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Lipid Metabolism of the Arterial Wall in Thromboangiitis Obliterans (Buerger's Disease)*

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Summary. The uptake and incorporation of [I^{14}-C] oleic acid by diseased arterial intima removed by thrombendarteriectomy in 3 patients with Thromboangiitis obliterans (TAO) was studied.

The diagnosis of TAO had been established by clinical, angiographic and histological criteria.

The uptake and distribution of the label was found very similar in TAO and normal intima and differed considerably from atherosclerotic intima, from fatty streaks as well as from fibro-fatty lesions.

In fatty streak lesions the incorporation of [I^{14}-C] oleic acid into phospholipid, triglyceride and cholesterol ester was significantly increased compared to TAO, normal intima and umbilical artery.

In TAO the distribution of labelled lipids between subcellular fractions of the arterial intima was also studied and, as in normal intima, most of the cholesterol ester were found membrane-bound whereas in fibro-fatty lesions the bulk of the cholesterol ester was present in the lipid skin fraction.

The incorporation of [I^{14}-C] oleic acid into different phospholipids was highest in atherosclerotic intima while no significant differences were found between normal intima and TAO.

These data suggest a different pathogenesis of TAO and atherosclerosis.

Key words: Peripheral vascular disease — Atherosclerosis — Lipid synthesis — Cholesterol ester — Phospholipid — Subcellular fractions — [I^{14}-C] Oleic acid.

Zusammenfassung. Aufnahme und Einbau von [I^{14}-C] Ölsäure in die thrombangiitisch veränderte Intima wurde an Gefäßzylindern untersucht, die bei 3 Patienten im Rahmen einer Thrombendarterektomie entnommen worden waren. Bei diesen Patienten war die Diagnose Thrombangiitis obliterans (TAO) auf Grund klinischer, angiographischer und histologischer Befunde gestellt worden.

Der Einbau des Isotops in die verschiedenen Lipide der Gefäßwand war bei TAO und normaler Intima sehr ähnlich und unterschied sich deutlich von dem Einbau in atherosklerotische Intima und zwar sowohl in Fettstreifen als auch in Atherome.

Bei Fettstreifen war der Einbau von [I^{14}-C] Ölsäure in Phospholipide, Triglyzeride und Cholesterin-Ester gegenüber der TAO, normaler Intima und Nabelschnurarterien signifikant erhöht. Die Verteilung der markierten Lipide auf verschiedene subcelluläre Fraktionen der Intima war in dem untersuchten thrombangiitischen Gewebe jener der normalen Intima vergleichbar: Die Cholesterinveresterung fand ganz überwiegend membrangebunden statt, während bei Atheromen die markierten Cholesterin-Ester vor allem in der überstehenden Fettschicht nachweisbar waren.

Der Einbau des Isotops in verschiedene Phospholipide war bei atherosklerotischer Intima wesentlich höher als in den anderen untersuchten Geweben. Zwischen normaler und thromboangiitischer Intima fanden sich keine signifikanten Unterschiede in der Verteilung der markierten Phospholipide.

Diese Befunde legen eine eigenständige, von der Atherosklerose unabhängige Pathogenese der Thromboangiitis obliterans nahe.

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Thromboangiitis obliterans (TAO), Buerger's disease, is an uncommon, occlusive vascular disease which primarily affects the small and medium sized arteries and veins of the extremities. The disease is inflammatory and segmental and occurs almost exclusively in young men who are cigarette smokers. Mostly it is associated with episodes of migratory thrombophlebitis but it is not associated with diabetes mellitus, hypercholesterolaemia or heart disease which might be a source of emboli (McKusick *et al.*, 1962).

In the past this concept of TAO as a diagnostic entity in the clinical or pathological sense has been contested (Ratschow, 1961; Eisen, 1966), because it was not being encountered clinically and thourough examination of unselected, amputated limbs did not reveal the characteristic microscopical changes. For these reasons it was considered to be indistinguishable from atherosclerosis or idiopathic arterial thrombosis (Wessler *et al.*, 1960).

