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Injury and repair of smaller muscular and elastic arteries

A light microscopical study on the different healing patterns of rabbit femoral and carotid arteries following dilatation injuries by a balloon catheter

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Summary. 26 rabbits of the Danish country strain were subjected to mechanical dilatation injury of the left femoral and carotid arteries with Fogarty's embolectomy catheters F2 and F3 respectively. The rabbits were killed 2, 7, 14 and 28 days after the dilatation injury and the arteries examined histologically. Initially both of the arteries exhibited necrosis of the media and infiltration of the vessel wall with neutrophils and mononuclear cells. From day 7, intimal thickening was observed in both types of arteries, progressing in thickness during the later stages. However, thrombosis occurred in the majority of the carotid arteries, whereas this was only infrequently seen in the femoral arteries. In all of the dilated arteries, the elastic laminas were stretched or fragmented and never regained their normal appearance. In the carotid artery, giant cells accumulated around the fragmented elastin and calcified areas, located primarily at the intima-medial border. These changes were never observed in the femoral artery. At the twenty-eight days stage, proliferation of the smooth muscle cells more or less led to restitution of the media in the femoral artery, whereas the carotid artery showed medial restitution only to a lesser extent. The similarities between the injured carotid artery and human temporal arteritis, and the utility of the model as an animal model for the study of temporal arteritis are underlined.

Key words: Carotid artery – Dilatation injury – Femoral artery – Rabbit – Temporal arteritis

Introduction

Injury and repair of arteries have for long been considered important for the initiation and pathogenesis of arterial diseases e.g. arteriosclerosis and different types of arteritides. In arteriosclerosis, primary attention has been paid to the endothelial damage and the secondary intimal thickening (Ross and Glomset 1977). Consequently, attempts have been made to perform experiments with selective endothelial abrasion carried out with various methods, to study the repair processes of the endothelium and subendothelium under different conditions. Care has normally been taken to avoid more profound lesions of the underlying media, although some injury is always present (Schwartz et al. 1975; Fishman et al. 1975; Ross and Glomset 1977; Christensen et al. 1979; Reidy and Schwartz 1981; Ramsay et al. 1982; Clowes et al. 1983; Clowes and Schwartz 1985; Reidy and Silver 1985).

In some human arterial diseases, degenerative and inflammatory medial changes are, however, severe and frequently combined with an extensive intimal thickening and thrombosis. This is true of giant cell arteritis. In this disease, deterioration of the internal elastic membrane, accumulation of giant cells around the fragmented membrane, and inflammation and repair processes throughout all layers of the vessel wall are seen (Albert et al. 1982). The arteries involved in giant cell arteritis are of the muscular and the elastic type.

The present study was undertaken to compare the arterial response to severe injury in a muscular and a smaller elastic artery with the findings in giant cell arteritis (Lorenzen et al. 1987). Furthermore, the alterations in the smaller arteries were compared with our previous studies on injury and repair in the rabbit aorta (Lorenzen 1963; Helin et al. 1971a, b; Garbarsch 1976).

Material and methods

In 26 rabbits of the Danish country strain, 2–3 months of age and weighing about 2.5 kg, dilatation of the left common carotid artery and the left femoral artery was performed. In all

