAMP. This underlines the sensitivity of human smooth muscle cells to inhibition by cAMP. The failure of IBMX to sustain elevated cAMP concentrations for 14 days probably explains why the thymidine index over the last 24 hours of culture was not affected. The decline in cAMP concentrations may be because the production of prostacyclin (a likely stimulator of endogenous cAMP concentration in veins) decreases during culture (AA Soyombo, AC Newby, unpublished observations). The inhibitory effect of 8-Br-cGMP occurred at concentrations tenfold lower than in previous studies[29-31] and just sufficient to cause vascular relaxation. This implies that vasodilator concentrations of endothelium-derived nitric oxide exert a tonic inhibitory control on proliferation of human vascular smooth muscle cells that may be lost after vein grafting.[45]

How far can these data be extrapolated to the clinical situation? Organ culture generates a rapid intimally directed proliferation of vascular smooth muscle cells, as seen in early vein grafts. In organ culture, as in vivo and as opposed to isolated cell culture models, vascular smooth muscle cells are initially in a contractile state and are in close proximity to endothelial cells and extracellular matrix components. In organ culture, proliferation is stimulated by serum rather than by endogenous or platelet-derived mitogens, as in vivo. However, serum provides a similar combination of mitogens (including platelet-derived growth factor and basic fibroblast growth factor) to those likely to be present in vein grafts. Furthermore, the inhibitors studied here are likely, from previous work, to be effective irrespective of the pathway of mitogenesis.[46]

The results of this study imply therefore that vein graft intimal thickening can be ameliorated by

avoiding surgical preparative injury. Several simple protocols have been described to avoid medial injury caused by overdistention by using either a pressure-limiting device[13] or arterial pressure[47, 48] when checking the competence of side-branch ligatures. Endothelial disruption can be reduced by avoiding manipulation that leads to abrasion of the intimal surface.[17] Avoidance of injury was shown to improve early patency in a pig model.[42] Conversely, distention of vein was shown to promote graft atherosclerosis by increasing cholesterol and lipoprotein deposition in grafts of hypercholesterolemic primates.[49] In the absence of a definitive clinical trial, the present study adds to the weight of experimental evidence favoring the use of atraumatic methods of vein preparation.

Postoperative pharmacologic intervention, as well as optimal surgical preparative technique, is needed to improve long-term patency. Only lipid-lowering therapy has so far been shown to decrease lesion formation in vein grafts.[50] Potent inhibitors of proliferation of vascular smooth muscle cells have not been available for clinical studies, however. The results of this study indicate that cAMP and cGMP pathways are possible therapeutic targets. Although neither 8-Br-cAMP nor IBMX could be used in man, agents with similar actions are available for clinical studies. Stable analogs of prostacyclin and selective cAMP phosphodiesterase inhibitors (e.g., milrinone) have been shown to exert vasodilator effects,[51] mediated by elevation of cAMP concentration in vascular smooth muscle cells. It will be important to establish, in animal studies initially, whether significant inhibition of proliferation of vascular smooth muscle cells in vein grafts can be achieved without excessive vasodilatation or other side effects such as positive inotropy.[51] On the basis of our data, nitrovasodilators or specific cGMP phosphodiesterase inhibitors may also be useful. A particularly exciting therapeutic possibility is raised by the recent cloning of nitric oxide synthase.[52] Transfer of this gene (for example, in liposomes or by retrovirus) into saphenous vein at the time of grafting could lead to continuous nitric oxide production and localized inhibition of intimal thickening.

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