Iodine-131-Labeled Fibronectin: Potential Agent for Imaging Atherosclerotic Lesion and Thrombus

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Fibronectin is known to interact with fibrin, collagen, etc. We have labeled fibronectin with ^{131}I, and measured its accumulation in the deendothelialized lesion in the rabbit aorta to evaluate it as a candidate for imaging atherosclerotic lesions and thrombi. Accumulation of [^{131}I] fibronectin in the deendothelialized lesion was apparent at 48 hr, and increased at 72 hr after injection of the agent. Our results indicate that radiolabeled fibronectin may be a useful tracer for imaging early atherosclerotic lesion and thrombus.

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Early detection of atheroma and thrombus is of great clinical interest with regard to current therapeutic attempts to reduce deterioration and the incidence of thrombosis. The sites of early lesions of atherosclerosis are regions where the permeability of the wall to plasma proteins is increased, at least in part due to alteration or damage of the endothelium (*1*). Recently, fibronectin was reported to be abundant in atherosclerotic lesions of the intima, especially in developing fibrous plaques, and also abundant in experimentally induced atherosclerotic lesions (*2,3*). Fibronectin is a glycoprotein consisting of two disulfide-bonded subunits, each of molecular weight ~220,000, which is present in plasma (cold insoluble globulin) and is also produced in both soluble and insoluble forms by many types of cells. It interacts with proteoglycans, and with collagen and fibrin to which it is covalently cross-linked by Factor XIII (*4,5*).

In the present study, we evaluated the accumulation of iodine-131- (^{131}I) labeled fibronectin in the deendothelialized lesion of the rabbit aorta, which has many characteristics in common with the initial fibroproliferative lesion of human atherosclerosis (*6*). In addition, the availability of scintigraphic visualization of atherosclerotic lesion and thrombus using the agent is discussed.

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MATERIALS AND METHODS

Preparation of ^{131}I-Labeled Fibronectin

Human fibronectin used for this investigation was provided. (The Green Cross Corporation, Osaka, Japan) The purity of the protein was checked with a high performance liquid chromatography (HPLC) (Hitachi, Tokyo, Japan) using TSK G3000 protein column, and was found to be 99.0% (Dimer 96.7%, Monomer 2.3%). Iodine-131 NaI (DuPont MEN Research Products, Boston, MA) and 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril (IODO-GEN) (Pierce Chemical Co., Rockford, IL) were obtained from commercial sources. IODO-GEN (10 mg) was dissolved in 2 ml of chloroform and 50 μg of IODO-GEN was plated onto the surface of a glass reaction vessel by drying under a stream of N_2 gas at room temperature. Fibronectin (500 μg) was mixed with carrier free $Na^{131}I$ (300 μCi) and 300 μl of 0.05*M* phosphate-buffered solution (PBS), pH 7.4, in the reaction vessel and incubated at room temperature for 15 min (*7*). Radioiodination was terminated by removal of the mixture from the reaction vessel. Radioiodinated fibronectin was separated from unbound ^{131}I on a Sephadex G25 column using 0.05*M* PBS, pH 7.4. The radioactivity and protein concentration of each gel-filtrated fraction were measured by using a dose calibrator (Aloka, Mitaka-Shi, Tokyo, Japan) and uv absorbance spectrophotometer (Shimadzu, Kyoto, Japan) at 280

nm, respectively. The labeling efficiency was 82.1 ± 10.3% (mean ± s.d.) and the specific activity was 0.4 – 0.6 mCi/mg.

Deendothelialization of Abdominal Aorta

Male, Japanese, white rabbits weighing 2.8 ± 0.3 kg (mean ± s.d.) were used in the study. Rabbits were anesthetized with 25 mg/kg of intravenous sodium pentobarbital. A 4 F Fogarty catheter was inserted into the aorta via the left femoral artery. When the tip of the catheter reached the abdominal aorta, 20 cm from the insertion site of the femoral artery, the balloon was inflated to a pressure of ~700 mmHg. While maintaining this pressure, the balloon was pulled back slowly to 10 cm, and then allowed to collapse. The collapsed balloon was passed down to its original position. This procedure was repeated six times before the catheter was withdrawn. The femoral artery was then ligated. Verification of the deendothelialization of the aorta was performed by endothelial staining, using Evans Blue (9 mg/kg) injected intravenously, just after the deendothelialization (1 hr before sacrifice). Formation of thrombus on the deendothelialized surface was ascertained by fibrin staining, using the phosphotungstic acid hematoxylin (PTAH) staining method of Mallory, 2 hr after the deendothelialization.

