Effect of Iridium 192 Radiation on Thromboatherosclerotic Plaque in the Rabbit Aorta

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THE WELL-KNOWN antiatherogenic effect of cortisone administration in the cholesterol-fed rabbit is due primarily to its capacity to inhibit intimal hyperplasia in this animal.[1] Inhibition of medial hyperplasia also probably accounts for the antiatherogenic effect observed[2] in rabbits whose aortas were exposed to radiation from iridium Ir 192 wire prior to cholesterol feeding. In animals exposed to such radiation there was a conspicuous inhibition in the development of the type of atherosclerosis ordinarily observed in an aorta subjected to the abrasive effect resulting from a wire (vinyl-covered) dwelling 48 hours in its lumen.[3]

Although cortisone retarded or prevented the development of diet-induced atherosclerosis in the cholesterol-fed rabbit, it was not found to be equally effective in retarding the hyperplastic phase of a thromboatherosclerotic process, even when this process was present in the same animal whose dietary-induced atherosclerotic response was being markedly altered by the administration of the drug. In view of this last finding, it seemed important to us to determine what effect, if any, prior exposure of the aorta to radiation from ^{192}Ir might have upon the development of this same type of thromboatherosclerotic lesion. The results of such study furnish the subject for this present report.

Methods

The thoracic and abdominal segments of the aortas of 20 mature, male, albino rabbits (2,800 to 3,800 gm) were exposed for 48 hours to radiation from a 20-cm length of iridium Ir 192 wire (Iridiotope) sheathed in vinyl tubing, exactly as described in a previous study.[2] For control purposes, a similar length of nonradioactive iridium wire was placed in the aortas of 20 additional rabbits for 48 hours. The total average radiation emitted by the wire was estimated to be approximately 1,050 mg hours equivalent of radium. If the aortic wall is assumed to be 0.75 mm from the central axis of the wire, then the aortas of the irradiated rabbits received 1,570 roentgens. Fourteen days after removal of the wires, all 40 rabbits were reoperated on under pentobarbital sodium and ether anesthesia and a magnesium-aluminum alloy thrombogenic coil (3 cm in length) was inserted into each abdominal aorta immediately distal to the exit of the left renal artery, according to previously described methods.[4] For additional controls, five normal stock-fed rabbits also bore an inserted alloy coil. Except for these latter five, all 40 remaining rabbits then were fed a cholesterol (2%) and cottonseed oil (2%) enriched diet for ten weeks at which time they were killed. Five of the rabbits exposed to radiation and two of the rabbits exposed to inert wire died during the first week following insertion of the thrombogenic coil. Thirteen rabbits exposed to radiation and 16 rabbits exposed to inert wire survived the ten-week period of cholesterol feeding. All of the five stock-fed rabbits bearing only the thrombogenic coil survived. Blood samples were obtained immediately before, and then at four and ten weeks after the beginning of the cholesterol ingestion. These were analyzed for their plasma cholesterol content.[5]

At autopsy the entire aorta of each rabbit exposed to inert or radioactive wire was opened, inspected, and its amount of gross atherosclerosis graded.[5] Blocks of tissue from the aorta were obtained from (1) the area immediately below the semilunar valves but above any point possibly reached by the wire, and (2) the thoracic and abdominal area which once had been exposed to the wire. Sections cut from these blocks were stained with Sudan IV. In addition, the proximal 10-cm section of each aorta was analyzed for cholesterol content as described previously.[6] The thromboatherosclerotic plaques induced by the magnesium-aluminum alloy coils also were inspected and sections removed for microscopic study. The plaques were then removed from the aorta and analyzed for their cholesterol content. The aortas and thrombosclerotic plaques of the five stock-fed rabbits were similarly treated.

Submitted for publication April 6, 1965.