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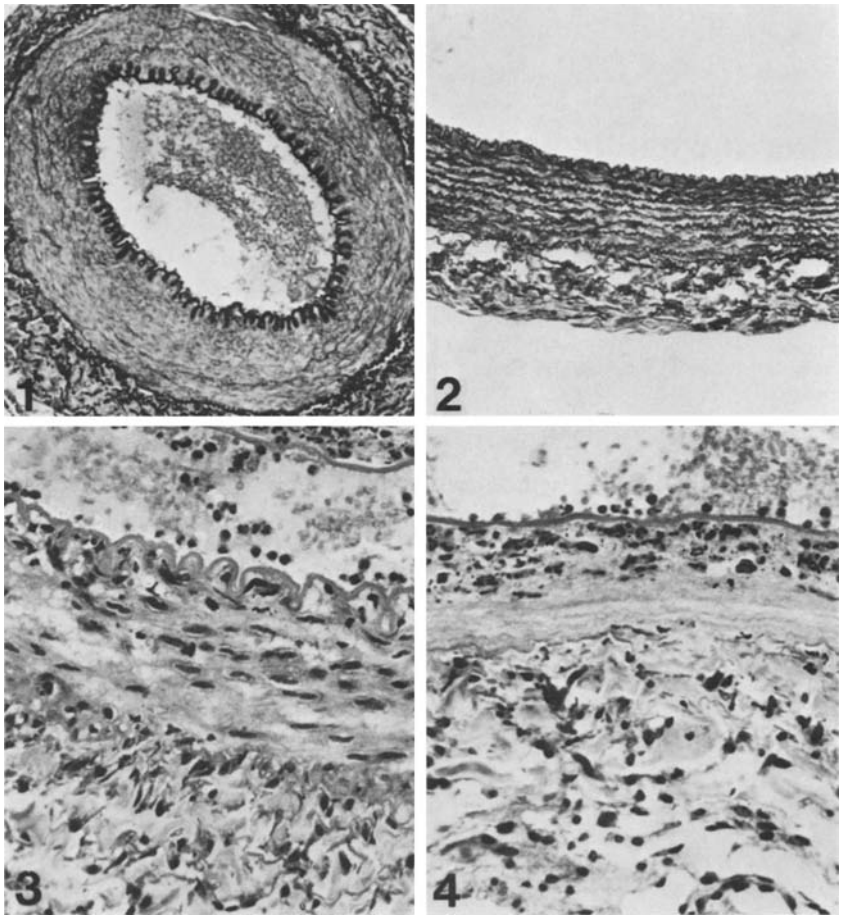

Fig. 1. Control femoral artery. Orcein staining (×350)

Fig. 2. Normal carotid artery. Orcein staining (×350)

Fig. 3. Femoral artery 2 days after dilatation injury. Luminal alignment of monocytes along the internal elastic lamina. H.E. staining (×900)

Fig. 4. Femoral artery 2 days after dilatation injury. The luminal media infiltrated with polymorphonuclear granulocytes. H.E. staining. (×900)

operations the animals were anaesthetized with sodium pentobarbital (50 mg/kg body weight, intravenously). The left femoral artery was exposed at knee level. Through an incision, a Fogarty embolectomy catheter 2F was introduced and advanced to the external iliac artery. The balloon was filled with 0.05 ml water and the catheter redrawn 3 times before removal, after which the artery was ligated. Then the left external carotid artery was exposed through a midline incision in the neck. A Fogarthy embolectomy catheter 3F was introduced, and dilatation of the common carotid artery was performed similarly after inflation of the balloon with 0.075 ml water. The left external carotid artery was ligated, and the wounds were closed. The right femoral artery was ligated at knee level and used as undilated control artery.

After 2, 7, 14 and 28 days the femoral and the common carotid arteries were removed bilaterally in 6, 6, 6 and 7 animals respectively, and the animals killed by intravenous injection of 200 mg sodium pentobarbital. In order to avoid interference of the incision and the ligature, 1 $^1/_2$ cm of the arteries next to the ligature were discarded.

The arteries were fixed in 0.5% cetylpyridinium chloride in 4% formalin (Williams and Jackson 1956), before dehydration and embedding in paraffin. Five μm sections were cut, and the following methods of staining applied:
1. Haematoxylin and eosin, 2. van Gieson staining for collagen fibers, 3. Orcein staining for elastic fibers (Lillie and Fullmer 1976), 4. 0.1% toluidine blue in 30% ethanol for glycosaminoglycans (Kramer and Windrum 1955), 5. Alcian blue 8GX (Gurr), 0.3%, at pH 1 and 2.5 for glycosaminoglycans (Pearse 1968), 6. The Calcium Red methods for calcium (McGee-Russel after Pearse (1972). 7. Mallory's phosphotungstic acid hematoxylin (PTAH) technique for fibrin and myofilaments (Lillie and Fullmer 1976).

Results

Microscopic anatomy

The control arteries

The right femoral arteries appeared undamaged, and were unchanged throughout the observation period. The media had circumferentially oriented smooth muscle cells amongst which delicate dispersed elastic fibers were observed. The intimal endothelial cells rested apparently directly on the internal elastic lamina, which was distinct and continuous (Fig. 1) apart from fenestrae, which connected the subendothelium with the media. A distinct subendothelial layer could be seen using light microscopy. The adventitia consisted of loosely arranged connective tissue with collagen fibers and dispersed elastic fibers. A well-developed external elastic lamina separated the media from the adventitia.

