Effect of broad-spectrum matrix metalloproteinase inhibition on atherosclerotic plaque stability

Jason L. Johnsona, Regina Fritsche-Danielsonb, Margareta Behrendtb, Annika Westin-Erikssonb, Håkan Wennbo b, Margareta Herslofb, Marie Elebringb, Sarah J. Georgea, William L. McPheatb, Christopher L. Jacksona,*

a Bristol Heart Institute, University of Bristol, Level 7, Bristol Royal Infirmary, Bristol BS2 8HW, United Kingdom
b AstraZeneca R&D, Mölndal, Sweden

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Abstract

Objectives: Matrix metalloproteinases (MMPs) form a large family of enzymes that collectively can degrade all components of the extracellular matrix, and there is widespread interest in developing MMP inhibitors for the prevention of atherosclerotic plaque rupture. We have therefore investigated the effects of a broad-spectrum MMP inhibitor, RS-130830, on plaque development and stability. This compound inhibits a wide range of MMPs at concentrations below 20 nmol/L.

Methods: Apolipoprotein E knockout mice were fed a Western diet. Dietary administration of RS-130830 commenced at the same time as fat-feeding and continued for 8, 12, 26 or 36 weeks. To investigate the effect of RS-130830 on established plaques, mice were fed high-fat diet for 16 weeks before initiation of drug treatment and were terminated 20 weeks after this.

Results: Broad-spectrum MMP inhibition was associated with a significant increase in plaque area, but there was no change in the incidence of plaque rupture. There were unfavourable changes in phenotypic characteristics associated with plaque instability, such as an increased lipid content and decreased collagen content.

Conclusions: These data suggest that broad-spectrum MMP inhibition RS-130830 does not have a beneficial effect on atherosclerosis in the apolipoprotein E knockout mouse model, and indicate that more selective compounds would be preferable.

Keywords: Plaque rupture; Atherosclerosis; Matrix metalloproteinases; Animal models

1. Introduction

Extracellular matrix degradation is tightly regulated within the normal vessel wall through a balance between proteinases and their endogenous inhibitors. However, within the atherosclerotic plaque the balance may become shifted towards matrix degradation, particularly at the rupture-prone shoulder regions of the fibrous cap where accumulating macrophages and phenotypically altered smooth muscle cells secrete a plethora of proteinases, including matrix metalloproteinases (MMPs) [1–5]. It has been proposed that these enzymes contribute to plaque rupture and thus, hypothetically, that inhibition of MMPs within the atherosclerotic plaque may prevent disruption and its clinical sequelae.

Numerous studies have attempted to dissect the roles of MMPs in atherosclerotic plaque development and instability, with the aid of synthetic MMP inhibitors. However results have been contradictory. In a recent study doxycycline, a widely used antibiotic with non-selective MMP inhibitory properties [6] was given to fat-fed LDL receptor knockout mice [7]. After 28 days of treatment, no significant effect was observed on aortic atherosclerosis in either angiotensin II-augmented or saline-infused mice. A similar study reported that administration of a hydroxamic acid-based broad-spectrum MMP inhibitor had no beneficial
effect on atherosclerotic plaque development in the aortas of high-fat-fed LDL receptor knockout mice [8]. However, in two independent prospective, double-blind, randomised, placebo-controlled clinical trials in patients with symptomatic coronary [9] and carotid [10] artery disease, treatment with doxycycline appeared to exert encouragingly beneficial effects. Doxycycline treatment reduced the activity of MMP-9 [9] and the expression of MMP-1 [10] in endarterectomy samples. Additionally, decrease in the plasma levels of the inflammatory markers C-reactive protein and interleukin-6 were observed [9]. Collectively, these findings indicate that doxycycline may retard proteolysis and inflammation in atherosclerotic lesions and thus result in their stabilisation. However, in these two preliminary studies, no effect on morphological characteristics or clinical end-points was observed.

We have previously reported that the effects on atherosclerosis of knocking out various MMPs in apolipoprotein E (apoE) knockout mice are quite divergent [11]. This suggests that some MMPs support lesion growth and destabilisation, whereas others suppress lesion growth and promote lesion stability. The effects of treatment with a broad-spectrum MMP inhibitor are thus likely to be dependent on its degree of inhibition of specific MMPs, as well as the stage of lesion progression when treatment is initiated. The present study therefore aims to test these hypotheses by determining the effect of a broad-spectrum hydroxamic acid-based MMP inhibitor, RS-130830, on brachiocephalic artery atherosclerotic lesion development and stability in fat-fed apoE knockout mice.