The question of the existence of TAO as an entity has been investigated since by clinical and pathological studies (Williams, 1969; Leu *et al.*, 1973), and it has been found, that there is a significant difference in the prognosis for patients given a diagnosis of TAO and those given a diagnosis of atherosclerosis obliterans (McPherson *et al.*, 1963).

Recently the term Buerger's syndrome was widely accepted for a constellation of the mentioned clinical findings while the pathological specificity of this disease continued to be disputed (Wessler, 1969), because etiologic or pathogenetic mechanisms are not yet known or understood.

If the pathogenesis of TAO was related to atherosclerosis, similarities in the lipid metabolism of the arterial wall should be expected. In atherosclerosis the lipid metabolism of the arterial wall is altered in a very characteristic way, the phospholipid- and cholesterol ester synthesis being increased considerably compared to the normal intima (Wahlquist *et al.*, 1969; Horsch *et al.*, 1973). In the present study, the lipid metabolism of the arterial intima affected by TAO is investigated and compared to the lipid metabolism of atherosclerotic and normal intima in order to establish the different pathogenesis of TAO from atherosclerosis.

Material and Methods

Three male patients, aged 26, 32 and 43 years respectively, all cigarette smokers (over 20 cigarettes/day) with rest pain and, in two of them, with migratory phlebitis, were submitted to reconstructive surgery of the femoral artery. Angiography had revealed in all cases well delineated vessel walls and sudden occlusions of the femoral artery with corkscrew or tree root collaterals and several other peripheral occlusions. In the abdominal aorta no calcifications could be detected.

Diabetes mellitus, hyperlipidemia, autoimmune- and heart disease had been exluded. In all three patients the diagnosis of Thromboangiitis obliterans was assumed by clinical criteria and, in two cases, histological examination of the tissue confirmed this diagnosis.

The endarterectomy specimens measured 2.9, 3.8 and 2.4 cm respectively, they were cleaned of blood and connective tissue, split longitudinally and incubated within 30 min after removal.

Normal and atherosclerotic human femoral arteries were removed within 30 min of clinical death, umbilical arteries were obtained within the same time interval. The arteries were used as segments of 5 cm length, cleaned of adventitial tissue and split longitudinally while moistened with saline.

The TAO specimens were all removed from the upper femoral artery and are anatomically comparable to the intima-media preparations from normal and atherosclerotic femoral artery segments.

Incubations were carried out for 4 hours at 37° C in 10 ml of equal volumes of human blood group 0 serum and tissue culture medium 199[1], containing 10 μCi of [I^{14}-C] oleic acid[2]. [I^{14}-C] labelled oleic acid was added to the incubation medium combined with the albumin in the serum.

After incubations, the arteries were washed thoroughly several times in ice cold 0.9% NaCl solution and the intima with parts of the media was stripped off, homogenized in a mortar, extracted with chloroform methanol (2:1/V:V) and the resulting extract washed by the method of Folch *et al.* (1957).

In the subcellular fraction studies the same procedure was adopted, except that the tissues were homogenized in 0.9% NaCl solution using a Kontes glass homogenizer, centrifuged at 1,000 × *g* for 5 min to remove cell debris and connective tissue and the resulting supernatant was then centrifuged at 100,000 × *g* for 1 hour at 2° C in a preparative ultracentrifuge using a swinging bucket (Spinco, Model L 2/65B Rotor SW 39 L). This resulted in three fractions: a lipid layer floating on the surface (the lipid skin), a clear supernatant (SN) and a particulate fraction (P 100). These fractions were separated and extracted individually as described.

The dry defatted weight of the tissues was determined after extraction. Aliquots of the lipid extracts from all the fractions were separated into phospholipid, triglyceride, fatty acid, cholesterol and cholesterol ester by thin layer chromatography using silica gel G (Merck) and n-hexane-diethyl ether-acetic acid (100:38:3, U/V/V) as the developing solvent.