Accumulation of [^{131}I]Fibronectin on Deendothelialized Lesion

Iodine-131 fibronectin (120 ± 60 μCi, mean ± s.d.) was injected intravenously via a marginal ear vein at 2 hr after the deendothelialization. Blood (1.0 ml) was collected in heparin via the other marginal ear vein at intervals after injection to observe the clearance of [^{131}I] fibronectin. In order to prevent thyroid uptake of ^{131}I, 1 ml of 1% saline solution of potassium iodide was given intravenously, 30 min before the experiments. The radioactivity of each blood sample was counted in a well counter (Shimadzu, Kyoto, Japan) and expressed as percent of radioactivity of the baseline sample.

At 24 hr (n = 5), 48 hr (n =5), or 72 hr (n = 5) after the injection of [^{131}I]fibronectin, the animals were killed and the aorta, including normal (control) and deendothelialized (lesion) segments, was removed. The blood sample (B) as a background was taken by cardiac puncture just before killing. The radioactivity of each segment and blood sample was measured in a well counter and expressed in counts per min/g tissue (cpm/g). The degree of [^{131}I]fibronectin accumulation in the deendothelialized lesion was assessed as the ratio (cpm/g/cpm/g) of the lesion (L) with respect to the blood (L/B ratio) and the ratio (cpm/g/cpm/g) of the control (C) with respect to the blood (C/B ratio), and also as the difference in the optical density of the autoradiogram. Furthermore, for exploring the nonspecific binding of plasma protein on deendothelialized lesion, we examined the uptake of [^{131}I]HSA (human serum albumin) on the vessel wall at 24 hr after injection in two rabbits.

RESULTS

Figure 1 shows a longitudinal section of the internal surface of the aorta after deendothelialization, stained in vivo by Evans Blue. As the deendothelialized portion of the aorta was stained blue (dark gray in the black and white photograph), the endothelium of the aorta was clearly stripped off by the deendothelialization procedure. Accumulation of platelet-fibrin thrombus on a similarly deendothelialized surface of the aorta was observed as a light blue stain (gray in the black and white photograph) by PTAH staining on Figure 2.

The clearance of [^{131}I]fibronectin in rabbit was measured and its half life was 15.2 ± 2.8 hr (mean ± s.d.).

The results of the [^{131}I]fibronectin accumulation study in each animal are shown in Table 1. The L/B ratio increased gradually while the C/B ratio was almost constant. There was a statistical difference between the L/B and C/B ratio at each time interval ($p < 0.05$; Mann-Whitney U test). The autoradiogram of the longitudinal section of the aorta, which was removed at 48 hr after the injection of [^{131}I]fibronectin, clearly shows differences in optical density values between the lesion and the control sample (L/C = 10.0) (Fig. 3).

Table 2 shows organ distribution of [^{131}I]fibronectin. Although the tissue radioactivity per g of the lung, liver, kidney, and heart were lower than that of blood at 48 hr after injection, the radioactivities of lung, liver and kidney at 72 hr were higher than that of blood, especially the liver.

DISCUSSION

Our results clearly demonstrated an abundant accumulation of [^{131}I]fibronectin in the deendothelialized lesion of the rabbit aorta after a single injection of the agent. With respect to the thrombus model of this study, Stemerman et al. (*6*) reported that with endothelial

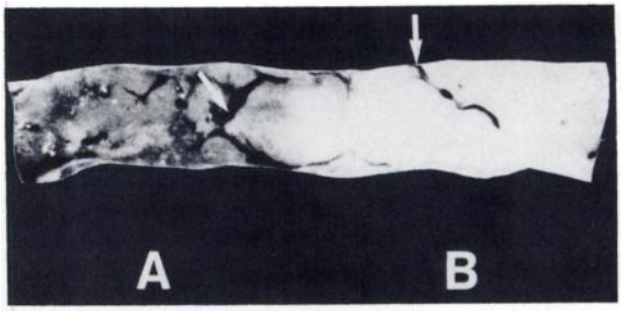

FIGURE 1
Longitudinal section of the internal surface of the aorta stained in vivo by Evans blue. The left half of the aorta (A) shows the lesion, and the right half (B) the normal portion. Only the lesion (A) was stained, while the adherent blood is seen in streaks (arrows).

