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The gangliosides of adult human aorta: intima, media and plaque

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The composition, structure and localization of gangliosides of aorta taken from subjects who had died after myocardial infarction were studied. Individual gangliosides were purified by high-performance liquid chromatography and high-performance thin-layer chromatography and were characterized on the basis of their chromatographic mobility, carbohydrate composition, neuraminidase hydrolysis and methylation analysis. The main aortic gangliosides were identified as G_{M3}, G_{M1}, G_{D3}, G_{D1a} and G_{T1b}. Significant differences in the ganglioside composition of intima and media were detected and the ganglioside profile of atherosclerotic plaques was found to differ markedly from that of unaffected intima. The latter was characterized by high content of G_{D3}, a ganglioside thought to be associated with membrane permeability, cell interaction, adhesiveness and growth and to supress unspecific immune responses. Possible implications of the results in low-density lipoprotein binding to the arterial wall and in immunological changes induced by atherosclerotic lesions are discussed.

Recently we found that several antigens reacting with monoclonal antibodies raised against aortic cell subpopulations are glycosphingolipids [2]. Earlier it has been shown that there is a threefold increase of gangliosides in the aorta during atherosclerosis [3]. Since gangliosides are important factors determining cell adhesiveness [4] and low-density lipoprotein binding [5] (and the literature cited therein), which both have been suggested to be involved in the development of atherosclerotic lesions (see for example [6]), the ganglioside composition of and localization in human aorta is of considerable interest. Nonetheless, information on this subject is sparse [3, 7, 8]. With exeption of G_{M3} aortic gangliosides were identified only on the basis of their chromatographic mobility and no attempts to differentiate between intimal and medial glycolipids were undertaken. Here we present the results of a detailed study of the gangliosides of adult human aorta, its uninvolved intima, atherosclerotic plaques and underlying media.

MATERIALS AND METHODS

Tissue

The human thoratic aortas from 40–60-year-old men and women who had died of myocardial infarction were obtained at autopsy within 1.5–3 h after sudden death. Atherosclerotic lesions (mainly atherosclerotic plaques) occupied, as a rule, about 50% of the total inner surface of the aorta. Adventitia together with one third of the outer media were stripped away and the remaining parts of aortic material were washed and frozen until analysis. The grossly normal areas and the uncomplicated atherosclerotic plaques were cut out and inner intima consisting of an elastic-hyperplastic and connective tissue sublayers was separated mechanically along the secondary internal intimal limiting membrane from media and attached intimal musculoelastic sublayer. The accuracy of separation of the inner intima and media was controlled macroscopically and microscopically as described earlier [9, 10].

Extraction and purification of gangliosides

Aortic tissues (300 g wet tissues) were homogenized and extracted with chloroform/methanol (2:1 and 1:2, v/v; 3 l and 1.5 l respectively) according to [11]. The total lipids were treated for 1 h at 40 °C with 0.1 M KOH in methanol. The mixture was neutralized with 0.35 M acetic acid in methanol and evaporated. The dry residue was dissolved in 20 ml water and subjected to dialysis through a cellophane membrane against distilled water for 24 h at 4 °C. The content of the dialysis bag was evaporated, the dry residue was dissolved in 500 ml of chloroform/methanol/water (30:60:8, v/v) and applied onto a column (3 × 25 cm) packed with 10 g DEAE-Sephadex A-25 (Pharmacia, Sweden, 40–100 mesh) as described in [12]. The gangliosides were separated into monosialo-, disialo- and trisialo-ganglioside fractions.

In an analogous fashion gangliosides were extracted from separated grossly normal intima, atherosclerotic plaque as well as underlying media (4–5 g, 25–30 g, 12–14 g, 18–20 g of wet tissue respectively). The total lipids were treated with KOH, neutralized and subjected to dialysis as indicated above. Then the lipids were dissolved in 20–50 ml chloroform/methanol/water (30:60:8, v/v) and applied onto a column (1.5 × 20 cm) packed with 2 g DEAE-Sephadex A-25 in (acetate form). The neutral lipids were eluted with the same mixture, gangliosides were eluted with chloroform/methanol/0.8 M ammonium acetate (60:30:8, v/v) and were analyzed by HPTLC.

The ganglioside fractions were further fractionated by HPLC on a column (4.6 × 250 mm) packed with silica-gel

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Abbreviations. The designation of gangliosides follows the IUPAC-IUB recommendations [1]: G_{M3}, II^3NeuAcLacCer; G_{M1}, II^3NeuAcGgOse$_4$Cer; G_{D3}, II^3(NeuAc)$_2$LacCer; G_{D1a}, II^3,IV^3-(NeuAc)$_2$GgOse$_4$Cer; G_{T1b} II^3(NeuAc)$_2$$IV^3$NeuAcGgOse$_4$Cer.