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Fig 1.—Two aortas (*left*) of rabbits fed excess cholesterol for ten weeks, beginning two weeks after 48 hours' exposure to indwelling intra-aortic vinyl tubing stiffened with iridium Ir 192 wire and two aortas (*right*) of rabbits similarly treated except that vinyl tubing had contained an inert wire. Note marked suppression of atherosclerotic process in thoracic and abdominal segments of aortas which has been exposed to ^{192}Ir. Circular pattern of atherosclerosis observed in control aortas is typical of type of atherosclerosis induced by mechanical abrasion from tubing.

Results

When the aortas of the rabbits exposed to radiation were compared with those of rabbits exposed only to inert wire, it was evident (Fig 1) that the aortic atherosclerotic process had been markedly inhibited in the rabbits whose aortas approximately 12 weeks previously had been exposed for 48 hours to ^{192}Ir. A much more extensive process was observed in the thoracic and abdominal segments of the aortas exposed only to the inert wire, while the majority of the aortas exposed to ^{192}Ir showed, in analagous aortic segments, only limited areas of slightly elevated, grey-white tissue. The average gross grading of the 13 aortas exposed to radiation (Table) was 0.8, compared with a grading of 3.5 in the 16 controls (aortas exposed to nonradioactive wire) ($P<0.001$). The average cholesterol content of aortic segments from the 13 aortas exposed to radiation was 4.9 gm/100 gm, as compared to 9.6 gm/100 gm in similar segments from the 16 aortas exposed to nonradioactive wire ($P<0.001$).

Microscopically, there was also evident inhibition of the atherosclerotic process in those segments of the aorta which had been exposed to the ^{192}Ir. In accord with our previous observations,[2] the superficial layer containing sparsely distributed pleomorphic cells with occasional sudanophilia (color plate, Fig 1) again was observed in the sections taken from the aortic segments exposed to the ^{192}Ir. This very limited process contrasted with the intense hyperplasia and lipid infiltration observed in sections taken from analagous areas in aortas exposed to only inert wire. In these latter aortas (color plate, Fig 2) the hyperplastic tissue composed of smooth muscle fibers induced initially by the vinyl tubing itself [3] was observed to be infiltrated by abundant

Effect of I^{131} Radiation Upon Aortic and Thromboatherosclerosis

				Aorta		Plaque	
	No. of Rabbits	Average Weight (Gm)	Average Serum Cholesterol (Mg/100 Ml)	Grade Atherosclerosis (0-4)	Cholesterol (Gm/100 Gm)	Dry Weight (Mg)	Cholesterol (Gm/100 Gm)
Rabbits Exposed to Iridium Ir^{192} and Fed Cholesterol Diet							
13		3,406	975	0.8	4.9	15.4	23.1
	Range	(3,195-3,840)	(513-1,370)	(0-1.5)	(3.0-8.2)	(6-43)	(20.0-31.2)
	SE		±82	±0.13	±0.34	±3.5	±1.1
Control Rabbits Fed Cholesterol Diet							
16		3,390	1,025	3.5	9.6	22.4	22.7
	Range	(2,871-3,818)	(67-1,566)	(1-4)	(4.6-15.1)	(6-42)	(13-28.9)
	SE		±74	±0.3	±0.71	±4.5	±0.8
Control Rabbits Fed Stock Diet							
5		3,415	50	0	0.6	8.0	1.1
	Range	(2,905-3,740)	(30-65)		(0.5-0.7)	(4-14)	(0.8-1.2)
	SE		±2		±0.1	±2.1	±0.1

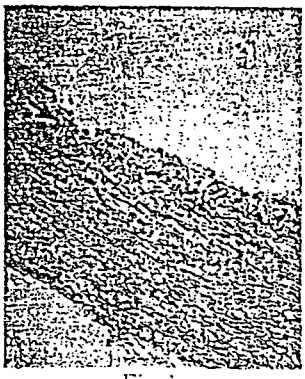

Fig 1.

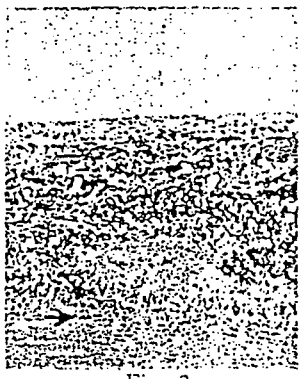

Fig 2.