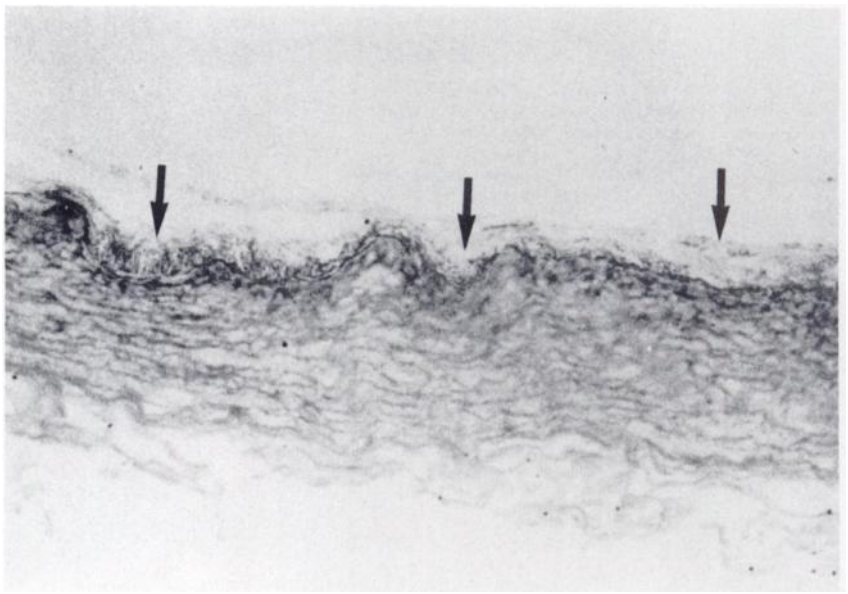

FIGURE 2
Fibrin staining of the deendothelialized aorta. The upper part of the figure shows the lumen of the aorta. The lesion, with fibrin and platelet deposition, stained light blue (gray in this black and white photograph). The arrows point to the fibrin and platelet deposition.

TABLE 1
Results of [^{131}I]Fibronectin Accumulation Study

Rabbit no.	Time postinjection (hr)	Blood (cpm/g)	Control (cpm/g)	Lesion (cpm/g)	C/B*	L/B†	L/C
1	24	30.8×10^3	15.1×10^3	57.2×10^3	0.49	1.86	3.79
2	24	10.4	4.06	50.1	0.39	4.82	12.3
3	24	9.59	5.13	25.7	0.53	2.68	5.01
4	24	8.55	2.76	38.4	0.32	4.49	13.9
5	24	3.72	1.04	17.6	0.28	4.73	16.9
				(mean ± s.d.)	0.4 ± 0.1	3.7 ± 1.4	10.4 ± 5.7
6	48	10.4×10^3	4.45×10^3	37.0×10^3	0.43	3.56	8.31
7	48	2.13	1.31	13.5	0.62	6.34	10.3
8	48	0.77	0.43	7.56	0.56	9.82	17.6
9	48	4.06	2.24	11.3	0.55	2.78	5.04
10	48	1.02	0.42	8.13	0.41	7.97	19.4
				(mean ± s.d.)	0.5 ± 0.1	6.1 ± 2.9	12.1 ± 6.1
11	72	4.99×10^3	1.44×10^3	45.9×10^3	0.29	9.20	31.9
12	72	1.69	0.57	17.7	0.34	10.5	31.1
13	72	1.10	0.53	5.33	0.48	4.81	10.1
14	72	1.25	0.35	5.96	0.28	4.77	17.0
15	72	1.32	0.75	8.01	0.57	6.07	10.7
				(mean ± s.d.)	0.4 ± 0.1	7.1 ± 2.6	20.2 ± 10.7

* Specific radioactivity ratio of control to blood.
† Specific radioactivity ratio of lesion to blood.