Zorbax-SIL using a linear gradient of 2-propanol/hexane/water (from 55:45:5 to 55:25:20, v/v) [13]. The fractions (0.5 ml) were collected and monitored by HPTLC on silica gel 60 plates (Merck, FRG) using chloroform/methanol/0.2% $CaCl_2$ (55:45:10, v/v/v) [14].

Analytical procedures

Gangliosides were quantitatively determined as described in [15].

Enzymatic degradation of gangliosides with *Vibrio cholerae* neuraminidase (Koch-Light, England) was carried out according to [16]. The hydrolysis products were analyzed by TLC on silica gel 60 plates using chloroform/methanol/0.2% $CaCl_2$ (55:45:10, v/v/v) for the gangliosides and chloroform/methanol/2.5 M NH_4OH (60:35:8) for the glycolipids. Sialic acids obtained by enzymatic hydrolysis were analyzed by TLC according to [17] using authentic *N*-acetyl- and *N*-glycolyl neuraminic acids (Sigma, USA) as reference substances.

Quantitative and qualitative determination of carbohydrates was carried out by GLC of the trimethylsilyl ethers of methylglycosides using a 1.5% OV-1 on Chromosorb G column (80–100 mesh), operated at 150–300 °C (6 °C/min) with mannitol as internal standard [18].

Gangliosides (up to 1 mg) were methylated according to [19, 20] and the methylation products were purified by chromatography on a column (10 × 1 cm) packed with silica gel L (40–100 μm). The permethylated gangliosides were eluted with chloroform/methanol (98:2, v/v). Mass spectra of these derivatives were recorded with a M-80A instrument (Hitachi, Japan) at evaporation temperature 300 °C and acceleration 70 eV.

Fatty acid methyl esters were prepared by acid methanolysis of the gangliosides followed by extraction with hexane, and were analyzed using a Pye-104 gas chromatograph equipped with a column (1800 × 4 mm) with 1.5% OV-1 on Chromosorb G (60–100 mesh) operated at 100–300 °C (8 °C/min).

Quantitative determination of ganglioside components was carried out as in [21]. The ganglioside mixtures (10–20 nmol sialic acid) were separated by HPTLC as described above. The chromatograms were treated with the resorcinol reagent and heated on a sand bath for 30 min at 110 °C. The content of gangliosides in the spots was obtained through colour intensity using a scanning densitometer (Opton, FRG) at 580 nm.

RESULTS

Gangliosides of whole aorta

The thin-layer chromatographic pattern of the gangliosides isolated from human aorta is shown in Fig. 1 and their relative abundance is presented in Table 1. The data of Fig. 1 reveal five major ganglioside fractions designated as GAI to GAV. On thin-layer chromatography most of these fractions migrated as partly resolved double bands due to the heterogenity of their fatty acid composition (Table 2) [22]. Except fraction GAIII the two bands of each fraction were analyzed together.

Fraction GAI. This ganglioside comigrated with human liver G_{M3} and was similar to the latter in carbohydrate composition (Table 3). On treatment with *V. cholerae* neuraminidase fraction GAI yielded *N*-acetylneuraminic acid and a desialylated compound which coincided chromatographically with lactosylceramide. Direct probe mass spectra of the permethylated derivative gave fragment ions corresponding to terminal *N*-acetylneuraminic acid [*m/z* 376, 73% and 344 (376–32), 100%], an ion with *m/z* 548, 88% corresponding to NeuAc–O–Hex and ions *m/z* 800, 19% and 768 (800–32), 26% corresponding to NeuAc–O–Hex–O–Hex–O and ions of other fragments suggesting the presence of C_{18} sphingosine and C_{18}–C_{24} fatty acids: *m/z* 1096, 1%; 1180 (*M*-253), 0.25%; *m/z* 1318, 0.15%; and 1397

