Composition of alkoxylipids of human heart and aorta

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ABSTRACT The major alkoxylipids of human heart and aorta are alkyl and alk-1-enyl diacyl glycerols, alkyl acyl and alk-1-enyl acyl glycerophosphoryl cholines, and the corresponding glycerophosphoryl ethanolamines. There are no pronounced differences in the composition of corresponding classes of alkoxylipids from heart, aorta, and other human tissues previously reported.

SUPPLEMENTARY KEY WORDS alkyl and alk-1-enyl diacyl glycerols · alkyl acyl and alk-1-enyl acyl glycerophosphoryl cholines · alkyl acyl and alk-1-enyl acyl glycerophosphoryl ethanolamines · cardiovascular tissues

NEUTRAL AND IONIC lipids derived from alkyl and alk-1-enyl glycerol ethers have been found in numerous human tissues (1). Thus, alkyl acyl and alk-1-enyl acyl glycerophosphatides have been demonstrated in the human heart (2, 3). More than 25 years ago alkyl glycerol ethers were isolated from lipid hydrolysates of aortae (4) and it was later shown that they occur in the aorta (5) as well as in depot fats (5–7) of adult humans, primarily as alkyl diacyl glycerols. Alkyl and alk-1-enyl diacyl glycerols, which occur in tissues of healthy adult individuals in about equal amounts, could not be detected in the heart and aorta of new-born babies (5, 7). Recently it has been demonstrated that the content of alkoxylipids in the aorta increases with age and with severity of atherosclerosis (8, 9), while the ratio of alkyl ethers to alk-1-enyl ethers remains constant (9). However, analyses of the two types of ether moieties in various lipid classes of human cardiovascular tissues have not been reported.

The present communication records the identification of various alkoxylipids in human hearts and aortae, and analyses of their constituent alkyl and alk-1-enyl moieties.

MATERIALS AND METHODS

Reference Compounds

Isopropylidene derivatives of alkyl glycerol ethers were prepared by the reaction of mesylates with isopropylidene glycerol (10). Saturated aldehydes were obtained by oxidation of mesylates (10) with dimethyl sulfoxide (11), and unsaturated aldehydes were synthesized via diazoketones and acetyl ketols (12).

Human Tissue Lipids

Hearts and aortae were obtained 8 hr after death from A, a woman 42 yr of age, who had died during surgery of a brain tumor, and B, a woman 35 yr of age, who had died of pneumonia. Pericardic fat was removed from the hearts and the adventitia was removed from the aortae. According to the definitions of the World Health Organization (13), the aortae were classified as Stage II.

Each of the hearts and aortic intimae was homogenized, the homogenate was extracted with chloroform–methanol (2:1, v/v), and the lipids were purified according to established procedures (14). Heart A, 174.0 g, yielded 6.9 g of lipids; heart B, 147.0 g, gave 6.2 g. Aorta A, 27.5 g, yielded 1.9 g of lipids; aorta B, 24.9 g, gave 1.3 g. The neutral lipids were extracted from the total lipids with hexane and the residual phospholipids and other polar lipids were dissolved in chloroform–methanol (1:1, v/v).

Isolation of Lipid Classes and Preparation of Derivatives

Neutral Lipids. About 50 mg of neutral lipids, dissolved in hexane, were applied in a streak to layers (0.5 mm thick) of Silica Gel G on plates, 20 × 20 cm, and chromatographed in hexane–diethyl ether–acetic acid 90:10:1 in tanks lined with filter paper (15). After

separation, various bands were visible on the layers without an indicator; others were detected under UV radiation (270 nm) after the plates had been sprayed with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol (15). Neutral lipid fractions were eluted from the adsorbent with several portions of diethyl ether and the eluates were filtered through sintered glass funnels. Small amounts of phospholipids present in the solution of neutral lipids remained at the starting lines. They were eluted and combined with the chloroform–methanol solutions of the polar lipids.