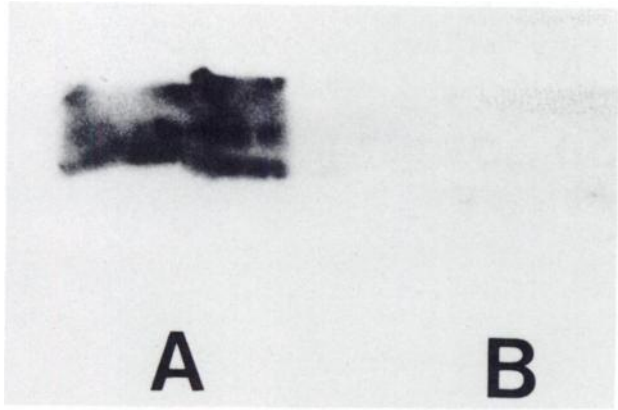

FIGURE 3
Autoradiogram of longitudinal section of the aorta, removed at 48 hr after [^{131}I]fibronectin injection. The left half (A) shows the lesion, and the right half (B) the normal portion. The difference in optical density between lesion and normal portion is apparent. The optical density ratio of lesion to control is 10.0.

removal, subendothelial connective tissue was exposed to the blood, and the thrombus formed was composed largely of platelets, with some fibrin, close to the lumen. They also demonstrated the similarity of the lesion to the fibromusculo-elastic lesion or preatherosclerotic intimal hyperplasia in man. Fibronectin is known to have affinity for a number of substances normally occurring in the deendothelialized lesion, such as fibrinogen, fibrin, and native and denatured collagen (*8*). Although several mechanisms might be involved in the adhesive action of [^{131}I]fibronectin to the lesion, the agent accumulates in the lesion mainly because of an interaction with fibrin and collagen. In the [^{131}I]HSA accumulation study, mean C/B and L/B ratios were 0.46 (range 0.29–0.63) and 0.69 (range 0.63–0.75), respectively. Since the L/B ratio of [^{131}I]HSA was much less than that of [^{131}I]fibronectin, in the present study, the accumulation of [^{131}I]fibronectin in the lesion is attributable mainly to specific binding to fibrin and collagen.

In the [^{131}I]fibronectin accumulation study, the L/B ratio increased with time while the C/B ratio was almost constantly < 1.0. The L/B ratio at 48 hr and 72 hr after injection were 6.1 ± 2.9 and 7.1 ± 2.6 (mean ± s.d.). Scully et al. (*9*) proposed that a thrombus/blood ratio of >3 was needed for positive scintigraphic imaging. Our data satisfied this criterion, so [^{131}I]fibronectin appears to be a suitable radiopharmaceutical for the scintigraphic detection of early atherosclerotic lesion and thrombus. From the results of organ distribution, the relative tissue uptake of [^{131}I]fibronectin, compared with blood, was higher at 72 hr after injection than at 48 hr, especially in the liver. Considering the background of the liver, imaging by the gamma camera at 48 hr after the radiopharmaceutical administration might be optimum. We are now carrying out studies with indium-111-labeled fibronectin, as it has an advantage over ^{131}I as a radiolabel for fibronectin, including lower radiation dosimetry per μCi administration and higher sensitivity and resolution of the gamma camera.

In conclusion, radiolabeled fibronectin may be an appropriate radiopharmaceutical for early detection of intravascular atherosclerotic lesions and thrombi.

TABLE 2
Organ Distribution of [^{131}I]Fibronectin in Rabbits*

Organ	Specific radioactivity % of blood	
	After 48 hr	After 72 hr
No. of rabbits	n = 3	n = 3
Blood	100%	100%
Lung	62 (15)	122 (24)
Liver	87 (15)	420 (212)
Kidney	69 (30)	127 (47)
Heart	38 (7)	62 (7)

*Results expressed as mean percentage (range) of tissue radioactivity per g/blood radioactivity per g.

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