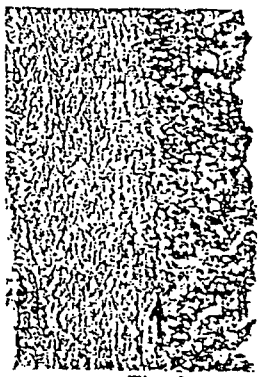

Fig 3.

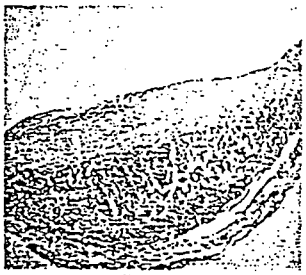

Fig 4.

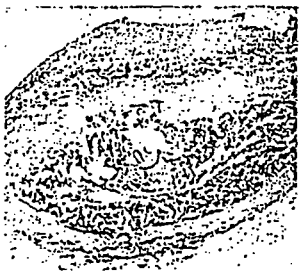

Fig 5.

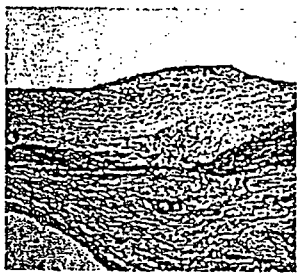

Fig 6.

Fig 1.—Photomicrograph of aorta of rabbit fed excess cholesterol for ten weeks, beginning two weeks after exposure of this segment of the aorta for 48 hours to iridium Ir 192 wire sheathed in vinyl tubing. Note the narrow fringe of new growth (*above arrow*) upon the luminal surface and the lipid accumulation (red) in this tissue and in the tunica media. The inhibitory effect of ^{192}Ir radiation can easily be seen when this aorta is compared to those shown in Fig 2 and 3. Sudan IV; × 160.

Fig 2.—Photomicrograph of aorta of rabbit treated exactly as that described in Fig 1 except this segment of the aorta has been exposed to inert wire sheathed in tubing. The hyperplastic tisue (*above arrow*) here is much more extensive than that seen in Fig 1 and also Fig 3. Note that compared with the aorta shown in Fig 3, the hyperplastic tissue here, despite the lipid infiltration appears uniform, the cellular portion consisting almost entirely of spindle-shaped smooth muscle cells. Sudan IV; × 160.

Fig 3.—Photomicrograph of a segment of the same aorta as shown in Fig 1 which was taken from an atherosclerotic area (in ascending portion of the aorta just beyond the semilunar valves) which had never been exposed to the iridium wire or its vinyl tubing. This is the usual type of aortic "spontaneous" atherosclerotic process (*above arrow*) observed in the cholesterol-fed rabbit. Even at this time (ie, after ten weeks of cholesterol feeding), most of the cells are luminal foam cells rather than smooth muscle cells, thus giving the tissue an irregular, unpatterned type of architecture. This irregularity of pattern and relatively modest total hyperplasia is characteristic of the "spontaneous" plaques. Sudan IV; × 160.

Fig 4.—Photomicrograph of the thromboatherosclerotic plaque in the same aorta as shown in Fig 1. The media (*below*) is markedly encroached upon, thinned and infiltrated with lipid. The fibrous cap of the plaque (*top*), as is usually observed, is relatively free of lipid. Compared to the plaque shown in Fig 5, this plaque shows essentially the same size, cellular structure and lipid accumulation. Sudan IV; × 40.

Fig 5.—Photomicrograph of the thromboatherosclerotic plaque in the same aorta shown in Fig 2. Despite the difference observed between the type of atherosclerosis similarly induced in this aorta by mechanical abrasion from the wire sheathed in vinyl tubing and the atherosclerosis similarly induced in the aorta exposed to ^{192}Ir, their respective aortic thromboatherosclerotic plaques are similar in every discernible detail. Sudan IV; × 40.