Alkyl and alk-1-enyl diacyl glycerols and free aldehydes were recovered together from the adsorbent layer between the lower edge of the steryl ester fraction and the upper half of the triglyceride fraction. These classes of compounds were resolved on layers of Silica Gel G by double development with hexane–diethyl ether 95:5 (16). The alkyl and alk-1-enyl diacyl glycerols, which migrated between the aldehyde fraction and the leading portion of the triglyceride fraction, were recovered. The two classes of neutral alkoxylipids were rechromatographed twice or thrice until free aldehydes could not be detected and the amount of triglycerides was reduced substantially.

Alcoholysis of the mixture of alkoxylipids and triglycerides with methanol–HCl (17) yielded dimethyl acetals (from alk-1-enyl diacyl glycerols), alkyl glycerol ethers (from alkyl diacyl glycerols), methyl esters, and glycerol. The ether-soluble products of methanolysis were separated in toluene on layers of Silica Gel G. The fractions of methyl esters (R_f 0.55), dimethyl acetals (R_f 0.25), and alkyl glycerol ethers (R_f 0.00) were each recovered with water-saturated diethyl ether.

Mixtures of alkyl glycerol ethers were allowed to react with acetone containing a small amount of $HClO_4$ (18). The isopropylidene derivatives were isolated (R_f 0.60) by chromatography on Silica Gel G in hexane–diethyl ether 9:1 and analyzed by gas–liquid chromatography.

Hexane solutions of the dimethyl acetals were applied as a streak to adsorbent layers that had been exposed to HCl vapors so that the acetals were hydrolyzed. The plates were developed with toluene and the aldehydes (R_f 0.52) were eluted, rechromatographed on Silica Gel G, and analyzed by gas–liquid chromatography.

Phospholipids. The choline and ethanolamine glycerophosphatides of hearts A and B were each isolated by chromatography of the polar lipid fraction on 20 × 20 cm layers of Silica Gel H, 0.5 mm thick, about 30 mg of polar lipids being applied per plate for chromatography in chloroform–methanol–water 65:25:4 in tanks lined with filter paper (19). The various fractions were detected as white bands after the plates had been sprayed with distilled water. The fractions of choline glycerophosphatides (R_f 0.15)[1] and ethanolamine glycerophosphatides (R_f 0.40) were eluted with chloroform–methanol–water 30:50:20 (20).

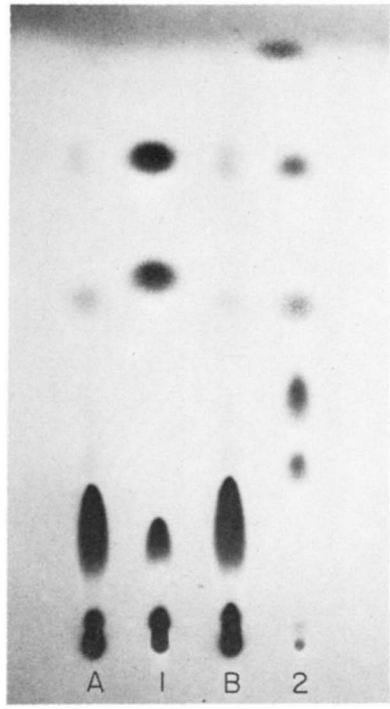

FIG. 1. Thin-layer chromatogram of the neutral lipids from two hearts on Silica Gel G in hexane–diethyl ether 95:5, developed twice (16). Charred after spraying with chromic–sulfuric acid. *A*, lipids of heart A; *1* (from bottom to top) oleic acid, cholesterol, triolein, methyl oleate, and cholesteryl oleate; *B*, lipids of heart B; *2* (from bottom to top, disregarding two small spots at origin) octadecyl dioleoyl glycerol, alk-1-enyl diacyl glycerols from ratfish liver, palmitaldehyde, octadec-9-enyl stearate, and octadecene.

The choline and ethanolamine glycerophosphatide fractions were each reduced with $LiAlH_4$ (21) to give alkyl glycerol ethers, alk-1-enyl glycerol ethers, fatty alcohols, and glycerol. The ether-soluble products of hydrogenolysis were applied to adsorbent layers that had been exposed to HCl vapors in order to hydrolyze the alk-1-enyl glycerol ethers. The plates were developed with hexane–diethyl ether–acetic acid 70:30:1 and the aldehydes (R_f 0.75) were eluted, rechromatographed, and analyzed. The plates were then developed with hexane–diethyl ether–acetic acid 50:50:1; the alkyl glycerol ethers (R_f 0.15) were eluted and converted to their isopropylidene derivatives, which were purified and analyzed.

