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The influence of chylomicron remnants on endothelial cell function in the isolated perfused rat aorta

David J. Grieve, Michael A. Avella, Jonathan Elliott, Kathleen M. Botham *

Department of Veterinary Basic Sciences, Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, UK

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Abstract

A system for the perfusion of the isolated rat aorta which allowed the study of both the uptake of chylomicron remnants by the artery wall and their effects on endothelial function was developed. Perfusion for 2 h with 125I-labelled native or oxidised (by treatment with copper sulphate) chylomicron remnants showed that small amounts became associated with the artery wall (0.111 ± 0.034 and 0.216 ± 0.082 ng protein/mg tissue, respectively). Tests on endothelial function were carried out in vessel rings prepared after perfusion of the aortas in the presence or absence of chylomicron remnants for 2 h. After perfusion of the vessels with oxidised chylomicron remnants, the maximum response to phenylephrine (PE) was significantly increased (from 0.34 ± 0.06 to 0.51 ± 0.04 g/mg tissue; \( P < 0.05 \)), while the maximum % relaxation to carbachol (CCh) was significantly decreased (from 91.6 ± 2.4 to 71.5 ± 7.2; \( P < 0.05 \)) and the response to S-nitroso-N-acetylpenicillimine (SNAP) was unaffected. Perfusion with native chylomicron remnants showed a tendency to induce similar effects, although the changes observed did not reach statistical significance. As the lipoproteins were not present in the solution bathing the vessel rings during these tests, these effects can be attributed to perfusion of the aortas with chylomicron remnants, despite only small quantities being associated with the artery wall. The results suggest that oxidised chylomicron remnants influence vascular endothelial function by interfering with the L-arginine-nitric oxide (NO) pathway. The observed potentiation of contraction to PE may be due to inhibition of the basal release of NO or to the release of contractile factors. These findings support a role for dietary lipoproteins in the modulation of endothelial cell function which occurs in the pathogenesis of atherosclerosis. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Chylomicron remnants; Copper oxidation; Endothelium-derived relaxing factor; Perfusion of the isolated rat aorta

1. Introduction

It has been known for some time that plasma low density lipoprotein (LDL) is involved in the atherogenic process [1]. LDL has been shown to accumulate in the intima of normal arteries [2] and to be present in foam cells of early fatty streaks in both humans and experimental animals [3,4]. LDL has also been shown to be taken up in vitro in the perfused rabbit aorta and in vivo in both the rabbit and rat aorta [5–7].

In the past, less attention has been paid to the role of chylomicrons and chylomicron remnants, which transport dietary lipid to the liver, but there is now increasing evidence that chylomicron remnants may be involved in atherogenesis. Chylomicron remnants have been shown to accumulate in both the rabbit and rat aorta in vivo [6,8,9] and apolipoprotein E-containing lipoproteins have been found in the human aortic intima [10]. In addition, it has been demonstrated that chylomicron remnants are rapidly phagocyted by

Abbreviations: CCh, carbachol; EDTA, ethylenediaminetetra-acetic acid; KHS, Krebs–Henseleit solution; LDL, low density lipoprotein; MDA, malondialdehyde; NO, nitric oxide; PBS, phosphate-buffered saline; PE, phenylephrine; SNAP, S-nitroso-N-acetylpenicillamine; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid.

* Corresponding author. Tel.: +44 171 4685274; fax: +44 171 3881027.
macrophages [11]. Furthermore, patients with type III hyperlipoproteinemia, characterised by a defect in apolipoprotein E such that there is impaired binding to receptors, have been reported to have an increased incidence of vascular disease [12]. Postprandial chylomicron remnant concentrations have also been found to be elevated in coronary artery disease [13] and in renal dialysis patients, a condition associated with accelerated atherosclerosis [14]. Finally, patients with familial hyperchylomicronaemia develop premature atherosclerosis, despite their low circulating LDL concentrations [15].

It is well established that atherosclerosis and hypercholesterolaemia cause impaired endothelium-dependent relaxation, and this is thought to be due to cholesterol accumulation in the artery wall [16]. Native LDL has been shown to inhibit endothelium-dependent relaxation in rabbit aorta and porcine coronary artery [17,18] by interfering with the endogenous L-arginine-nitric oxide (NO) pathway [19]. However, a number of other studies have concluded that oxidation of LDL in the artery wall [20] is critical to the subsequent inhibition of endothelium-dependent relaxation [21]. Indeed, dietary antioxidants such as β-carotene and α-tocopherol have been shown to restore endothelial function in hypercholesterolaemic animal models [22].

