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Smooth Muscle Cell Proliferation in the Arterial Intima after Stretch Injury: Relationship between the Severity of Stretching and Intima Hyperplasia in New Zealand White Rabbits

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Abstract: We examined histologically whether the severity of arterial stretch injury is related to the degree of subsequent intimal hyperplasia. In six male New Zealand White rabbits, the common carotid artery was hyperextended with a 3F Fogarty balloon catheter. Two weeks later, no proliferative change was evident in the intima in the most hyperextended portion. Paradoxically, however, intimal hyperplasia due to smooth muscle cell proliferation was observed in the moderately extended portions. The intimal hyperplasia appeared to be exacerbated where the arterial stretching was more severe. It is concluded that the severity of arterial stretch injury is closely related to the intimal proliferation of smooth muscle cells.

Key words: intimal hyperplasia, smooth muscle cell proliferation, stretch injury

Angioplasty with a balloon for treatment of arterial sclerotic lesions is widely used in patient with ischemic heart disease, arteriosclerosis obliterans and other vascular diseases, but restenosis following angioplasty is observed in 30–40% of cases, posing a great clinical problem [1, 7]. This restenosis is partly due to vascular wall injury caused by inflation of the balloon itself, which may lead to intimal hypertrophy due to migration of smooth muscle cells and their subsequent proliferation [12]. Extensive studies aimed at preventing the neointimal hypertrophy following vascular injury

have therefore been performed [8, 10, 15, 17].

It has been shown that intimal hyperplasia due to smooth muscle cell proliferation is generally caused by stimulation in the form of intimal denudation or mechanical extension [2–5, 13], but the relationship between the severity of vascular extension and the degree of intimal proliferation following angioplasty has not been fully elucidated. The present study was therefore performed to histologically examine whether the severity of arterial extension is related to the degree of subsequent intimal hyperplasia.

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Studies were carried out on six male New Zealand White rabbits (body weight; $2,653 \pm 56$ g, between 80 and 90 days old), which were purchased from a commercial vendor (Kuroda Pure Line Animals Co., Japan). All the animals were housed in individual cages in an animal room maintained at a temperature of $22 \pm 2^\circ\text{C}$, humidity of $55 \pm 10\%$ and 10/hr fresh air changes with 12 : 12 hr light/dark cycles. Under anesthesia with ketamine (50 mg/kg, i.m.), atropine (0.05 mg/kg, s.c.) and medetomidine (0.05 mg/kg, s.c.), the right common carotid artery (external diameter; 2.0 ± 0.2 mm) was exposed and incised transversely. Then a 3F

Fogarty balloon catheter was inserted 2.5 cm from the incision in the central direction, and inflated to increase the arterial diameter to 4.5 mm for 3 sec, as shown in Fig. 1. After deflation, the balloon was pulled out and vascular suturing was performed. It has been reported that the total number of smooth muscle cells in the intima reaches its maximum in two weeks after intimal denudation [5]. The animals were therefore sacrificed under anesthesia two weeks later, and the right common carotid arteries were collected. To prepare specimens of arterial regions subjected to various wall tensions as a result of balloon inflation, samples were

Fig. 1. It is seen here that the right common carotid artery is extended to a maximum 4.5 mm external diameter with a 3F Fogarty balloon catheter inserted in the central direction. S_1 is the portion extended to a 4.5 mm arterial external diameter, S_2 and S_3 are the proximal and the distal segments from S_1 , respectively. The 2 mm shown is the mean external diameter of the carotid artery before extension.

Fig. 2. A light micrograph of a transversely sectioned S_1 . Thrombus and residual intima containing smooth muscle are observed in the lumen. M: Media, A: Adventitia, HE stain. $\times 132$.

isolated from the maximally extended portion (Segment 1; S_1), the proximal region from S_1 (Segment 2; S_2) and the distal region from S_1 (Segment 3; S_3), as shown in Fig. 1. These samples were fixed in 10% buffered neutral formalin solution, and 3 sections ($6\text{ }\mu\text{m}$) were prepared from each sample and stained with hematoxylin-eosin for histological examination. The cross-sectional areas of the neointima and media were measured with a computerized image analyzer

(Cosmozone Is, Nikon). The mean values for the measured areas in these 3 sections (S_1 , S_2 and S_3) were used to compare the differences among the samples. We used the left common carotid artery from the same animal as a normal control, but did not represent it as the intact sample.