Gas–Liquid Chromatography

The instrument, an F & M Scientific Hewlett–Packard model 5750, was equipped with a flame ionization de-

[1] This fraction includes small amounts of diacyl glycerophosphoryl serines. Alkyl acyl and alk-1-enyl acyl glycerophosphoryl serines could not be detected.

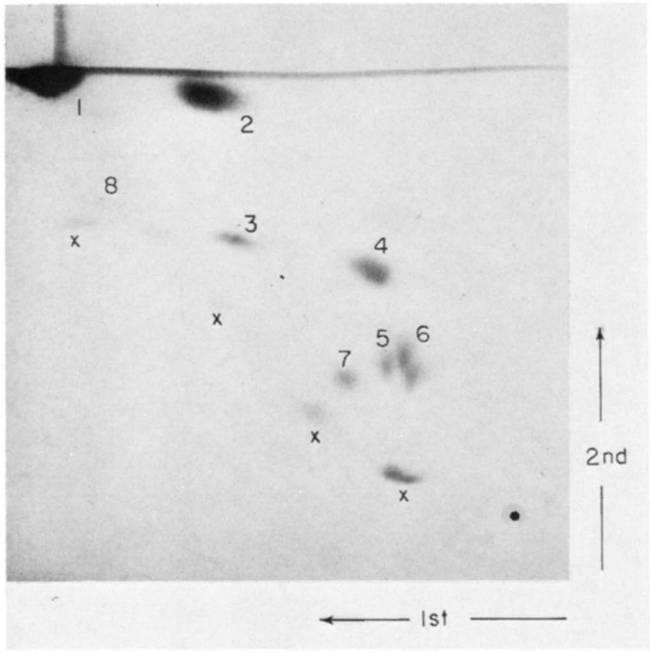

FIG. 2. Thin-layer chromatogram of the phospholipids and other polar lipids from aorta B on Silica Gel H in chloroform–methanol–water–conc. ammonia 130:70:8:0.5 (1st direction), and chloroform–acetone–methanol–acetic acid–water 100:40:20:20:10 (2nd direction) (30). Indicators: molybdenum blue reagent (31) for phospholipids, α-naphthol reagent (32) for glycolipids, and charring after spraying with chromic–sulfuric acid solution. *1*, triglycerides and cholesterol; *2*, fatty acids; *3*, ethanolamine glycerophosphatides; *4*, choline glycerophosphatides; *5*, serine glycerophosphatides and ethanolamine lysoglycerophosphatides; *6*, sphingomyelins; *7*, inositol glycerophosphatides; *8*, cardiolipins; *X*, glycolipids of unknown structures.

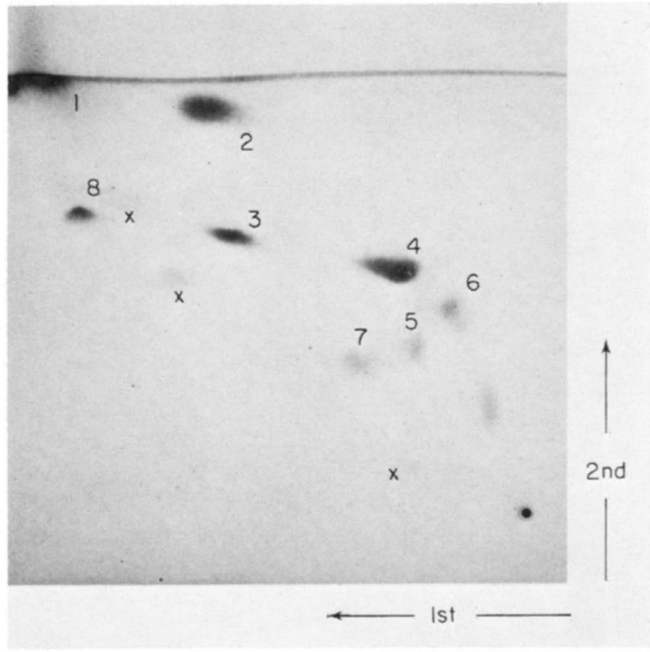

FIG. 3. Thin-layer chromatogram of the phospholipids and other polar lipids from heart B. (Experimental conditions as in Fig. 2.)

tector. Helium, at a flow rate of 70 ml/min, served as carrier gas.