Recent work in our laboratory has suggested that chylomicron remnants can also influence endothelial function in the artery [23]. Incubation of native chylomicron remnants with freshly isolated rat aortic rings was shown to cause inhibition of endothelium-dependent relaxation and potentiation of endothelium-dependent contraction. We have also found that prior oxidation of the chylomicron remnants by incubation with copper sulphate caused a marked potentiation of the effects observed with the native particles [24]. In these experiments, however, the adventitial and cut surfaces of the vessel rings, in addition to the luminal surface, were exposed to the lipoprotein which was present in the bathing solution throughout the studies.

For this reason, we have developed a system for perfusion of the artery, which mimics the in vivo situation much more closely. The aim of the present study was to investigate whether perfusion of the aorta with chylomicron remnants leads to effects on endothelial cell function which persist after the lipoprotein is removed from the solution bathing the artery wall. In order to obtain chylomicron remnants having physiological properties, it is necessary to prepare them in vivo, as they are normally rapidly taken up from the blood by the liver. Because of the technical difficulties involved in the collection of sufficient chyle and the number of animals required for this type of experiment, in terms of size and cost the rat is uniquely suitable for their preparation. Previous work has suggested that chylomicron remnants are taken up by the rat aorta in vivo to a similar extent as they are by the rabbit aorta in vivo [8,9], and as it is important to use tissues from homologous species in studies with lipoproteins, the rat was chosen as the animal model in the present study. Although it is well established that the rat is resistant to development of hypercholesterolaemia, atherosclerotic lesions have been shown to occur if circulating cholesterol concentrations are raised experimentally [25]. The experiments were carried out using native chylomicron remnants and those which had been oxidised by exposure to copper sulphate. Uptake was determined using 125I-labelled lipoproteins, and endothelial function was tested using vessel rings prepared after perfusion of the aortas in the presence or absence of chylomicron remnants.

2. Materials and methods

Male Wistar rats (300–350 g) were used throughout this study. They were housed under constant climatic conditions (temperature 21–22°C, relative air humidity 50 ± 5%, constant 12 h daylength) with free access to food and water.

2.1. Preparation of chylomicrons and chylomicron remnants

Chylomicrons and chylomicron remnants were prepared as previously described [26]. Rats were tube-fed with 1.5 ml corn oil, supplemented with α-tocopherol (4 mg/ml) as an antioxidant. After ≈ 1 h, anaesthesia was induced (sodium pentobarbitone; 60 mg/kg body weight i.p.), the thoracic duct was cannulated with polyethylene tubing (external diameter 1.52 mm) and the chyle was collected overnight into a tube containing 2 mg ampicillin as a preservative. Following collection, the chyle was layered under NaCl solution (d 1.006 g/ml) and centrifuged in a fixed-angle rotor for 6 × 10⁵ g/min at 12°C. The top fraction containing large chylomicrons (particle diameter > 100 nm) was harvested and ampicillin in 0.9% saline was added (final concentration 100 μg/ml). For the in vivo preparation of chylomicron remnants, rats were anaesthetised (sodium pentobarbitone; 60 mg/kg body weight i.p.) and a functional hepatectomy was carried out by ligation of all vessels supplying the liver and intestines. Chylomicrons containing 40 μmol triacylglycerol and 50 mg glucose were administered into the ileolumbar vein and after 45 min, blood was withdrawn from the bifurcation of the aorta and allowed to clot. The serum was obtained by centrifugation, and the chylomicron remnants were isolated by ultracentrifugation for 6 × 10⁷ g/min at 12°C. The top 1–1.5 ml was then layered under NaCl (d 1.006 g/ml) and centrifuged for 3 × 10⁷ g/min at 12°C. The top 1–1.5 ml from this centrifuga-
tion containing the purified chylomicron remnants was harvested and 100 mg/ml ampicillin was added. The preparations were then stored at 4°C until required. Free and total cholesterol and triacylglycerol concentrations of the chylomicrons and chylomicron remnants were determined using a commercially available kit (Boehringer Mannheim GmbH, Mannheim, Germany).