Table. 1 Microscopic changes in the rabbit femoral and carotid arteries 2, 7, 14 and 28 days after a mechanial dilatation injury

	2 Days	1 Week	2 Weeks	4 Weeks
Carotid artery				
Injured elastin	6/6[a]	6/6	6/6	7/7
Intimal thickening	0/6	5/6	6/6	7/7
Medial necrosis	6/6	6/6	6/6	7/7
Thrombosis	0/6	1/6	3/6	5/7
Occlusion	0/6	1/6	2/6	5/7
Giant cells	0/6	0/6	0/6	7/7
Femoral artery				
Injured elastin	6/6	6/6	6/6	7/7
Intimal thickening	0/6	3/6	6/6	7/7
Medial necrosis	6/6	6/6	6/6	7/7
Thrombosis	0/6	1/6	1/6	1/7
Occlusion	0/6	1/6	1/6	1/7
Giant cells	0/6	0/6	0/6	0/6

[a] Number of animals with changes of the arteries out of total number

The right carotid artery exhibited some of the same characteristics as the femoral artery. The principal difference between the two vessels was the medial elastic tissue which, in the carotid artery was abundant, often forming elastic membranes similar to those of the aorta (Fig. 2), although not as distinct and well-defined.

In both arteries acidic glycosaminoglycans could be demonstrated with toluidine blue and Alcian blue staining. These glycosaminoglycans were observed between the smooth muscle cells and elastic fibers, and more prominently in the carotid than the femoral artery.

The dilated arteries

Two days after dilatation the changes were similar in the carotid and femoral arteries. The endothelial layer was absent. Granulocytes and mononuclear cells were located along both sides of the internal elastic membrane (Fig. 3). The membrane was stretched and the arteries were dilated. In the media extensive necrosis was observed with leucocytic accumulation in the luminal zone (Fig. 4). In the adventitia, infiltration with mononuclear cells was prominent.

Seven days after dilatation the changes were also similar in the two arteries, and in one carotid and one femoral artery the lumen was occluded by thrombosis. In 3 out of 6 femoral and 5 out of 6 common carotid arteries (Table 1), an intimal thickening had developed, containing smooth muscle cells, some neutrophils and mononuclear cells, delicate fragments of collagen fibers and elastin. Endothelial cells were absent. In one carotid and one femoral artery, disruption of the internal elastic membrane was noticed. The membrane in general was smooth. Leucocyte infiltration was still prominent, and several mitotic figures were seen in the intima and media.

Fourteen days after dilatation, 3 carotid and 1 femoral artery were partially obliterated by thrombosis (Table 1; Fig. 7). The intimal thickening had increased, occasionally with considerable amounts of fibrin included as observed with PTAH staining, and containing smooth muscle cells, some mononuclear cells, collagen fibers, elastic fibers and glycosaminoglycans. In the media, the smooth muscle cells had become more numerous, but necrotic foci were still present and accumulations of glycosaminoglycans were observed. The leucocyte infiltration was regressing.

At *28 days* the intimal hyperplasia had increased further. In the femoral artery the necrotic medial areas were replaced by smooth muscle cells as evaluated by the few medial necroses, but several gaps existed in the internal elastic lamina through which confluence occurred between the intimal and medial smooth muscle cells (Figs. 5 and 6). One femoral and 5 carotid arteries were obliterated by an organized thrombus with collagen fibers and glycosaminoglycans, and invaded by intimal cells, but only traces of fibrin were observed when compared with the 14 days group.

In the carotid arteries, fragmentation and calcification of the disrupted elastic membranes were frequently seen, and the medial restoration was far less than in the femoral artery with the occurrence of larger necrotic areas. Numerous foreign-body giant cells were located near the changed elastic membranes (Fig. 8).

Discussion

The pattern of injury and repair in the carotid and the femoral arteries showed a number of similarities. This is true of the intimal thickening, the stretching and fragmentation of the internal elastic membrane, the alterations in the media, the accumulation of glycosaminoglycans among the smooth muscle cells, and the appearance of leucocytes in the luminal media and in the adventitia in the initial stages of the repair process.