Alkyl glycerol ethers were analyzed as isopropylidene derivatives (18), whereas aldehydes were chromatographed as such (6). The isopropylidene derivaties of alkyl glycerol ethers were resolved at 200°C on a column, 150 cm long, 0.4 cm I.D., filled with 10% diethyleneglycol succinate (Applied Science Laboratories, Inc.) on Anakrom A, 60–70 mesh (Analabs, Inc.). Aldehydes were separated on the same column at 175°C.

Fractions were assigned by comparison of their retention times with those of synthetic reference compounds and by argentation chromatography of the samples followed by gas–liquid chromatography of subfractions having uniform degree of unsaturation. The relative amounts of the fractions were calculated by triangulation of peak areas on the recording charts.

RESULTS AND DISCUSSION

The major lipid classes of neutral lipids in heart and aorta of man are sterol esters, triglycerides, and free sterols. In addition, small amounts of alkyl and alk-1-enyl diacyl glycerols and free aldehydes could be detected in lipid extracts of these tissues. As an example, Fig. 1 shows a thin-layer chromatogram of the neutral lipids of two hearts.

The human aorta is rich in sphingomyelins, choline glycerophosphatides, and ethanolamine glycerophosphatides, whereas the heart contains large proportions of cardiolipins in place of sphingomyelins. Figs. 2 and 3 show thin-layer chromatograms of the polar lipids of an aorta and a heart, respectively.

Both choline and ethanolamine glycerophosphatides of human cardiovascular tissues contain diacyl, alkyl acyl, and alk-1-enyl acyl compounds. It is well known that chromatography on silica gel does not resolve the alkoxy glycerophosphatides from the corresponding diacyl glycerophosphatides.

We have reduced the alkyl diacyl glycerols, choline glycerophosphatides, and ethanolamine glycerophosphatides of two hearts with lithium aluminum hydride, isolated the constituent alkyl glycerol ethers of each of the three classes of alkoxylipids, and analyzed them by gas–liquid chromatography of the isopropylidene derivatives. They consist exclusively (Table 1) of saturated and monounsaturated compounds, hexadecyl glycerol ether being the major constituent in each class. Similar compositions have been reported for the alkyl diacyl glycerols (6, 7) and alkyl acyl glycerophosphatides (22) of other human tissues.

The aldehydes released by acid-catalyzed hydrolysis from alk-1-enyl diacyl glycerols, alk-1-enyl acyl choline

TABLE 1 COMPOSITION OF THE ALKYL CHAINS OF ALKOXYLIPIDS IN TWO HUMAN HEARTS (A AND B)

Alkyl Chains*	Alkyl Diacyl Glycerols		Alkyl Acyl CGP		Alkyl Acyl EGP	
	A	B	A	B†	A	B
	%		%		%	
12:0			1.9	6.1	1.2	3.9
13:0 br			1.9	6.7	tr.	3.6
13:0	tr.	tr.	tr.	tr.	2.9	tr.
14:0 br					1.9	tr.
14:0	tr.	tr.	1.5	5.6	1.5	4.8
15:0 br	tr.	tr.	tr.	2.1	tr.	4.7
15:0	tr.	tr.	1.7	4.4	1.0	4.9
16:0 br	tr.	tr.				
16:0	35.3	42.2	50.5	29.0	41.9	39.8
17:0 br‡	tr.	tr.	tr.	3.4	tr.	3.8
17:0	tr.	tr.	tr.	2.4	tr.	2.5
18:0 br			tr.	tr.	tr.	tr.
18:0	39.0	33.5	21.5	16.5	31.9	19.9
18:1	25.7	24.3	21.0	23.8	17.7	12.1
18:2	tr.	tr.	tr.	tr.	tr.	tr.

*Designated by number of carbon atoms:number of double bonds. br, branched. The following compounds were also found in trace (<1%) amounts: 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 22:0, 22:1, 22:2.