2. Methods

2.1. Animals

ApoE homozygous knockout mice were kindly provided by Dr. J. Breslow (Rockefeller University, New York, New York). The strain background of the animals was 71% C57BL/6, 29% 129, as determined by fingerprinting of tail-tip DNA. The housing and care of the animals and all the procedures used in these studies were performed in accordance with the guidelines and regulations of the University of Bristol and the United Kingdom Home Office. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Matrix metalloproteinase inhibitor

RS-130830 is an orally active hydroxamic acid MMP inhibitor with potent activity against a variety of human MMPs, including the collagenases MMP-8 (IC50 = 0.9 nmol/L) and MMP-13 (IC50 = 0.5 nmol/L); the gelatinases MMP-2 (IC50 = 0.3 nmol/L) and MMP-9 (IC50 = 0.4 nmol/L); the membrane-type metalloproteinase MMP-14 (IC50 = 15 nmol/L); the stromelysin MMP-3 (IC50 = 9.5 nmol/L); and the metalloelastase MMP-12 (IC50 = 0.7 nmol/L). It is inactive against human MMPs-1 and -7.

RS-130830 is also active at nanomolar levels against murine MMPs, with an IC50 of 0.1 nmol/L against murine MMP-9 and 3.5 nmol/L against murine MMP-12.

2.3. Pharmacokinetic analysis

Pharmacokinetic analysis was performed to assess whether adequate plasma levels of drug were achieved, and also to assess the stability and absorption of the compound when mixed with high-fat diet. RS-130830, in the form of a finely divided powder, was admixed with the diet to a final concentration of 240 mg/kg of diet, calculated to produce a final dose of 40 mg/kg of bodyweight/day. Ten male and ten female apoE knockout mice received this diet for 7 days, and then two males and two females were terminated at 08:00, 14:00, 20:00, 00:00, and 04:00 h. At termination, a heparinised blood sample was taken by
cardiac puncture, and plasma levels of RS-130830 were determined by high-performance liquid chromatography.

2.4. Experimental design

ApoE knockout mice, aged 8 weeks at the start of the study, were fed a high-fat diet containing RS-130830 at a concentration of 240 mg/kg of diet. Control mice received high-fat diet only. Every 2 weeks, three randomly selected groups of four mice were weighed and the concentration of RS-130830 in the diet adjusted as necessary to maintain a dose of 40 mg/kg of bodyweight/day. Mice were terminated after 8, 12, 26 or 36 weeks of feeding. Male mice were used for the 8 week study, and female mice for the longer time periods. To investigate the effect of RS-130830 on established plaques, female mice were fed high-fat diet for 16 weeks before initiation of RS-130830 administration and were terminated 20 weeks after this. The experimental design is summarised in Fig. 1.

2.5. Plasma lipid profile

Heparinised plasma samples were collected at termination and plasma lipid profiles were determined as described previously [12].

2.6. Termination

Animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone before exsanguination by arterial perfusion via the abdominal aorta with phosphate-buffered saline (PBS) at a constant pressure of 100 mm Hg, with outflow through the incised jugular veins. This was followed by constant pressure perfusion with zinc–HCl fixative (0.05% (w/v) calcium acetate, 0.5% (w/v) zinc acetate, 0.5% (w/v) zinc chloride, 0.1 mol/L Tris–HCl, pH 7.4). The brachiocephalic artery was removed from each animal.

2.7. Histochemistry

Serial 3 μm paraffin sections of the proximal brachiocephalic artery were histochemically stained with elastin van Gieson or picrosirius red.

2.8. Morphometric analysis

All measurements were made on elastin-stained sections, and one vessel cross-section was quantified per mouse. Analysis was performed using a computerised image analysis system (Image Pro Plus, Media Cybernetics, Carlsbad, USA). The lengths of the internal and external elastic laminae were recorded. These were used to derive the media area, by assuming them to be the circumferences of perfect circles. The plaque area was measured directly, and was subtracted from the area enclosed by the internal elastic lamina to derive the patent lumen area.