Phospholipids were separated into their individual components by thin layer chromatography on silica gel (Camag) using the method of Skipski *et al.* (1964). A mixture of known phospholipids was added as internal standard in order to detect the phospholipid spots after separation.

The lipid spots were visualized by spraying with 0.2% dichloro-fluorescin and scraped directly into counting vials. The radioactivity[3] was determined directly using the dioxane-water scintillator of Snyder (1964). ^{14}C label applied to the plate was quantitatively recovered. Protein was determined by the method of Lowry *et al.* (1951).

Statistics

Data are presented as means ± SEM. The significance was determined by the Student's *t*-test.

Results

The uptake of [I^{14}-C] oleic acid and the amounts incorporated into phospholipid, triglyceride and cholesterol ester by the various arterial tissues are shown in Table 1 for the three experiments carried out in each group.

The highest incorporation into all lipid fractions is found in the atherosclerotic artery while the incorporation of the label is significantly lower in TAO and normal intima ($p<0.01$). In both, the distribution of label between the different lipids is very similar and differs from the distributions in atherosclerotic arteries. The lowest incorporation is seen in umbilical arteries.

In all arteries the relatively highest incorporarion occurs into phospholipids, the lowest into cholesterol ester, but there is significantly more cholesterol ester labelled in atherosclerotic lesions than in both TAO and normal intima.

1 TCM 199, Difco Lab. Detroit, Michigan, USA.

2 [I^{14}-C] oleic acid, specific activity 59,7 mCi/mMol. Radiochemical Center, Amersham, Buchler.

3 Packard liquid scintillation counter TRI CARB.

Table 1. Uptake and incorporation of (I^{14}-C) oleic acid into various lipids by human arterial wall (dpm incorporated/mg dry defatted weight/10^6 dpm in incubation medium)

	Phospholipid	Triglyceride	Cholesterol ester
Umbilical artery	46.8 ± 2.82	16.2 ± 4.47	1.6 ± 0.05
Normal intima	49.43 ± 12.58	45.4 ± 6.79	6.2 ± 1.48
Thromboangiitis obliterans	41.6 ± 4.56	26.9 ± 1.09	6.7 ± 0.58
Atherosclerotic intima (Fatty streaks.)	299.7 ± 100.38	122.3 ± 20.21	46.10 ± 28.28

$n = 3$ for each group.

In normal intima and TAO the incorporation into phospholipid and cholesterol ester respectively is identical and differs significantly ($p<0.005$) from atherosclerotic intima (fatty streak lesions), where especially the esterification of cholesterol is increased.

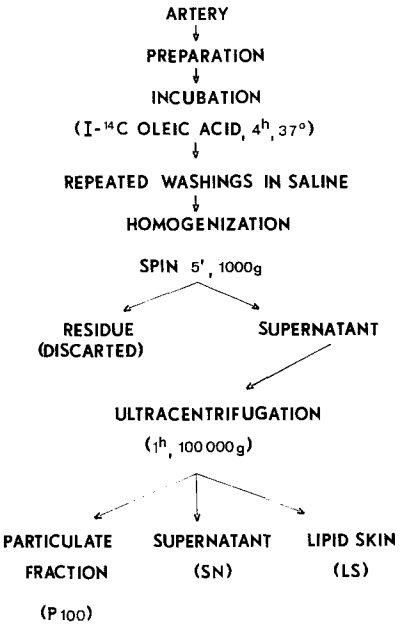

Fig. 1. Diagram for the subcellular fractionation of arterial tissue

In Fig. 1 the experimental procedure for the separation of the subcellular fractions is presented and the distribution of labelled phospholipids, triglyceride and cholesterol ester between these subcellular fractions is given in Table 2 for arterial intima in TAO, normal and atherosclerotic artery.