Fig 6.—Photomicrograph of the aortic thrombosclerotic plaque of rabbit fed stock diet for ten weeks. Lipid deposit is minimal because this rabbit remained normocholesteremic. Note the rupture of the media and the ingrowth of cells from the adventitia. These cells appear to be fibrocytes and eventually compose the greater part of the plaque. Sudan IV; × 40.

amounts of lipid. Finally, the retarded atherosclerotic process observed in the segments of aortas exposed to ^{192}Ir also contrasted strongly with the profuse dietary-induced atherosclerosis (color plate, Fig 3) which was frequently observed in these same aortas in the area immediately subjacent to the semilunar values which had been exposed neither to radiation from the wire nor to abrasion from its vinyl sheath.

On the other hand, the thromboatherosclerotic plaques induced in the rabbits exposed to ^{192}Ir did not appear to differ from those induced in the control rabbits exposed to the nonradioactive iridium wire (Fig 2). Both types of plaques, unlike those in the aortas of the stock-fed rabbits, were equally and severely infiltrated with lipid. The average weight and also the cholesterol content of the plaques of the rabbits exposed to ^{192}Ir also were approximately the same (Table) as those of the plaques of rabbits exposed to the inert wire. The plaques of both series in turn were considerably heavier and far richer in cholesterol than the plaques induced in the stock-fed rabbits.

Histologic examination (color plate, Fig 4 and 5) also failed to differentiate the plaques of the cholesterol-fed rabbits exposed to ^{192}Ir from those of rabbits exposed to inert wire. Plaques from both these groups exhibited the same amount of basal deposition of lipid. The plaques of the stock-fed rabbits, of course, exhibited no sudanophilia. Perhaps of equal importance, the structure as well as the provenance of the tissue making up all these plaques was observed to be quite different from that of the tubing-induced hyperplastic tissue. Unlike the latter, the thrombus-induced plaques consisted chiefly of dense fibrous tissue which had its origin in the tunica adventitia (color plate, Fig 6). Thus, tubing-induced hyperplasia had its origin in and reflected the structure of the media, whereas thrombus-evoked new tissue arose from and reflected the cytoarchitecture of the adventitia.

Comment

We have reported previously[3] that exposure of the rabbit's aorta to wire-stiffened vinyl tubing for 48 hours caused immediate

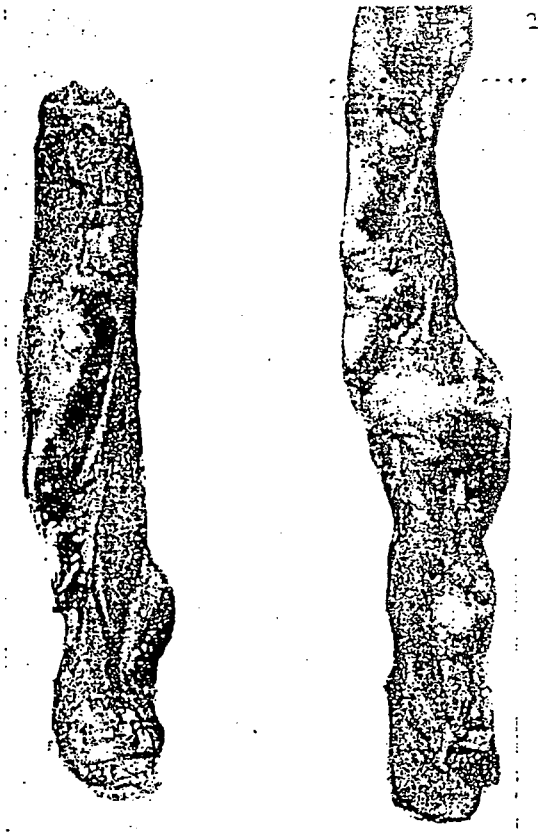

Fig 2.—Aortic thromboatherosclerotic plaque (*left*) of a rabbit fed excess cholesterol for ten weeks, beginning two weeks after 48 hours' exposure to ^{192}Ir wire and plaque (*right*) of rabbit similarly treated except exposed to inert wire. Note that in contrast to aortas shown in Fig 1, lipid accumulation and development of plaques are essentially similar.

