Relationship between Glycosaminoglycans and Pregnancy-Induced Sclerosis in Bovine Uterine Arteries

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(Received 28 April 1988/Accepted 2 July 1988)

ABSTRACT. The relationship between glycosaminoglycans (GAG) and the pregnancy-induced sclerosis of the bovine uterine artery was histochemically studied. Both sulfated and non-sulfated GAG were observed in the arteries. As advancing the intimal thickening and elastosis, GAG seemed to increase in amount in the thickened intima and the inner half of the media. Digestion with *Streptomyces* hyaluronidase slightly altered the alcian blue stainability of the uterine arteries, whereas digestion with chondroitinase ABC apparently reduced the alcianophilia of the arteries. It is, therefore, assumed that the sulfated GAG such as chondroitin sulfates might correlate with advancing the pregnancy-induced sclerosis.—**KEY WORDS:** cow, GAG, pregnancy-induced sclerosis, uterine artery.

Jpn. J. Vet. Sci. 50(5): 1055–1059, 1988

During past many years, attention has been paid to the estrus- or pregnancy-induced sclerosis occurring in the blood vessels of female reproductive organs [1, 2, 8]. The main findings of the sclerosis were the intimal thickening and the increase and degeneration of elastic elements (elastosis). It has been described that the uterine arterial wall becomes more stratified with each gestation, especially in the cow and sow [5, 15].

Glycosaminoglycans (GAG) together with elastin and collagen are believed to be essential in maintaining the structure and functional integrity of the normal arterial wall [7, 10, 16]. The GAG have also been postulated to play important roles in the pathogenesis of atherosclerosis [11, 14]. There have been much described histochemically and biochemically about GAG in bovine vessel walls, especially aorta [4, 6, 16]. There have been, however, only a few of brief descriptions of GAG accompanying with the pregnancy-induced sclerosis of the uterine arteries. Thus the histochemical investigation was conducted on the bovine

uterine arteries. In this study, the relationship between the sclerosis and the GAG of the bovine uterine arteries is described.

MATERIALS AND METHODS

The materials consisted of 59 nonpregnant Holstein cows. They were in good health and the reproductive organs were visibly and palpably normal. Based on the number of parturition, they were divided into four groups (Table 1).

The main trunk of uterine artery was designated as a portion 2–3 cm prior to its

Table 1. Materials and number of parturition

Group	Number of parturition ^{a)}	Number of cows
I	0	13
II	1– 2	6
III	3– 6	25
IV	7–12	15

a) Number of parturition of cows with unknown history was estimated from the number of the corpus albicans [5].

first bifurcation. Tissue samples were collected at a slaughter, fixed in 2% calcium acetate-10% formalin or in 10% buffered neutral formalin, and routinely processed into paraffin blocks. Paraffin sections (3–6 μm) were stained with hematoxylin-eosin and Weigert's resorcin-fuchsin for histological observation.

For the demonstration of arterial GAG [12], the following histochemical methods were employed: alcian blue (pH 2.5); alcian blue (pH 1.0); alcian blue (pH 2.5) after digestion with *Streptomyces* hyaluronidase (Seikagaku Kogyo Co., Japan) and alcian blue (pH 1.0, 2.5) after digestion with chondroitinase ABC (Seikagaku Kogyo Co., Japan) [9, 13, 17].

RESULTS

Varying amounts of GAG could be histochemically demonstrated in the bovine uterine arteries. GAG were mainly found in the tunica intima and the inner half of the tunica media.

In group I, uterine arteries showed the structure of normal muscular type (Fig. 1a). Only a few amounts of GAG were observed adjacent to the internal elastic lamina (Fig.