In the normal intima (mean of 3 experiments) more than 50% of the label incorporated into cholesterol ester is associated with the particulate fraction and only a relatively small proportion of it is present in the floating lipid layer. In contrast, in the atherosclerotic intima (fibrofatty lesions, mean of 3 arteries)

Table 2. Percentage distribution of labelled phospholipid, triglyceride and cholesterol ester between subcellular fractions of human femoral arteries

	Phospholipid	Triglyceride	Cholesterol ester
	Thromboangiitis obliterans		
P 100	81.4	86.7	93.6
SN	16.3	6.2	—
LS	2.3	7.1	6.4
	Normal intima		
P 100	78.8 ± 18.8	63.6 ± 8.2	58.6 ± 2.5
SN	20.8 ± 19.1	9.8 ± 5.2	21.0 ± 12.7
LS	0.4 ± 0.64	26.6 ± 6.1	20.4 ± 11.9
	Atherosclerotic intima[a]		
P 100	77.1 ± 14.4	36.5 ± 15.3	37.9 ± 20.0
SN	18.2 ± 10.6	10.9 ± 4.5	10.4 ± 6.7
LS	4.7 ± 6.4	52.6 ± 18.5	51.7 ± 20.2

P 100 = Particulate fraction at 100,000 × *g*. SN = Supernatant at 100,000 × *g*. LS = Lipid skin at 100,000 × *g*. $n = 3$ for normal and atherosclerotic intima.
[a] Fibrofatty lesions.

Table 3. Uptake and incorporation of [I^{14}-C] oleic acid into different phospholipids by human arterial wall (dpm incorporated/mg dry defatted weight/10^7 dpm in incubation medium)

	LL[a]	SM	L	PI	PE
Umbilical artery	2.9 ± 0.21	5.7 ± 0.07	279.0 ± 39.6	134.0 ± 8.48	44.0 ± 0.7
Normal intima	3.4 ± 0.84	6.85 ± 2.86	258.0 ± 38.18	69.1 ± 3.46	34.55 ± 0.31
Thromboangiitis obliterans	0.9 ± 0.63	2.6 ± 0.28	341.5 ± 35.7	111.5 ± 10.25	56.0 ± 12.0
Atherosclerotic intima[b]	2.15 ± 1.52	6.15 ± 2.22	440.5 ± 23.68	156.5 ± 19.44	69.0 ± 4.94

$n = 3$ for each group.
[a] LL = Lysolecithin, SM = Sphingomyelin, L = Lecithin, PI = Phosphatidyl inositol, PE = Phosphatidyl ethanolamin.
[b] Fibrofatty lesions.

the labelled cholesterol ester is found predominantly in the lipid skin fraction[4]. In TAO the labelled cholesterol ester is present almost exclusively in the particulate fraction.

The distribution of labelled phospholipids between the subcellular fractions is very similar in all tissues studied while that of labelled triglycerides follows closely the pattern of the cholesterol ester.

4 These results have partly been published: Horsch, A. K. and Day, A. J., in Verh. dtsch. Ges. inn. Med. **80**, 950–952 (1974) and are given here for comparison with TAO.

Table 3 shows the incorporation of [I^{14}-C] oleic into different phospholipids of the arterial wall in umbilical artery, in TAO, in normal- and atherosclerotic intima. The distribution of the label is very similar for all tissues in the different phospholipids except for phosphatidyl inositol: The highest incorporation occurs in fibrofatty lesions, the lowest in normal intima while in TAO and umbilical artery there is almost twice as much phosphatidyl inositol synthesized as in normal artery ($p<0.05$). The ratio of lecithin:phosphatidyl inositol is lower in atherosclerotic intima (2.82) than in TAO (3.07) or in normal intima (3.74).