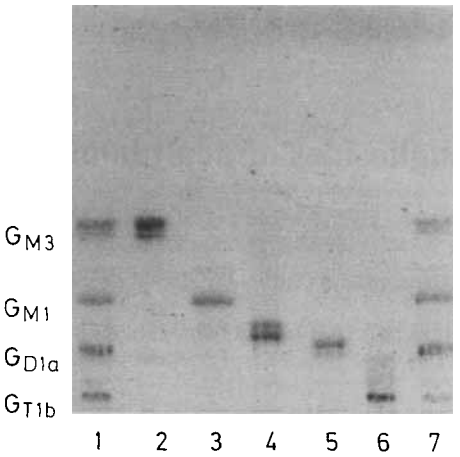

Fig. 1. *The main ganglioside fractions of whole human aorta.* Lanes 1 and 7, reference gangliosides; lane 2, fraction GAI (G_{M3}); lane 3, fraction GAII (G_{M1}); lane 4, fraction GAIII (G_{D3}); lane 5, fraction GAIV (G_{D1a}); lane 6, fraction GAV (G_{T1b})

Table 1. *Ganglioside composition of whole human aorta, intima, media and atherosclerotic plaque*
Values are expressed as a percentage of total calculated from the values of densitometric response, ± SEM. Two pooled samples were used, 12 aortas in each sample

Ganglioside	Whole aorta	Intima		Media	
		unaffected	plaque	unaffected	plaque
	%				
G_{M3}	81.7 ± 0.4	66.4 ± 3.0	93.4 ± 2.5	83.6 ± 0.5	69.6 ± 1.8
G_{M1}	4.6 ± 1.0	1.8 ± 1.0	1.7 ± 0.6	4.9 ± 1.2	3.8 ± 1.1
G_{D3}	5.0 ± 0.4	22.4 ± 1.5	2.6 ± 1.5	3.2 ± 1.1	11.2 ± 2.8
G_{D1a}	6.7 ± 0.5	1.9 ± 0.1	1.1 ± 0.5	6.9 ± 1.1	11.6 ± 2.2
G_{T1b}	2.1 ± 0.5	7.3 ± 2.4	1.4 ± 0.2	1.4 ± 0.4	3.8 ± 1.4

Table 2. *Fatty acid composition of aorta gangliosides*
The fatty acids were determined by GLC of the methyl esters

Fatty acid	Ganglioside				
	G_{M3}	G_{M1}	G_{D3}	G_{D1a}	G_{T1b}
	% total				
$C_{16:0}$	15	49	26	36	26
$C_{18:0}$	16	16	45	28	39
$C_{18:1}$	28	2	8	14	9
$C_{22:0}$	13	21	8	10	3
$C_{24:0}$	28	5	7	12	23
$C_{24:1}$	–	9	6	–	–

Table 3. *Molar ratios of carbohydrates in the isolated gangliosides*
The carbohydrate components were determined by GLC, molar ratios were calculated with glucose as 1

Component sugars	Ganglioside fractions				
	GAI (G_{M3})	GAII (G_{M1})	GAIII (G_{D3})	GAIV (G_{D1a})	GAV (G_{T1b})
Glucose	1.00	1.00	1.00	1.00	1.00
Galactose	1.15	1.85	1.10	1.96	2.06
N-acetylgalactosamine	–	0.85	–	0.92	1.20
N-acetylneuraminic acid	0.99	0.89	1.80	2.04	3.04

(*M*-32), 0.125%. On the basis of these data fraction GAI was identified as hematoside (G_{M3}). Its fatty acid composition is shown in Table 2.

Fraction GAII. This comigrated with G_{M1} isolated from human brain (Fig. 1). It contained glucose, galactose, galactosamine and sialic acid in approximate molar 1:2:1:1 ratio (Table 3) and was neuraminidase-resistant. Although a more detailed analysis could not be performed due to the small amount of this fraction the above data tentatively identified it as II^3NeuAcGgose$_4$Cer (G_{M1}).

Fraction GAIII. This had an R_f value intermediate between those of brain gangliosides G_{M1} and G_{D1a} (Fig. 1). It contained glucose, galactose and sialic acid in a molar 1:1:2 ratio (Table 3). Neuraminidase treatment resulted in formation of *N*-acetylneuraminic acid and a neutral glycolipid which coincided chromatographically with lactosylceramide. Direct probe mass spectra of permethylated GAIII indicated the presence of ions: *m/z* 376, 72% and 344 (376–32), 100% corresponding to terminal *N*-acetylneuraminic acid; *m/z* 704 (768–64), 6.5% corresponding to NeuAc–O–NeuAc; *m/z* 941, 4.5%, *m/z* 909 (941–32), 5.5% corresponding to the terminal trisaccharide NeuAc–O–NeuAc–O–Hex. The two chromatographic bands of fraction GaIII were analyzed separately and yielded identical cleavage products. Evidently this fraction has the structure $(NeuAc)_2$LacCer corresponding to ganglioside G_{D3}.

