J. Biosci., Vol. 5, Number 2, June 1983, pp. 167-172.

Comparative study of major lipid constituents in human and monkey aorta

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MS received 28 December 1982

Abstract. The contents of total lipids, phospholipids, cholesterol, triglyceride and free fatty acid in intima and media of thoracic and abdominal segments of aorta from normal human subjects and rhesus monkeys were determined. An increase in total lipids in intima as compared to that of media was noted in both species. A comparison of lipid contents of thoracic and abdominal segments from both species revealed that abdominal segment contained significantly greater lipid. Further, human thoracic and abdominal aortic intima and media had higher content of cholesterol as compared to that of monkey aorta. The differences in lipid profile in aorta of these two species have been highlighted to provide a clue with respect to the differences in the prevalence and morphology of atherosclerosis as seen in monkey and man.

Keywords. Neutral and phospholipids; human and monkey aorta.

Introduction

Man and subhuman primates are phylogenetically related and present similarity in the distribution and morphological features of naturally occurring atherosclerosis (Strong and Tappen, 1965; Lindsay and Chaikoff, 1966; Chawla *et al.,* 1967; Chakravarti *et al.,* 1976; Chakravarti and Kukreja, 1981). However, frequency incidence and the prevalence of this disease in wild macaques and baboons is much lower than seen in human beings (Middleton *et al.,* 1967; Strong *et al.,* 1976; Chakravarti and Kukreja, 1981). The causes of this difference in the frequency incidence could be many, but among them the lower level of serum cholesterol in macaques as compared to that of man may play an important role (Portman and Alexander, 1966; Chawla *et al.,* 1967). Other factors like differences in arterial lipids and structural proteins between the two species may be contributing significantly to this difference. In view of the above, it was thought worthwhile to make a comparative study of normal profile of the aorta of man and rhesus monkey which may give a clue to the differences observed in the frequency incidence and biochemical characteristics of atherosclerosis in these two species.

Materials and methods

Collection of aorta samples and separation of intima and media

Aorta samples were collected from apparently normal healthy male rhesus

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monkeys and from 11 young male human subjects who died of street accidents. The body weight of rheusus monkeys ranged between 4-5 kg which gave an approximate estimate of age as 4 years and for human beings the age range was between 25-35 years. In monkeys, the clinical parameters including blood pressure, electrocardiogram, ocular fundus and serum lipids and lipoproteins were within normal limits. Tuberculin test was done to exclude tuberculosis. Parasitic infection was excluded by routine stool examination. The whole aorta was dissected out, opened longitudinally and examined with a hand lens. Those samples which did not reveal macroscopic and microscopic lesions were taken for this study. The upper 2/3rd of the thoracic aorta starting from first intercostal artery opening was removed to study the lipid profile. Similarly, the upper 2/3rd of the abdominal aorta starting from coeliac artery was taken for lipid study. The aorta samples were fixed on a waxed tray with endothelial surface facing upwards, and the intima was scrapped off with the back of a scalpel handle. In the case of monkeys, separation of intima from media was difficult since the intima was very thin, but in the case of humans the intima could be easily scrapped off from media (Smith *et al.,* 1967).

Extraction, separation and estimation of lipids

Lipid extraction from aorta samples was done by the method of Folch *et al.* (1957) using chloroform-methanol (2:1, v/v) mixture; the solvents were redistilled before use. Triglycerides, free fatty acids, free and esterified cholesterol were separated by using single dimension thin layer chromatography. Phospholipid fractions were separated by double dimension thin layer chromatography. Free and esterified cholesterol were estimated by the method of Zlatkis *et al.* (1953), triglycerides by Van Handel and Zilversmit (1957), phospholipid phosphorus by Bartlett (1959) and free fatty acids by the technique of Dole (1956).