2.9. Plaque lipid content

The lipid content of plaques was determined as described previously [12].

2.10. Morphological analysis

Sections stained for elastin were inspected for the presence or absence of acute plaque rupture, which was defined as a

<table>
<thead>
<tr>
<th>Study duration (weeks)</th>
<th>Mouse gender</th>
<th>Treatment group</th>
<th>Acute plaque ruptures</th>
<th>Buried fibrous caps (per mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Male</td>
<td>Control (22)</td>
<td>3 out of 22 mice (14%)</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS-130830 (37)</td>
<td>11 out of 37 mice (30%)</td>
<td>0.59 ± 0.11</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>Control (23)</td>
<td>2 out of 23 mice (10%)</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS-130830 (21)</td>
<td>2 out of 21 mice (10%)</td>
<td>0.39 ± 0.12</td>
</tr>
<tr>
<td>26</td>
<td>Female</td>
<td>Control (19)</td>
<td>0 out of 19 mice (0%)</td>
<td>1.58 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS-130830 (28)</td>
<td>6 out of 28 mice (21%)</td>
<td>1.21 ± 0.15</td>
</tr>
<tr>
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<td>Female</td>
<td>Control (21)</td>
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<td>1.54 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS-130830 (21)</td>
<td>1 out of 21 mice (5%)</td>
<td>1.32 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS-130830 (Delayed)</td>
<td>1 out of 12 mice (8%)</td>
<td>1.92 ± 0.34</td>
</tr>
</tbody>
</table>

There were no significant differences between treated groups and controls.

Table 1

Effect of MMP inhibitor treatment on plasma lipid concentrations

<table>
<thead>
<tr>
<th>Study duration (weeks)</th>
<th>Mouse gender</th>
<th>Treatment group</th>
<th>Total cholesterol (mmol/L)</th>
<th>LDL-cholesterol (mmol/L)</th>
<th>VLDL-cholesterol (mmol/L)</th>
<th>HDL-cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
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<td>8</td>
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<td>Control (22)</td>
<td>36.6 ± 2.4</td>
<td>8.7 ± 0.8</td>
<td>24.3 ± 1.2</td>
<td>0.9 ± 0.1</td>
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<tr>
<td></td>
<td></td>
<td>RS-130830 (37)</td>
<td>34.6 ± 2.1</td>
<td>9.9 ± 0.9</td>
<td>31.8 ± 3.8</td>
<td>0.9 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>12</td>
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<td>Control (23)</td>
<td>25.6 ± 1.7</td>
<td>7.8 ± 0.5</td>
<td>18.5 ± 1.6</td>
<td>0.8 ± 0.1</td>
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<tr>
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<td>22.4 ± 1.4</td>
<td>9.2 ± 0.9</td>
<td>16.6 ± 1.0</td>
<td>0.9 ± 0.1</td>
<td>2.2 ± 0.4</td>
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</table>

* p < 0.05 vs. Control.

Table 2

Effect of MMP inhibitor treatment on plaque stability

<table>
<thead>
<tr>
<th>Study duration (weeks)</th>
<th>Mouse gender</th>
<th>Treatment group</th>
<th>Acute plaque ruptures</th>
<th>Buried fibrous caps (per mouse)</th>
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<tr>
<td></td>
<td></td>
<td>RS-130830 (Delayed)</td>
<td>1 out of 12 mice (8%)</td>
<td>1.92 ± 0.34</td>
</tr>
</tbody>
</table>
disruption of the fibrous cap accompanied by intrusion of blood products into the core. The incidence of buried fibrous caps was also noted, structures invested with collagen and elastin and usually overlain with foam cells.

2.11. Immunohistochemistry

Serial 3 μm paraffin sections were dewaxed and rehydrated. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide. After blocking sections with 20% (v/v) goat serum in PBS, sections were incubated overnight at 4 °C with either a purified rat monoclonal antibody against mouse macrophages (Mac2) (BD Biosciences, Oxford, UK) at 3.12 μg/mL or mouse monoclonal antibody against α-smooth muscle actin (Sigma, Poole, UK) at 82 μg/mL in 1% (w/v) bovine serum albumin (BSA) in PBS. Sections were then incubated with the appropriate biotinylated secondary antibodies (Dako, High Wycombe, UK) diluted 1:200 in 1% (w/v) BSA in PBS, and then horseradish peroxidase-labelled Extravidin™ (diluted 1:400 in 1% (w/v) BSA in PBS). Colour was developed with 0.05% (diluted 1:400 in 1% (w/v) BSA in PBS). A negative control, where the primary antibody was replaced with either mouse or rat IgG at the same dilution, was always included.