In the S_1 specimen (Fig. 2), the lumen was markedly enlarged with a thin media, compared with those of S_2 (Fig. 3) and S_3 (Fig. 4), and intimal denudation was

Fig. 3. A light micrograph of a transversely sectioned S_2 , showing the intima (I), the elastica interna (arrowhead), the media (M) and the adventitia (A). Prominent intimal hypertrophy is recognized. HE stain. $\times 132$.

Fig. 4. A light micrograph of a transversely sectioned S_3 . The degree of intimal hypertrophy is less than that in S_2 (see Fig. 3). I: Intima M: Media, A: Adventitia, arrowhead: Elastica interna, HE stain. $\times 132$.

marked. In the S_2 and S_3 specimens, the neointima was thickened due to smooth muscle cell proliferation. The lumen in the S_1 specimen included partially organized thrombus, and smooth muscle cells were mixed in the residual intima. Because of intimal denudation and thrombus formation, it was difficult to measure the cross-sectional area in S_1 . Paired t -test was therefore performed to determine the differences between S_2 and S_3 in area. All values were expressed as the mean $\pm$ standard deviation, and statistical significance was defined as $p < 0.05$.

The cross-sectional area of the neointima was significantly greater in S_2 than in S_3 (0.11 ± 0.05 vs 0.03 ± 0.01 mm², $p = 0.0146$, $n = 6$, Table 1). In contrast, the cross-sectional area of the media showed no significant difference between S_2 and S_3 (0.51 ± 0.06 vs 0.45 ± 0.05 mm², $p = 0.1062$, $n = 6$).

Intimal smooth muscle cell proliferation is the most remarkable change observed following injury to the arterial endothelium [6, 14]. In the present study, however, no proliferative change was observed in the intima in S_1 . On the other hand, intimal hypertrophy due to smooth muscle cell proliferation was recognized in the S_2 and S_3 portions, and the degree of hyperplasia in S_2 was more severe than that in S_3 . Since the order of vascular external diameter was $S_1 > S_2 > S_3$ at the time of balloon inflation, the severity of wall tension was applied to $S_1 > S_2 > S_3$. These results indicate that the vascular stretch injury in S_1 , which was the most hyperextended portion, was too great to cause intimal regeneration and hypertrophy. In addition, it was inferred that if the severity of injury was below a certain degree, the stronger the stretch injury the more marked the neointimal proliferation that was caused. Reidy *et al.* [11] reported that in an extensive denudation model of the vascular intima in rabbits, intimal regeneration was incomplete, remaining static for 2 weeks after injury. Our finding that no intimal proliferation occurred in the vascular portion subjected to severe stretch injury accords with their report [11]. Also, the finding of no significant difference between S_2 and S_3 in the medial cross-sectioned area was compatible with the report by Struss *et al.* [16] that the medial cross-sectional area following arterial extension injury was constant for 12 weeks afterwards.

Physical stimulation in the form of smooth muscle hyperextension induces smooth muscle proliferation [5,

Table 1. Comparison of the differences between S_2 and S_3 neointimal areas

Rabbit No.	Neointimal area (mm ²)	
	S_2	S_3
1	0.154	0.036
2	0.070	0.024
3	0.190	0.028
4	0.054	0.024
5	0.125	0.029
6	0.068	0.037
Mean $\pm$ SD	0.11 ± 0.05	0.03 ± 0.01
$p = 0.0146$		

S_2 : The proximal segment from the most extended portion. S_3 : The distal segment from the most extended portion.

13]. Furthermore, it has been pointed out that many potent factors related to the proliferation and growth of smooth muscle cells are released from platelets, injured endothelium, smooth muscle cells and macrophages after injury by balloon inflation for angioplasty [9, 12]. It is possible that the more severe the arterial stretch injury is, the more the release of these intimal proliferative factors is promoted. Intimal hyperplasia of the carotid artery in the rabbit caused by smooth muscle cell proliferation following arterial stretch injury therefore progresses to a greater extent where vascular extension is stronger. However, stretch injury, if too extensive, does not lead to intimal hyperplasia. We conclude that the degree of stretch injury is closely related to intimal proliferation in the artery.

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