2.2. Iodination of chylomicron remnants

Chylomicron remnants were labelled using Na\(^{125}\)I (ICN Biomedicals, Aurora) and Iodogen (1.3,4,6-tetra-chloro-3\(a\),6\(\alpha\)-diphenylglycoluril) iodination reagent (Pierce, IL) as previously described [27]. A solution of Iodogen (100 \(\mu\)g/ml in chloroform) was slowly evaporated in a glass vial under a stream of nitrogen to leave a homogenous coat of reagent on the vessel wall. The chylomicron remnants were then placed in the vial with 0.5 mCi Na\(^{125}\)I and 1 M glycine buffer (10% of the lipoprotein volume), mixed rapidly for 5 min and then transferred to a Sephadex G50 column (Sigma, Poole, UK), acconditioned with phosphate buffered saline (PBS), to remove any unbound iodine. The fractions containing chylomicron remnants were collected, pooled and dialysed against 0.9% saline for 5 h. The proportion of \(^{125}\)I associated with protein was determined after precipitation with 10% tricarboxylic acid (TCA) and was found to be 89.9 ± 1.3% \((n = 4)\). The specific activity for labelled chylomicron remnants did not differ significantly between native (21.56 ± 3.14 cpm/ng protein, \(n = 4\)) and oxidised (18.27 ± 2.67 cpm/ng protein, \(n = 4\)) preparations.

2.3. Oxidative modification of chylomicron remnants

Chylomicron remnants were dialysed against 10 mM PBS, pH 7.4 for 5 h before incubation with a copper sulphate solution (final concentration, 10 \(\mu\)M) for 18 h at 37°C. At the end of the incubation period, oxidation was stopped by addition of ethylenediaminetetra-acetic acid (EDTA; final concentration, 2.6 mM). Lipoprotein oxidation was measured by the thiobarbituric acid reactive substances (TBARS) assay as previously described [20]. Tetraethoxypropane, which yields malondialdehyde (MDA) was used as a standard and results were expressed as nmol MDA/ml lipoprotein. The oxidised chylomicron remnants were then dialysed against 0.9% saline for 5 h to remove the copper sulphate.

2.4. Perfusion of the isolated rat aorta

The abdominal and thoracic cavities of a rat were entered under terminal general anaesthesia (sodium pentobarbitone; 60 mg/kg body weight i.p.) before the aorta was cannulated at the aortic arch and bifurcation with polyethylene tubing (external diameter 1.52 mm) and perfused with modified Krebs–Henseleit solution (KHS; composition (mM): NaCl 118, KCl 4.57, CaCl\(_2\) 1.27, KH\(_2\)PO\(_4\) 1.19, MgSO\(_4\) 1.19, NaHCO\(_3\) 25 and glucose 5.55). The mesenteric, coeliac and renal arteries were ligated and the vessel was dissected out of the carcass, washed with KHS and placed into a perfusion chamber. The re-circulating perfusion system consisted of a reservoir of 24 ml of the perfusate, gassed with 95% \(\text{O}_2\):5% \(\text{CO}_2\), a peristaltic pump (Harvard 1203), producing a flow rate of 2 ml/min, a bubble trap and pressure manometer. After the vessel had been perfused for 10 min with a perfusate consisting of 67% KHS and 33% bovine serum replacement, native or oxidised chylomicron remnants, containing 0.48 \(\mu\)mol cholesterol and 600 \(\mu\)g ampicillin in 6 ml 0.9% saline, were added to the perfusate and the vessel was allowed to perfuse for 2 h. This perfusion time was chosen as previous in vivo studies [6,8] have found that uptake of chylomicron remnants occurs within minutes and maximal uptake is achieved within 2 h. Furthermore, this period mimics the postprandial state as the half life of chylomicron remnants has been shown to be 14.1 ± 9.7 min [28].

2.5. Uptake of chylomicron remnants

Rat aortas were perfused as described above with iodinated native \((n = 4)\) or oxidised \((n = 4)\) chylomicron remnants. After 2 h the vessels were flushed with 10 ml KHS, cleared of all surrounding fat and connective tissue and cut into three 15 mm sections (representing cranial, mid and caudal aorta) which were then weighed and assayed for radioactivity in a gamma counter (Packard Cobra 5005). Non-specific binding of \(^{125}\)I was assessed by counting equivalent lengths of the polyethylene tubing and these values were subtracted from those of the perfused vessel sections.