1b, c). In group II, uterine arteries exhibited the slight intimal thickening and splitting internal elastic lamina (Fig. 2a). GAG were present in the inner half of the tunica media and between the split layers of internal elastic lamina (Fig. 2b, c). The amount of GAG seemed to be slightly more than that in group I. In group III, the tunica intima of uterine arteries thickened and constituted one-third the thickness of the arterial wall (Fig. 3a). The internal elastic lamina appeared to be one or more layers. GAG seemed to increase moderately in the thickened intima and the inner layer of tunica media (Fig. 3b, c). In group IV, the thickened intima approximated to half or two-thirds of the arterial wall in thickness. Several layers of elastic lamina appeared to be alike concentric rings in the thickened intima (Fig. 4a). Large amounts of GAG were found in interelastic spaces of the thickened intima (Fig. 4b, c).

The digestion with *Streptomyces* hyaluronidase slightly diminished the alcianophilia (pH 2.5) of the bovine uterine arteries (Fig. 5a). In contrast with this, the digestion with chondroitinase ABC apparently reduced or abolished the alcianophilia of the arteries (Fig. 5b).

Fig. 1. Three serial sections of bovine uterine artery in group I. $\times 20$. a: Weigert's, b: alcian blue (pH 2.5) and c: alcian blue (pH 1.0) stain.

Fig. 2. Three serial sections of bovine uterine artery in group II. $\times 15$. a: Weigert's, b: alcian blue (pH 2.5) and c: alcian blue (pH 1.0) stain.

Fig. 3. Three serial sections of bovine uterine artery in group III. $\times 15$. a: Weigert's, b: alcian blue (pH 2.5) and c: alcian blue (pH 1.0) stain.

DISCUSSION

It is generally accepted that the alcian blue (pH 1.0) stain is the histochemical method for identifying sulfated GAG such as chondroitin sulfates, and the alcian blue (pH 2.5) is for sulfated and non-sulfated GAG such as hyaluronic acid [9; 12, 13, 17]. The present study demonstrated that both sulfated and non-sulfated GAG were present in the bovine uterine arteries.

Previous studies have indicated that the

arterial GAG increase in the early stages of atherosclerosis and then gradually decrease with advancing the stages of atherosclerosis [3, 10]. In the present study, however, the arterial GAG seemed to increase with advancing the pregnancy-induced sclerosis. These results might be representative of the difference between the physiological sclerosis and the pathological one.

It is also accepted that *Streptomyces* hyaluronidase degrades hyaluronic acid, and chondroitinase ABC degrades hyaluronic acid, chondroitin and chondroitin sul-

Fig. 4. Three serial sections of bovine uterine artery in group IV. $\times 15$. a: Weigert's, b: alcian blue (pH 2.5) and c: alcian blue (pH 1.0) stain.

sclerosis.

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Fig. 5. a: Adjacent section to that of Fig. 3 stained with alcian blue (pH 2.5) following digestion with *Streptomyces hyaluronidase*. The alcianophilia is hardly altered by the enzyme digestion. b: Adjacent section to that of Fig. 4 stained with alcian blue (pH 1.0) following digestion with chondroitinase ABC. The alcianophilia is apparently weaker than that seen in Fig. 4c. $\times 15$.

fates [12, 17]. The present results of the enzyme digestion studies suggest that sulfated GAG such as chondroitin sulfates were increased as advancing the pregnancy-induced sclerosis. It is, therefore, suspected the ability of sulfated GAG to modulate the development of the pregnancy-induced

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要 約

ウシ子宮動脈におけるグリコサミノグリカン (GAG) と妊娠性硬変との関係：神谷新司・醍醐正之 (日本獣医畜産大学獣医解剖学教室)——ウシの子宮動脈壁における GAG について組織化学的に検索した。動脈壁 GAG は内膜および中膜にみられ、妊娠性硬変の進展とともに内膜肥厚部および中膜内層で増加する傾向が観察された。酵素消化試験により、増加する GAG が sulfated GAG であることが明らかとなったことから、ウシの子宮動脈にみられる妊娠性硬変の進展には GAG、とくに sulfated GAG が密接に関与することが推察された。