2.12. Measurement of apoptosis

Apoptotic cells were detected in plaques by in situ end-labelled (ISEL). Deparaffinised and rehydrated 3 μm slide-mounted sections of brachiocephalic artery were incubated at room temperature with proteinase K (5 μg/mL) for 15 min. After washing, the slides were incubated for 15 min at room temperature in a reaction mixture containing dATP, dCTP, dGTP, biotin-1,6-dUTP (all 0.01 mmol/L), DNA polymerase I (Klenow) large fragment (8 U/mL), and dCTP, dGTP, biotin-1,6-dUTP (all 0.01 mmol/L), DNA polymerase I (Klenow) large fragment (8 U/mL), 1 × Klenow reaction buffer (Promega, Southampton, UK). Endogenous peroxidase activity was inhibited in 3% hydrogen peroxide, and the sections were incubated for 30 min at room temperature with Extravidin™/horse radish peroxidase. The sections were then developed in 3,3′-diaminobenzidine (0.5%) and hydrogen peroxide (0.03%). The number of positive plaque cells was expressed as a percentage of the total plaque cell count.

2.13. Statistical analysis

Values are expressed as mean ± standard error of the mean (S.E.M.). Treatment group values were compared as statistically different by ANOVA followed by Tukey’s post hoc test (GraphPadPrism, San Diego, CA). Significant differences were indicated at *p < 0.05 unless otherwise stated.

Table 3

<table>
<thead>
<tr>
<th>Study duration (weeks)</th>
<th>Mouse gender</th>
<th>Treatment group (n)</th>
<th>Plaque area (×10^6 μm^2)</th>
<th>Media area (×10^4 μm^2)</th>
<th>Lumen area (×10^4 μm^2)</th>
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<td>Control (22)</td>
<td>42±3</td>
<td>68±3</td>
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<td>78±2*</td>
<td>135±5</td>
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<td>62±11</td>
<td>79±4</td>
<td>140±8</td>
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<td>81±13</td>
<td>85±2</td>
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<td>Control (19)</td>
<td>133±14</td>
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<td>129±11</td>
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<td>145±11</td>
<td>70±3</td>
<td>125±9</td>
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<td>36</td>
<td>Female</td>
<td>Control (20)</td>
<td>135±11</td>
<td>64±3</td>
<td>134±8</td>
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<td>RS-130830 (21)</td>
<td>179±13*</td>
<td>64±3</td>
<td>125±9</td>
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<td>Control (19)</td>
<td>178±12</td>
<td>69±3</td>
<td>123±11</td>
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<td>RS-130830 (Delayed) (12)</td>
<td>169±19</td>
<td>77±7</td>
<td>133±10</td>
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</table>

* p < 0.05 vs. Control.