2.6. Isolated vessel studies

When the aortas were to be subjected to pharmacological analysis, two vessels were perfused simultaneously, one with perfusate containing native or oxidised chylomicron remnants \((n = 6)\), as described above and the other (control perfusion, \(n = 6\)) with perfusate containing 600 \(\mu\)g ampicillin in 0.9% saline only. After 2 h the vessels were flushed with 10 ml KHS, cleared of any surrounding connective tissue and the thoracic portion cut into 3 mm ring segments. The rings were then suspended between two parallel stainless steel wires, taking great care to preserve the endothelial lining of the vessel, in organ bath chambers containing 10 ml KHS, gassed with 95% \(\text{O}_2\):5% \(\text{CO}_2\). One wire was fixed and the other was connected to an HSE 30 isometric force transducer. The signal from the force transducer was fed via an HSE bridge amplifier to
a Linseis 650 dual channel chart recorder. Three rings from each aorta were examined in the course of any one experiment. Vessel rings were held under 1 g resting tension and allowed to equilibrate for 1 h, during which time the KHS was changed every 5 min. Vessel segments were then contracted by exchanging the KHS for a depolarising KHS, where the NaCl had been replaced isotonically with KCl (118 mM). After 15 min, the depolarising KHS was replaced with KHS and resting tension re-established before the effects of the drugs were tested. Phenylephrine (PE; 3 μM) was then added to the bathing solution to cause supramaximal contraction of the vessel segments. When a stable increase in tone had been reached, cumulative concentration response curves were constructed to the relaxants carbachol (CCh; 10 nM to 0.1 mM) and S-nitroso-N-acetylpenicillamine (SNAP; 1 nM to 0.1 mM). In a further series of experiments, cumulative concentration relaxation response curves were constructed to PE (1 nM to 10 μM).

2.7. Materials

Carbamylcholine chloride (CCh) and L-phenylephrine hydrochloride (PE) were purchased from Sigma. S-nitroso-N-acetylpenicillamine (SNAP) was purchased from Research Biochemicals International (Natick, USA). All drugs were initially dissolved in deionised water (at 10 mM) and diluted in 0.9% w/v saline. All solutions were freshly prepared on the day of the experiment. Concentrations are expressed as the final concentration of each drug in the organ bath. Ethylenediaminetetra-acetic acid (EDTA; disodium salt) and trichloroacetic acid (TCA) were purchased from BDH Laboratory Supplies (Poole, UK) and 4-6-dihydroxyimidinate-2-thiol and 1,1,3,3-tetraethoxy-propane from Sigma. Bovine serum albumin fraction V powder, ampicillin and bovine serum replacement CPSR-3 were purchased from Sigma and corn oil and α-tocopherol from domestic suppliers.

2.8. Statistical analysis of data

Data from organ bath experiments were expressed either as increase in tension (g/mg tissue) or decrease in tension calculated as a percentage of the initial PE-induced tone, and were plotted against log agonist concentration. Cumulative concentration response curves for each vessel segment were fitted to the single site

Table 1
The uptake of native or oxidised chylomicron remnants by the perfused rat aorta

<table>
<thead>
<tr>
<th>Section</th>
<th>Native chylomicron remnants (ng protein/mg tissue)</th>
<th>Oxidised chylomicron remnants (ng protein/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 1</td>
<td>0.139 ± 0.056</td>
<td>0.276 ± 0.111</td>
</tr>
<tr>
<td>Section 2</td>
<td>0.130 ± 0.044</td>
<td>0.194 ± 0.087</td>
</tr>
<tr>
<td>Section 3</td>
<td>0.051 ± 0.021</td>
<td>0.173 ± 0.057</td>
</tr>
<tr>
<td>Whole vessel</td>
<td>0.111 ± 0.034</td>
<td>0.216 ± 0.082</td>
</tr>
</tbody>
</table>

The rat aorta was perfused for 2 h with 125I-labelled native or oxidised chylomicron remnants containing 0.48 μmol cholesterol and 600 μg ampicillin. Vessels were cut into three sections, each of 15 mm in length, taken from the cranial aorta (section 1), mid-aorta (section 2) and caudal aorta (section 3). Each value represents the mean ± S.E.M. of four animals.
response, A best fit values for EC50, the Hill slope of the concentration response curve. The EC50 3.2.