Results

Table 1 shows the values of different components of neutral lipid i.e. total cholesterol, free and esterified cholesterol, triglycerides and free fatty acids along with total lipids and phospholipids in both intima and media of human and monkey thoracic aorta. Total lipids in intima were greater than that in media of both species. The higher value of intimal total lipids in human thoracic aorta was mainly due to total, free and esterified cholesterol. Phospholipids of intima and media of human thoracic aorta did not show variation, but relative percentage of phospholipids to total lipids in media accounted for 42% as against 30% in intima. There was no significant difference in triglycerides and free fatty acids in intima and media of human thoracic aorta. In monkey thoracic aorta, the higher value of intimal total lipids as compared to that of media was due to increase in all fractions of lipids. A comparison of human and monkey thoracic intima and media aortic lipids revealed a significantly lower value of free and esterified cholesterol in monkey aorta.

The values of phospholipid fractions of human and monkey thoracic aorta are given in table 2. There was no significant difference in total phospholipids as well as phospholipid fractions in intima and media of human thoracic aorta. But in monkey thoracic aorta, total phospholipids were significantly less in media as

Table 1. Neutral lipids and phospholipids of human and monkey thoracic aorta.

	Human[a]		Monkey[b]	
	Intima (mg/100 gm wet tissue)	Media	Intima (mg/100 gm wet tissue)	Meida
Total lipids	1502±280	1109±271***	1381±202	923±123***
Phospholipids	496±122	476±109	580±178	461±152*
Total cholesterol	660±137	354±121***	346± 42	175± 45***
Free cholesterol	363±102	194± 59***	190±30.5	91± 22***
Esterified cholesterol	297±110	159± 58***	155±27.4	80± 20***
Triglycerides	301± 80	233± 89	372± 37	239± 84***
Free fatty acids	75± 19	66± 14	103± 24	64± 18***

Values are Mean ± S.E.

[a] n=11, [b] n=18

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 2. Human and monkey thoracic aorta phospholipid fractions.

	Human[a]		Monkey[b]	
	Intima (mg/100 g wet tissue)	Media	Intima (mg/100 g wet tissue)	Media
Total phospholipids	495±122	476±109	579±178	461±152
Phosphatidyl choline	121± 33	124± 27	163± 41	150± 32
Phosphatidyl ethanolamine	107± 24	102± 22	80± 30	72± 27
Sphingomyelin	132± 35	120± 27	170± 47	147±24.8
Lysophosphatidyl ethanolamine	27± 4.8	25± 4.2	30± 5.8	21± 5.2
Phosphatidyl serine	42±14.7	38± 9.7	38± 9.2	30± 5.8
Lysophospha-tydylcholine	38±10.3	34± 7.8	36± 8.2	30± 7.9

Values are Mean ± S.E.

* $P<0.05$, [a] $n=11$, [b] $n=18$.

compared to that of intima. The different fractions of phospholipids remained unaltered in intima and media. If we compare human and monkey thoracic aortal phospholipid fractions, there was significant increase in phosphatidylcholine and sphingomyelin in monkey thoracic aorta. However, a significantly lower value of phosphatidyl ethanolamine was found in monkey thoracic aorta as compared to that of human thoracic aorta.

So far as abdominal aorta is concerned a significant increase in total lipids of intima as compared to that of media was noted in both monkey and man (table 3) which was contributed by total cholesterol and triglycerides. If we

Table 3. Neutral lipids and phospholipids of human and monkey abdominal aorta.

	Human[a]		Monkey[b]	
	Intima	Media	Intima	Media
	(mg/100 g wet tissue)		(mg/100 g wet tissue)	
Total lipids	1630±292	1237±201***	1491±254	1123±232***
Phospholipds	538±118	525±114	611±158	527±123[NS]
Total cholesterol	717±130	383±109***	399± 97	235± 48***
Free cholesterol	394± 93	214± 81***	219± 82	129± 32***
Esterified cholesterol	322±103	166± 75***	175± 70	101± 27
Triglycerides	326±108	235± 72*	372±102	292± 81*
Free fatty acid	81± 23	68± 21	101± 22	78± 23**

Values are Mean ± S.E.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

[a] $n = 11$, [b] $n=18$.

compare the lipid profile of human and monkey abdominal aortic intima and media, it is observed that there is significantly lower value of total, free and esterified cholesterol in both intima and media of monkey as compared to the corresponding region of human abdominal aorta.