Discussion

The uptake and incorporation of [I^{14}-C] oleic acid into various lipids of the arterial wall was found to be similar in TAO and normal intima, but differed significantly from that in atherosclerotic lesions. In both fatty streaks and fibrofatty lesions considerably more label was incorporated into phospholipids and cholesterol ester than in arteries from TAO or in normal arteries.

One TAO specimen was submitted to subcellular fractionation and the results obtained resembled the normal rather than the atherosclerotic intima; more than 90% of the labelled cholesterol ester was present in the particulate fraction with little labelling of the supernatant.

In the normal intima there is little cholesterol esterification and the levels of cholesterol esterifying enzymes are low (Day *et al.*, 1968). In atherosclerotic lesions however cholesterol esters accumulate and the cholesterol esterifying activity is stimulated (Bowyer *et al.*, 1968; Proudlock *et al.*, 1972). In human fatty streak lesions the cholesterol ester synthesis is about twenty times higher than in the adjacent normal intima of the same artery (Horsch *et al.*, 1973).

The origin of the cholesterol ester appears to be both from filtration and deposition of serum cholesterol ester and from local esterification of serum cholesterol (Day *et al.*, 1970). The phospholipids are synthesized essentially within the arterial wall, but filtration from the serum also occurs to some extent (Shore *et al.*, 1955). Furthermore, the synthesis of these lipids seems to be associated with different metabolic pools. In subcellular fractions of atherosclerotic rabbit intima cholesterol esterification was primarily associated with the floating lipid skin fraction, whereas in normal intima esterification was much less and occurred predominantly in the particulate fraction. Phospholipidsynthesis was always associated with the particulate fraction and increased with the severity of the lesions (Proudlock *et al.*, 1973). Similar findings have been reported for human arteries (Horsch *et al.*, 1974).

In view of the limited amount of material available, no lipid determinations have been carried out. However, in similar work in the rabbit (Proudlock *et al.*, 1973) the incorporation of [I-^{14}C] oleic acid into cholesterol ester and phospholipid followed closely the distribution of these lipids chemically in the artery.

The increased incorporation into the respective lipid fraction of cholesterol ester or phospholipid in atherosclerotic intima cannot be interpreted of course, to represent increased turnover of these lipids since the specific activity has not been determined. In atherosclerotic intima, the high chemical content of cholesterol ester and phospholipid may itself produce a high incorporation of the [I-^{14}C]

oleic acid. Nevertheless, if one considers there is an initial common pool of fatty acid in the artery available for lipid synthesis, it can be asserted that the cpm incorporated into phospholipid and cholesterol ester respectively gives a measure of the micromoles of fatty acid incorporated. On this basis, the different uptake and incorporation of the label by the tissues studied is the result of different synthetic capacities of these tissues.

The incorporation of [I^{14}-C] oleic acid into phospholipid of the arterial wall in TAO resembles the incorporation of the label into umbilical arteries and is intermediate between that into the normal and atherosclerotic intima. With the development of the atherosclerotic lesion there is a shift from phosphatidylinositol— to lecithinsynthesis, but the significance of this changing pattern in phospholipidsynthesis is not clear (Newman *et al.*, 1966). In this regard there is no similarity in the phospholipidmetabolism in TAO with neither normal nor atherosclerotic arterial wall.

It would of course have been desirable to study more than three TAO specimens, but TAO is a rare disease and only a very limited number of these patients are amenable to surgery. However, the present results suggest an etiology for TAO distinct from atherosclerosis. The distinguishing feature of atherosclerosis is the intra- and extracellular accumulation of lipid within the lesion: such accumulation is absent in TAO. The lipid metabolism of the arterial wall in TAO is the same as in the normal intima.

The evidence for a distinct etiology of TAO in view of its better prognosis compared to atherosclerosis (McPherson *et al.*, 1963) may encourage a thorough differentiation of all individual data rather than a more general diagnosis of a peripheral vascular disease. Nevertheless atherosclerosis remains the major alternative in differential diagnosis of TAO and the diagnosis has still to be established on the basis of clinical findings.

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