Uptake of chylomicron remnants by the perfused

3.1. Measurement of lipoprotein oxidation

Incubation with copper sulphate for a period of 18 h resulted in TBARS values for the oxidised chylomicron remnants of 16.03 ± 2.80 nmol MDA/ml, which were significantly higher (P < 0.05, n = 3) than those obtained using freshly prepared chylomicron remnants (1.63 ± 0.60 nmol MDA/ml). Iodination of the native chylomicron remnants resulted in TBARS values of 0.45 ± 0.10 nmol MDA/ml which did not differ significantly from the controls (0.34 ± 0.05 nmol MDA/ml).

3.2. Uptake of chylomicron remnants by the perfused rat aorta

After iodination of the chylomicron remnants, the extent of labelling did not differ significantly between native and oxidised preparations (native 21.56 ± 3.14, oxidised 18.27 ± 2.67 cpm/ng protein, n = 4). The incorporation of native or oxidised chylomicron remnants into the artery wall after 2 h of perfusion are shown in Table 1. The radioactivity associated with three sections of the vessel, each 15 mm in length, representing the cranial, mid and caudal aorta was determined. Label from both native and oxidised chylomicron remnants was taken up, with the cranial aortic section tending to show a higher amount of incorporation. Uptake tended to be greater when the vessels were perfused with oxidised chylomicron remnants, although these changes did not reach statistical significance.

3.3. Effects of chylomicron remnants on endothelial function in the perfused rat aorta

After perfusion of the rat aorta with or without (control) native or oxidised chylomicron remnants, the response of isolated vessel rings to PE in the presence and absence of the lipoprotein was tested, and the results are shown in Fig. 1. Rings from aortas perfused with native particles tended to have a higher maximal response to PE, when compared to those from control vessels perfused without lipoproteins, although this difference failed to reach statistical significance (Fig. 1a; Table 2). Rings derived from rat aortas perfused with oxidised chylomicron remnants, however, showed a significant increase (P < 0.05) in their maximum response to PE when compared to those from vessels perfused in parallel with ampicillin only (Fig. 1b). The EC50 and Hill slope values, in experiments with either native or oxidised remnant particles, were not significantly different from the respective control values (Table 2).

Cumulative concentration relaxation response curves to CCh of rings perfused with or without chylomicron remnants are shown in Fig. 2. Segments from vessels which had been perfused with native chylomicron remnants showed no significant change in the maximum vasorelaxant response to CCh in comparison to those from aortas perfused in the absence of lipoprotein (Fig. 2a), although there was a trend towards a reduction. After perfusion of the aortas with oxidised chylomicron remnants, however, the maximum response was signifi-

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**Table 2**

Best fit values for concentration contraction response curves to PE after perfusion of rat aorta with chylomicron remnants

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Perfusion</th>
<th>EC50 (× 10⁻⁷ M)</th>
<th>Max. tension (g/mg)</th>
<th>Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.01 ± 0.13</td>
<td>0.32 ± 0.05</td>
<td>1.29 ± 0.10</td>
</tr>
<tr>
<td>1</td>
<td>+ Native remnants</td>
<td>0.92 ± 0.21</td>
<td>0.37 ± 0.04</td>
<td>1.22 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>1.36 ± 0.28</td>
<td>0.34 ± 0.06</td>
<td>1.21 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>+ Oxidised remnants</td>
<td>1.24 ± 0.67</td>
<td>0.51 ± 0.04*</td>
<td>1.18 ± 0.05</td>
</tr>
</tbody>
</table>

The rat aorta was perfused for 2 h with native (Expt. 1) or oxidised (Expt. 2) chylomicron remnants containing 0.48 µmol cholesterol and 600 µg ampicillin. Control perfusions in which ampicillin (600 µg) only was added were carried out in parallel as described in Section 2. After 2 h, rings segments were prepared and concentration contraction response curves were constructed to PE in the absence of the lipoproteins. Each value represents the mean ± S.E.M. of six animals. Significance limits: * P < 0.05 vs. corresponding control value.