These microscopic alterations are similar to those observed following different types of mechanical injury of the rabbit aorta (Baumgartner and Studer 1963; Björkerud 1969; Helin et al. 1971a, b; Garbarsch 1973). Similar changes are also found in human arteriosclerosis and temporal

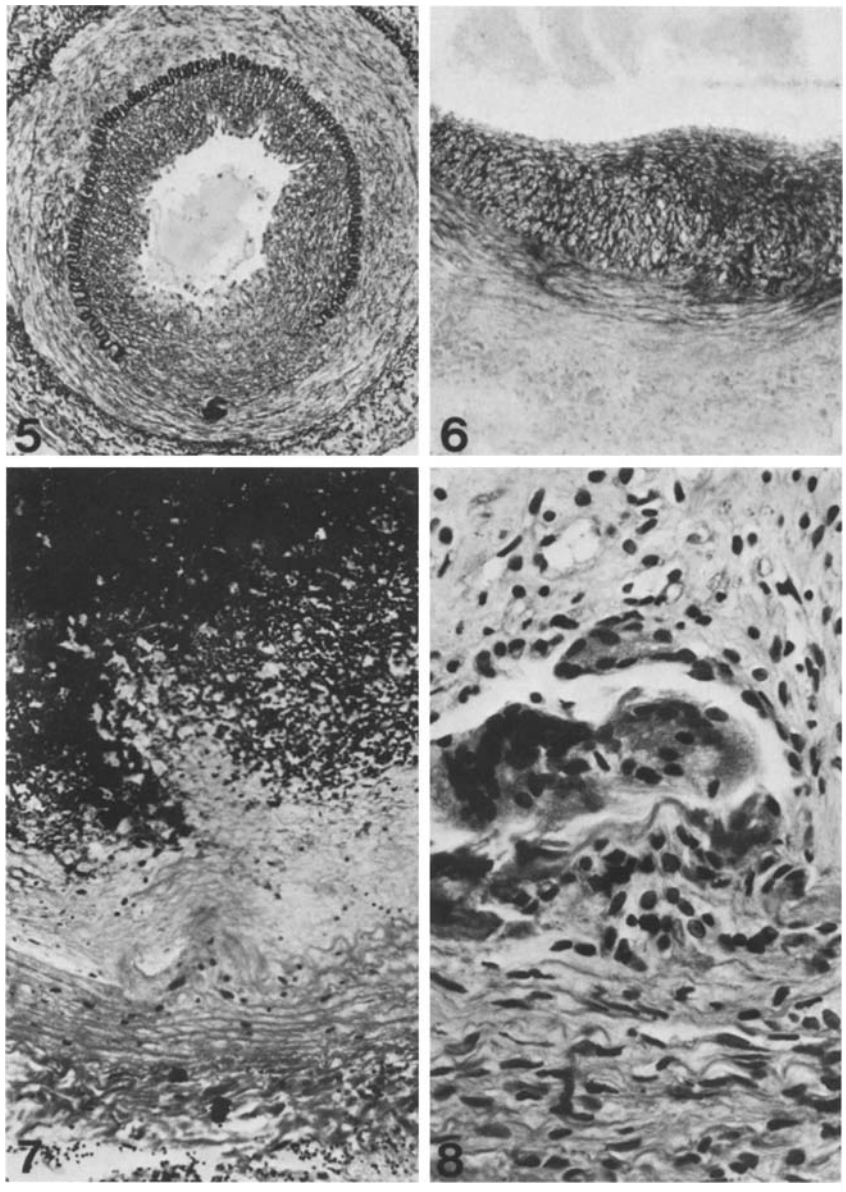

Fig. 5. Femoral artery 28 days after dilatation injury. The internal elastic lamina is in part lacking, and the media largely restored. Orcein staining. (×350)

Fig. 6. Femoral artery 28 days after dilatation injury. Glycosaminoglycans accumulate in the intima and the media. Alcian blue staining. (×350)

Fig. 7. Carotid artery 14 days after dilatation injury. A thrombus is observed in the lumen, and is invaded by intimal cells. PTAH staining. (×560)

Fig. 8. Carotid artery 28 days after dilatation injury. Giant cells gather around the intimal-medial border. H.E. staining (×1400)

arteritis (Parker et al. 1975; Park and Hazleman 1978; Albert et al. 1982; Banks et al. 1983) and reflect the common reaction pattern of the vessel wall to injury (Lorenzen 1963; Garbarsch 1976; Ross and Glomset 1977; Björkerud and Bondjers 1976).