† Part of the long-chain saturated alkyl glycerol ethers was lost during purification.

‡ Including 16:1.

TABLE 2 COMPOSITION OF THE ALK-1-ENYL CHAINS OF ALKOXYLIPIDS IN TWO HUMAN HEARTS (A AND B)

Alk-1-enyl Chains*	Alk-1-enyl Diacyl Glycerols		Alk-1-enyl Acyl CGP		Alk-1-enyl Acyl EGP	
	A	B	A	B	A	B
	%		%		%	
14:0	tr.	tr.	tr.	tr.	tr.	tr.
15:0 br	tr.	tr.	tr.	tr.	tr.	tr.
15:0	1.5	1.4	1.0	0.3	0.4	0.4
16:0 br	tr.	tr.	tr.	tr.	tr.	tr.
16:0	60.8	64.3	62.6	58.9	35.3	32.5
17:0 br†	3.0	3.3	tr.	6.1	tr.	tr.
17:0			tr.	tr.	3.2	4.0
18:0 br	tr.	tr.	3.5	1.4	tr.	0.7
18:0	20.4	19.8	16.8	16.4	38.9	43.0
18:1	14.3	11.1	14.2	14.6	20.5	18.0
18:2	tr.	tr.	1.9	2.3	1.6	1.4

* Designated by number of carbon atoms:number of double bonds. br, branched. The following compounds were found in trace (<1%) amounts: 18:3, 19:0, 20:0, 20:1, 20:2, 20:3, 20:4, 22:0, 22:1.

† Including 16:1.

glycerophosphatides, and alk-1-enyl acyl ethanolamine glycerophosphatides were analyzed by gas–liquid chromatography (Table 2). The alk-1-enyl chains of substituted diacyl glycerols and CGP of the heart are rich in 16:0, whereas those of EGP contain about equal proportions of 16:0 and 18:0. Table 3 shows that there are no great differences in the major alkyl moieties of the alkyl diacyl glycerols in heart, aorta, and depot fats. The same

TABLE 3 MAJOR CONSTITUENT ALKYL AND ALK-1-ENYL CHAINS IN ALKYL AND ALK-1-ENYL DIACYL GLYCEROLS IN VARIOUS HUMAN TISSUES*

	Aorta		Heart		Peri-nephric Fat (ref. 6)	Sub-cutaneous Fat (ref. 7)	
	A	B	A	B			
	%		%		%	%	
Alkyl chains							
16:0	41.2	26.8	35.2	42.2	36.2	30.3	40.0
18:0	29.0	34.8	39.0	33.4	40.6	40.2	30.7
18:1	29.8	38.4	25.7	24.2	23.2	29.5	29.3
Alk-1-enyl chains							
16:0	49.6	63.0	63.7	67.5	58.7	62.1	57.5
18:0	28.4	14.4	21.3	20.8	28.6	21.3	29.0
18:1	22.0	22.6	15.0	11.7	12.7	16.6	13.5

* Constituents occurring in small proportions are not considered.

is true for the major alk-1-enyl moieties of the alk-1-enyl diacyl glycerols of these tissues.

The compositions of the free aldehydes in hearts and aortae agreed in all details with those of the aldehydes obtained by acid-catalyzed hydrolysis from the total aldehydogenic lipids. We believe, therefore, that the major portion, if not all, of the free aldehydes in these extracts had been formed from alk-1-enyl ethers during autolysis of the tissues, during extraction of the total lipids or during storage of the lipid extracts.

Triglycerides and fatty acids in the diseased aorta are generally believed to originate primarily from the blood, whereas phospholipids are synthesized to a great extent in the aortic wall (23, 24). The sources of the neutral and ionic alkoxylipids are not known. As the compositions of the alkyl and alk-1-enyl diacyl glycerols in the aorta are similar to those of other healthy (6, 7) and diseased (22) tissues, and since these alkoxylipids occur in serum in small concentrations, it is reasonable to assume that they originate primarily from the blood. It is of interest that with increasing age the aorta accumulates not only various alkoxylipids but also other rather unusual lipids such as isoprenoid hydrocarbons (25, 26) and certain steroids other than cholesterol (5, 27, 28, 29).

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