logistic equation: Increase in tension = \( E_{\text{max}} \cdot A^n / (A^n + E_{\text{C50}}) \) by a modified Marquart procedure using Multitfit® (Day Computing, Cambridge, England) running on a Macintosh 475. \( E_{\text{max}} \) represents the maximum response, \( A \) is the concentration of drug used and \( n \) is the Hill slope of the concentration response curve. The best fit values for EC50, \( E_{\text{max}} \) and Hill slope for each vessel segment were used to calculate the means ± S.E.M. All significance limits were calculated using an unpaired Student’s t-test.
significantly lower than that observed after the corresponding control perfusion (Fig. 2b). Hill slope and EC$_{50}$ values obtained from the curves were not significantly affected by perfusion with either native or oxidised chylomicron remnants when compared to their corresponding control values (Table 3). When the relaxant effects of SNAP on rings derived from vessels perfused with native or oxidised chylomicron remnants were compared to their respective controls, no significant changes in any of the dose response curve parameters were observed (Fig. 3; Table 3).

4. Discussion

It is well established that atherosclerosis and hypercholesterolaemia cause alteration of endothelium-dependent vasomotion [16]. Previous studies have suggested that the accumulation of cholesterol from LDL within the artery wall may be involved in these effects but no one has investigated whether chylomicron remnants cause modulation of endothelial cell function, although there is evidence to suggest that these particles can be taken up by the artery wall [6,8].

In the present study, perfusion of the rat aorta for 2 h resulted in small amounts of chylomicron remnants becoming associated with the arterial tissue. Chylomicron remnants have previously been shown to be taken up in vivo by the rabbit and rat aorta [6,9] and by atherosclerotic lesions of the rabbit aorta [8]. Furthermore, macrophages, fibroblasts and smooth muscle cells, all of which are present in the artery wall and involved in the atherogenic process, have been found to metabolise chylomicron remnants [11,29,30]. The amount of label associated with the perfused artery in the present study is of the same order of magnitude as that demonstrated in previous studies [6,9]. Furthermore, these studies showed that the chylomicron remnants are incorporated into the intima and media of the vessel wall, so it is assumed that the associated radioactivity in our vessels is indicative of uptake into the tissue. Although the amount of label taken up by the vessel after perfusion for 2 h with chylomicron remnants was small, this did cause a modulation of endothelial function which persisted after the lipoprotein was removed from the solution bathing the artery wall.

We have shown previously that incubation with both native [23] and oxidised [24] chylomicron remnants inhibits CCh-induced relaxation in segments from the freshly isolated rat aorta. Other studies have shown that oxidised LDL attenuates relaxations in response to CCh in rat aorta and acetylcholine in rabbit aorta [31,32]. As we have found that CCh-induced relaxations of rat aorta are endothelium-dependent and completely inhibited by the NO synthase inhibitor, $N$-nitro-L-arginine (data not shown), and similar results have been reported for acetylcholine-induced relaxations of rat aorta [33], it is likely that activation of the L-arginine-NO pathway mediates CCh-induced relaxation of the rat aorta. The present study demonstrates that, after perfusion of the rat aorta with oxidised but not native chylomicron remnants, relaxation of the vessels to CCh is slightly, but significantly attenuated while the response to SNAP is unaffected. These findings, therefore, suggest that the oxidised chylomicron remnants cause inhibition of some stage of the receptor-activated L-arginine-NO pathway of the arterial endothelial cells. Furthermore, the response of the artery to the NO donor, SNAP was not changed by the oxidised lipo-

Fig. 2. Concentration relaxation response curves to CCh in endothelium-intact rat aortic rings. The ring segments were taken from vessels which had been perfused for 2 h with (a) native chylomicron remnants or (b) oxidised chylomicron remnants containing 0.48 μmol cholesterol and 600 μg ampicillin (■) or with 600 μg ampicillin only (control. ○). Relaxation is expressed as a percentage of the contraction induced by 3 μM PE. Each point represents the mean value of six animals and error bars show the S.E.M. The best fit values derived from these concentration response curves are shown in Table 3.
protein, indicating that the effect is not on the vasodilator action of NO at the level of the vascular smooth muscle cell.