denudation of the aortal lining endothelium and probable frequent fragmentation of its internal elastic lamina. These particular injuries led to a characteristic type of hyperplasia in which smooth muscle cells and elastic fibrils derived from the tunica media predominated. Moreover, these lesions appeared to persist for many months[3] during which they quickly became atherosclerotic if the animal was fed excess cholesterol. However, if such aortas had been exposed to ^{192}Ir at the time of denudation, both the hyperplastic and atherosclerotic processes were inhibited markedly.[2]

In the present experiments, a second form of atherosclerosis also was induced (ie, thromboatherosclerosis). This type of atherosclerosis, unlike that induced by exposure of the aorta to vinyl tubing, was not composed of smooth muscle cells and elastic fibrils[3] but of densely packed fibrous tissue. This latter tissue, moreover, was observed

to arise not from the tunica media but from the tunica adventitia. Also, unlike tubing-induced atherosclerosis, the thromboatherosclerotic plaque was not inhibited in its development or lipid accumulation by prior exposure of its site of aortic origin to ^{192}Ir.

Why does earlier exposure to ^{192}Ir afford protection of the aorta against one form of atherosclerosis, ie, the one induced by the wire sheathed in vinyl tubing, and not against another form of atherosclerosis, ie, the thromboatherosclerotic plaque? Certainly, it cannot be due to the fact that the injury induced by the vinyl tubing is more quickly recovered from than that induced by the thrombogenic coil because the lesion induced by the vinyl tubing persists as an active process and is quite susceptible to atherosclerotic involvement for many months, as our earlier study [3] revealed. We believe that this difference in protection afforded by prior irradiation is due to the fact that the tubing-induced plaques (whose development is inhibited) are composed chiefly of smooth muscle cells coming from the tunica media as shown in an earlier study.[3] However, the tissue making up the thromboatherosclerotic plaque is chiefly composed of connective tissue or fibroblasts stemming directly from the tunica adventitia (color plate, Fig 6). The proliferation of these cells do not appear to be affected by prior exposure to ^{192}Ir.

Therefore, inhibition of an atherosclerotic process by inhibition of hyperplasia can occur only if the inhibiting agent, such as radiation from ^{192}Ir or cortisone administration, is capable of suppressing the hyperplasia of the particular cell components making up that particular atherosclerotic process. Thus, cortisone was found [1] to inhibit the development of the "spontaneous" type of plaque (ie, the one usually occurring in the rabbit simply fed cholesterol and not subjected to any other procedure) by preventing the hyperplasia and metaplasia of the original lining endothelial cells into luminal foam cells.[8] However, cortisone administration did not significantly diminish the cellular development of a thromboatherosclerotic process, and as a consequence the plaques resulting were almost similar in their size and lipid content [1] to those of rabbits not treated with cortisone. Exposure of the aorta to radiation from ^{192}Ir, as the present studies demonstrate, was similarly unable to alter the cellular development of thromboatherosclerotic plaques, even though another cellular type of atherosclerosis was being inhibited in the same aortas. It is therefore probable that, if interference with the cellular development of an atherosclerotic plaque ever becomes a mode of therapy, the sensitivity of the particular tissue comprising most of the plaque to the agent to be employed must be assayed.

Summary

Prior exposure of a segment of the rabbit's aorta to radiation from iridium 192, markedly inhibits subsequent development of atherosclerosis in the exposed segment of aorta when the animal is fed excess cholesterol, but it was not found capable of inhibiting the development of thromboatherosclerosis in the same segment. This difference in inhibitory effect appears to be due to the difference in the provenance and composition of the cellular components making up the two types of atherosclerosis.

This work was aided by grants from the National Institutes of Health, National Heart Institute (HE-00119), and the Life Insurance Medical Research Fund.

The Burroughs Wellcome Fund underwrote the cost of color reproductions used in this article.

Generic and Trade Name of Drug

Iridium Ir 192——*Iriditope.*

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