Twenty-eight days after the dilatation injury 3 remarkable features distinguished the carotid artery from the femoral artery: a greater tendency to luminal obliteration by thrombi in different stages of organization, an occurrence of numerous foreign body giant cells around calcified elastic membranes and a less pronounced medial regeneration. Occlusive thrombosis was seen in the carotid as well as in the femoral artery, but more frequently in the carotid artery at the 30 days stage. This is in contrast to the findings in the rabbit aorta (Baumgartner and Studer 1963; Björkerud 1969; Helin et al. 1971a, b) and the rat common carotid artery following milder mechanical dilatation injuries (Fishman et al. 1975; Clowes et al. 1978; Clowes et al. 1983). The differences in the reaction patterns may be due to differences in animal species, the type of the artery and the techniques applied to produce vascular injury. Endothelial denudation induces platelet adherence and the formation of microthrombi (Baumgartner 1972; Fishman et al. 1975; Bylock and Bondjers 1981; Richardson et al. 1984), but the neointima formed by the smooth muscle cell proliferation fol-

lowing injury is not intrinsically thrombogenic (Groves et al. 1979). Other factors must therefore have influenced the formation of thrombosis in the present study e.g. connective tissue elements or necrotic smooth muscle cells (Richardson et al. 1984).

Changes in blood flow (Gertz et al. 1981) and the ligature near the common carotid artery may further have facilitated the thrombus formation and occlusion of the arterial lumen (Buck 1961; Guyton and Karnovsky 1979; Bhawan et al. 1977). However, ligation of the undilated femoral arteries never resulted in thrombosis, whereas thrombosis occurred in a few dilated femoral arteries. Consequently, it seems unlikely that the ligature per se was the causative agent. Furthermore, thrombosis has not been reported following milder mechanical dilatation of the rat common carotid artery, in which introduction of the catheter also was carried out through the external carotid artery (Fishman et al. 1975; Clowes et al. 1978; Clowes et al. 1983). Differences in the structures of the femoral and the carotid artery may explain the differences in reaction patterns in the vessel wall of the two arteries. The abundance of elastic tissue, with fragmentation and calcification of the elastic membranes, is probably responsible for the formation of giant cells reflecting a "foreign body" reaction in the carotid artery (Friedman et al. 1975). Giant cells are also characteristic of the aortic reaction to injury (Helin et al. 1971a, b; Björkerud and Bondjers 1976). Moreover, injury of the aortic wall also shows the same general inflammatory and repair processes as found in the carotid artery, including fibrosis and accummulation of glycosaminoglycans (Helin et al. 1971b; Garbarsch 1973). These alterations contrast in part with those observed in the femoral artery, in which the profound initial damages were followed by a more complete medial regeneration as larger necrotic areas never occurred in the media 28 days after injury. This has previously been reported after milder injuries to the rabbit femoral artery than those inflicted by us (Potvliege and Bourgain 1979).

The present study also presented features in common with the morphology of human *temporal arteritis*. Histopathologically, this disease is characterized by a granulomatous panarteritis in which the inflammatory cells mainly consist of macrophages, lymphocytes, and giant cells primarily located at the intimal-medial junction around fragments of the altered internal elastic membrane (Parker et al. 1975; Lorenzen et al. 1987). Another characteristic is the marked intimal thickening, which may be overlaid by an orgainizing thrombus often occluding the lumen. The intimal thickening is more pronounced than normally observed in the senescent non-arteritic vessels. Patchy degeneration and drop-out of smooth muscle cells are also prominent in human temporal arteritis (Albert et al. 1982).

The resemblance between human temporal arteritis and the injured common carotid artery in the rabbit makes the model suitable for certain types of studies with relevance to human giant cell arteritis, for instance the effect of glucocorticoids on the arterial healing process.

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