It is possible that the impaired CCh-induced relaxations observed after perfusion with oxidised chylomicron remnants may simply be due to the greater degree of tone produced by PE in the treated compared to the control preparations. However, the following lines of evidence argue against this suggestion. Firstly, relaxations to SNAP did not differ between rings from control vessels and those perfused with oxidised chylomicron remnants, even though the initial tone was greater in the treated vessel rings. Secondly, in a previous study we constructed concentration relaxation response curves to CCh after vessel tone had been raised with different concentrations of PE [34]. Vessel rings were contracted with a submaximal (0.3 μM) or supra-maximal (3 μM) dose of PE, but the maximum % relaxation of the control vessel segments was found not to differ (85.2 ± 5.7 and 88.7 ± 2.6, respectively). We conclude, therefore, that the observed inhibition was due to a direct effect of the chylomicron remnants on CCh-induced relaxation.

Perfusion of the arteries with oxidised, but not native, chylomicron remnants also caused significant potentiation of contraction to PE. In previous studies we have shown that incubation with both native [23] and oxidised [24] chylomicron remnants potentiates PE-induced contraction in the freshly isolated rat aorta. We demonstrated that this effect was endothelium-dependent, as contractions of endothelium-denuded vessels were not enhanced by the lipoproteins. Furthermore, we found that contractions to depolarising KHS were not significantly different between control vessels and those perfused with chylomicron remnants (data not shown), and that mechanical removal of the endothelium did not significantly alter the contractile response to depolarising KHS (data not shown), supporting the results of previous work [35]. Taken together, these findings suggest that the potentiation of PE-induced contraction by oxidised chylomicron remnants is endothelium-dependent. In other studies, oxidised LDL has been shown to potentiate contractions to PE in rat aorta and rabbit femoral artery [31,36]. The potentiation of PE-induced contraction in the rat aorta was inhibited by the NO synthase inhibitor, L-NG-nitroarginine [31], suggesting that oxidised LDL inhibits the basal release of NO. Evidence in support of this idea has come from findings that oxidised LDL causes a decrease in NO synthase mRNA levels [37] and that basal production of NO by rat aorta can modify the responses seen to PE [38]. In contrast, our previous work has shown that the potentiation of PE-induced contraction, found when freshly isolated rat aorta segments were incubated with native chylomicron remnants, was increased rather than inhibited, by the addition of the NO inhibitor Nω-nitro-L-arginine [23], indicating that chylomicron remnants may not cause their effect by inhibition of the basal release of NO but by another mechanism. It is possible that oxidised remnant particles stimulate the release of an endothelium-dependent contractile factor. Such a factor, endothelin, has been shown to be produced in response to oxidised LDL in porcine vessels [39] and was found in increased concentrations in atherosclerotic human arteries [40]. Furthermore, superoxide and a cyclo-oxygenase product, both of which have also been proposed as endothelium-dependent contractile factors, have been found in increased concentrations in hypercholesterolaemic rabbit vessels [41,42]. The mechanism by which chylomicron remnants cause the potentiation of PE-induced contraction is the subject of an ongoing study.

Overall our results suggest that oxidised chylomicron remnants tend to become associated with the artery wall of the perfused rat aorta more readily than native chylomicron remnants, and have a more marked effect on tone.

Table 3

Best fit values for concentration relaxation response curves to CCh and SNAP after perfusion of rat aorta with chylomicron remnants