Table 4 contains the values of phospholipid content of abdominal aorta of monkey and man. A comparison of phospholipid content of human and monkey abdominal aorta revealed significantly higher content of phosphatidylcholine and sphingomyelin and with lower content of phosphatidyl ethanolamine in intima and media of monkeys as compared to that of human. Further, if we compare lipid constituents of human and monkey thoracic with abdominal aorta, the latter shows a higher lipid content in both species.

Discussion

From this study, certain similarities and dissimilarities in the lipid composition of human and monkey aorta have been elucidated. The present findings of lipid

Table 4. Human and monkey abdominal aorta phospholipid fractions.

	Human		Monkey[b]	
	Intima	Media	Intima	Media
Total phospholipids	538±118	525±114	611±158	530±123
Phosphatidyl-choline	131± 32	124± 29	174± 40	159± 38
Phosphatidyl ethanolamine	115± 28	118± 34	75± 24	81± 24
Sphingomyelin	129± 32	112± 24	160± 38	140± 27
Lysophosphatidyl ethanolamine	49± 4.3	47±11.2	36± 8.9	31± 8.2
Phosphatidyl-serine	44± 7.8	49± 9.3	43±17.2	37± 5.8
Phosphatidyl-inositol	16± 4.3	12± 4.2	17± 5.6	13± 3.2
Lysophosphatidyl choline	41± 8.9	43±10.2	50±10.2	40± 9.8

Values are Mean ± S.E.
[a] n=11, [b] n=18.

profile of intima and media of thoracic and abdominal aorta from human subjects are in conformity with previous reports (Buck and Bossiter, 1951; Smith, 1960; Bottcher *et al.,* 1960; Insull and Bartsch, 1966). There is no report in literature regarding the lipid profile of different layers and segments of monkey aorta. The neutral lipids were present in greater amount in intima of both thoracic and abdominal aorta as compared to the media in both man and monkey. This comparison supports the validity of Anitschkow's hypothesis (Anitschkow, 1925) that intima is freely permeable to the lipids of plasma and the intima wall exerts no selective action on these lipids. No significant difference could be observed in phospholipids of intima and media in both thoracic and abdominal aorta of both these species. However, relative percentage of phospholipids to total lipids, revealed higher level of this in media as compared to intima in both species, which could be the result of increased local synthesis (Besterman and Gillet, 1971). There was more lipid in abdominal aorta as compared to thoracic aorta in both species, and this fact may explain the higher incidence of atherosclerosis in abdominal aorta (McGill *et al.,* 1964; Geer and Malcon, 1965). A comparison of lipid constituents of intima and media of thoracic and abdominal aorta of man with that of monkey revealed significantly lower value of free and esterified cholesterol in monkey aorta. This may be one of the factors which may explain lower incidence and extent of spontaneously occurring atherosclerosis in monkey (27%) (Chakravarti and Kukreja, 1981) as compared to man (80-90%).

Phospholipid fractions of human and monkey aorta revealed significantly higher level of phosphatidyl-choline and sphingomyelin in monkey thoracic and abdominal aortic intima and media as compared to corresponding areas in human aorta. As phosphatidylcholine is known to stabilize the hydrophobic lipids in solution and prevents its deposition in tissues and that it has a strong lipotropic action, atheroma formation can be effectively inhibited. The level of phosphatidyl ethanolamine was significantly decreased in intima and media of both thoracic and abdominal segments of monkey aorta as compared to that of human. The higher value of phosphatidyl ethanolamine in human aorta appears to be the result of increased vascular wall lipogenesis which may predispose the vessel to intravascular mural thrombosis, since phosphatidyl ethanolamine is known to promote platelet aggregation and fibrin formation (Turner *et al.,* 1963).

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