Fraction GAIV. This chromatographically coincided with brain G_{D1a} (Fig. 1). It contained glucose, galactose, galactosamine and sialic acid in approximate molar ratio of 1:2:1:2 (Table 3). On treatment with *V. cholerae* neuraminidase GAIV gave a sialic acid containing glycolipid whose migration rate corresponded to G_{M1}. On the basis of these results GAIV was identified as II^3,$IV^3(NeuAc)_2$GgOse$_4$-Cer (G_{D1a}).

Fraction GAV. This comigrated with human brain G_{T1b} (Fig. 1) and contained glucose, galactose, galactosamine and sialic acid in approximate molar ratio of 1:2:1:3 (Table 3). Treatment with *V. cholerae* neuraminidase resulted in formation of *N*-acetylneuraminic acid and a ganglioside which chromatographically corresponded to G_{M1}. We conclude that fraction GAV represents $II^3(NeuAc)_2$,IV^3NeuAcGgOse$_4$Cer (G_{T1b}).

Gangliosides of intima and media

The ganglioside composition of grossly normal intima, atherosclerotic plaque and underlying media is shown in Table 1 and Fig. 2. As can be seen from these data, the ganglioside profiles of the intima and the underlying media differ significantly. In uninvolved zones the most important differences between intima and media are related to the content of G_{D3} and G_{M3}. In the media the G_{M3} level is higher than in the intima, whereas the latter is characterized by a high content of G_{D3} which is only a minor component in the media. Intimal atherosclerotic plaques are characterized by significant changes in the ganglioside composition: as compared to unaffected intima G_{D3} decreases sharply and the content of G_{M3} increases from 67% to 93%. At the same time in the media under plaque the ganglioside profile remains similar to that of the media from grossly normal areas.

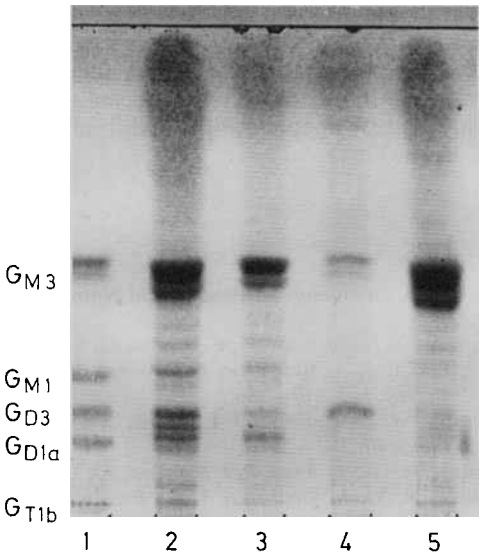

Fig. 2. *Thin-layer chromatography of gangliosides of human aorta.* Lane 1, standards; lane 2, media under plaque; lane 3, unaffected media; lane 4, unaffected intima; lane 5, atherosclerotic plaque. Conditions are given in the text

DISCUSSION

The results of the present study revealed significant differences in the ganglioside composition of intima and media. The intima was characterized by relatively high content of G_{D3}, a ganglioside which is considered as an antigen characteristic of many fast-growing undifferentiated fetal or malignant cells (see [23] for a review and also [24–28]). Usually G_{D3} is very low or absent in human normal extraneuronal tissues and serum. The reason for the elevation of smooth muscle cell proliferation during development of atherosclerotic lesions are not known but there might be an analogy to the loss of contact inhibition by tumor cells which has shown to be associated with significant changes in the surface gangliosides (reviewed in [4]). Possibly gangliosides are implicated also in the uncontrolled proliferation of aortic smooth cells.

Some other possible biological consequences of the observed differences in the ganglioside composition of atherosclerotic intima, media and plaque may also be considered.

Recently it has been established that G_{D3} and G_{M3} but not gangliosides of other types are active suppressors of non-specific immune responses [29, 30]. Taking into account the high level of G_{D3} in the uninvolved intima and of G_{M3} in atherosclerotic plaque, exposition of these gangliosides due to injury of the endothelium could lead to changes in the activity of immunocompetent cells in the neighborhood of atherosclerotic lesions.

Finally it should be noted that plasma membrane gangliosides have been found to enhance cell binding and uptake of low-density lipoproteins [5]. Therefore the altered ganglioside patterns of plaque as compared to unaffected tissue may play a role in low-density-lipoprotein-derived lipid accumulation in atherosclerotic aorta.

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