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Agonist</th>
<th>Perfusion</th>
<th>PE contraction (g/mg)</th>
<th>EC50 (× 10^-7 M)</th>
<th>% Max. relaxation</th>
<th>Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CCh</td>
<td>Control</td>
<td>0.27 ± 0.04</td>
<td>5.37 ± 1.24</td>
<td>88.7 ± 2.6</td>
<td>1.45 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>CCh</td>
<td>+ Nat. rem.</td>
<td>0.27 ± 0.04</td>
<td>5.54 ± 1.22</td>
<td>80.8 ± 5.7</td>
<td>1.14 ± 0.04</td>
</tr>
<tr>
<td>1</td>
<td>CCh</td>
<td>Control</td>
<td>0.27 ± 0.05</td>
<td>6.57 ± 2.86</td>
<td>91.6 ± 2.4</td>
<td>1.29 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>CCh</td>
<td>+ Oxy. Rem.</td>
<td>0.35 ± 0.07</td>
<td>6.26 ± 0.83</td>
<td>71.5 ± 7.2*</td>
<td>1.20 ± 0.08</td>
</tr>
<tr>
<td>1</td>
<td>SNAP</td>
<td>Control</td>
<td>0.23 ± 0.03</td>
<td>2.04 ± 0.56</td>
<td>99.8 ± 0.6</td>
<td>1.13 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>SNAP</td>
<td>+ Nat. rem.</td>
<td>0.28 ± 0.05</td>
<td>3.06 ± 0.94</td>
<td>100.9 ± 0.9</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>1</td>
<td>SNAP</td>
<td>Control</td>
<td>0.29 ± 0.06</td>
<td>4.62 ± 2.12</td>
<td>101.3 ± 2.0</td>
<td>1.42 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>SNAP</td>
<td>+ Oxy. Rem.</td>
<td>0.36 ± 0.06</td>
<td>6.09 ± 3.13</td>
<td>101.2 ± 1.8</td>
<td>1.16 ± 0.07</td>
</tr>
</tbody>
</table>

The rat aorta was perfused for 2 h with native (Nat. rem.; Expt. 1) or oxidised (Ox. rem.; Expt. 2) chylomicron remnants containing 0.48 μmol cholesterol and 600 μg ampicillin. Control perfusions in which ampicillin (600 μg) only was added were carried out in parallel as described in Section 2. After 2 h, ring segments were prepared and contracted with 3 μM PE before concentration relaxation response curves were constructed to CCh and SNAP.

Each value represents the mean ± S.E.M. of six animals. Significance limits: * P<0.05 vs. corresponding control value.
on endothelial cell function. Similarly, oxidative modification of LDL, which is known to be important in its atherogenicity, is believed to be necessary for its effects in inhibiting endothelium-dependent relaxation [21,32]. Our results, however, indicate that oxidised LDL and oxidised chylomicron remnants may exert their influence on endothelial function by different mechanisms, and this is under further investigation in our laboratories.

In conclusion, we have found that small amounts of both native and oxidised chylomicron remnants become associated with the perfused rat aorta. After perfusion with oxidised chylomicron remnants, the maximum response of the vessels to PE was significantly increased and maximum % relaxation to CCh was significantly decreased, while SNAP induced relaxations were unaffected. Native chylomicron remnants showed a tendency to induce similar effects although these did not reach statistical significance. These results suggest that oxidised chylomicron remnants influence vascular endothelial function by interfering with the L-arginine-NO pathway. The potentiation of contraction to PE caused by the oxidised remnant particles may not be due to inhibition of the basal release of NO, but, alternatively, to the release of endothelium-dependent contractile factors. The effects were observed in the absence of lipoprotein in the solution bathing the artery wall, even though only small amounts of lipoprotein became associated with the vessels during the perfusion period. This demonstrates that oxidised chylomicron remnants not only modulate endothelium-dependent relaxation and contraction when they are present in the circulation but also that these effects persist after the lipoprotein is removed from the system. These results indicate that lipoproteins of dietary origin may have a role in the modulation of endothelial cell function which occurs in the pathogenesis of atherosclerosis, and demonstrate that the perfused rat aorta provides a useful model to study the potential atherogenic mechanisms of chylomicron remnants.

Fig. 3. Concentration relaxation response curves to SNAP in endothelium-intact rat aortic rings. The ring segments were taken from vessels which had been perfused for 2 h with (a) native chylomicron remnants or (b) oxidised chylomicron remnants containing 0.48 μmol cholesterol and 600 μg ampicillin or with 600 μg ampicillin only (control, □). Relaxation is expressed as a percentage of the contraction induced by 3 μM PE. Each point represents the mean value of six animals and error bars show the S.E.M. The best fit values derived from these concentration response curves are shown in Table 3.

Acknowledgements

We thank the Medical Research Council for their financial support.

References

[6] Mamo JCL, Wheeler JR. Chylomicrons or their remnants penetrate rabbit thoracic aorta as efficiently as do smaller macromolecules, including low-density lipoprotein, high-density lipoprotein, and albumin. Coron Artery Dis 1994;5:695–705.


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