Table 4

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<tr>
<th>Study duration (weeks)</th>
<th>Mouse gender</th>
<th>Treatment group (n)</th>
<th>Collagen (%)</th>
<th>Lipid (%)</th>
<th>Apoptosis (%)</th>
<th>Smooth muscle (%)</th>
<th>Macrophages (%)</th>
<th>Macrophage/smooth muscle ratio</th>
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<td>Male</td>
<td>Control (22)</td>
<td>24.1±3.3</td>
<td>21.6±3.4</td>
<td>3.1±1.2</td>
<td>13.0±1.9</td>
<td>34.4±4.2</td>
<td>2.5±0.4</td>
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<td>19.1±2.3</td>
<td>17.0±2.3</td>
<td>4.5±1.8</td>
<td>11.0±1.6</td>
<td>35.9±2.5</td>
<td>8.3±1.9</td>
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<td>Control (23)</td>
<td>16.9±3.6</td>
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<td>28.5±4.5</td>
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<td>6.8±1.9</td>
<td>7.1±1.6</td>
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<td>34.0±3.8</td>
<td>38.0±3.6*</td>
<td>ND</td>
<td>3.9±1.1</td>
<td>3.9±1.1</td>
<td>1.2±0.3</td>
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<td>20.6±2.9</td>
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<td>3.9±0.9</td>
<td>4.7±1.1</td>
<td>1.7±0.9</td>
</tr>
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<td>Control (20)</td>
<td>31.2±3.1</td>
<td>26.9±2.4</td>
<td>ND</td>
<td>1.4±0.3*</td>
<td>4.1±1.3</td>
<td>4.8±1.6</td>
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<td>RS-130830 (21)</td>
<td>31.8±3.1</td>
<td>27.8±2.6</td>
<td>ND</td>
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<td>2.9±1.4</td>
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<td>Control (19)</td>
<td>36.5±2.4</td>
<td>31.8±2.0</td>
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<td>0.5±0.1*</td>
<td>1.4±0.5</td>
<td>7.3±3.2</td>
</tr>
<tr>
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<td>RS-130830 (Delayed) (12)</td>
<td>36.5±2.4</td>
<td>31.8±2.0</td>
<td>ND</td>
<td>0.5±0.1*</td>
<td>1.4±0.5</td>
<td>7.3±3.2</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. Control.
Fig. 2. Haematoxylin and eosin staining of representative control (Panels A, C, E, G and I) and RS-130830-treated (Panels B, D, F, H and J) apolipoprotein E knockout mouse brachiocephalic atherosclerotic plaques after receiving high-fat diet for 8 weeks (Panels A and B), 12 weeks (Panels C and D), 26 weeks (Panels E and F) or 36 weeks (Panels G, H, I and J). Treatment with RS-130830 commenced either at the same time as high-fat feeding (Panels B, D, F and H), or after 16 weeks of high-fat feeding (Panel J). The scale bar in Panel A represents 200 μm and is applicable to all panels.
Fig. 3. Immunostaining for α-smooth muscle actin in representative control (Panels A, C, E, G and I) and RS-130830-treated (Panels B, D, F, H and J) apolipoprotein E knockout mouse brachiocephalic atherosclerotic plaques after receiving high-fat diet for 8 weeks (Panels A and B), 12 weeks (Panels C and D), 26 weeks (Panels E and F) or 36 weeks (Panels G, H, I and J). Treatment with RS-130830 commenced either at the same time as high-fat feeding (Panels B, D, F and H), or after 16 weeks of high-fat feeding (Panel J). The scale bar in Panel A represents 200 μm and is applicable to all panels.
with their controls using the computer programs InStat and Prism (both GraphPad Software, San Diego, California, USA). Only animals surviving to scheduled termination were included in the main analyses; animals that died prematurely were used only for survival analysis, by the Kaplan–Meier method. For the comparison of group means, a check was first made for similar variances; if this was passed then an unpaired two sample two-tailed Student’s \( t \)-test was carried out, or one-way analysis of variance if there were more than two groups. If the variances were significantly different, then an unpaired two sample two-tailed \( t \)-test with Welch’s correction was used for two groups. Contingency data (presence of acute plaque rupture) were analysed by Fisher’s Exact test. Discontinuous data (incidence of buried fibrous caps) were analysed using the Mann–Whitney test. In all cases, statistical significance was concluded where \( p < 0.05 \).

### 3. Results

#### 3.1. RS-130830 plasma analysis

The concentration of RS-130830 in the plasma of male and female apoE knockout mice fed a high-fat diet for 1 week, containing RS-130830 at a concentration of 240 mg/kg of diet, averaged 601 ± 99 nmol/L across the time course. The lowest concentration (observed at 08:00 h) was 290 ± 55 nmol/L in plasma; the peak concentration was observed at 00:00 h and was 883 ± 288 nmol/L. Therefore, this feeding regime and drug administration protocol were adopted for all studies.

In male animals treated for 8 weeks the terminal plasma concentration of RS-130830 was 311 ± 45 nmol/L. In female mice treated for 12 weeks the terminal plasma concentration was 499 ± 50 nmol/L and at 26 weeks was 417 ± 51 nmol/L. Treated animals with plasma levels below 50 nmol/L were excluded from the analysis; there were six such animals in total.

#### 3.2. Effect of RS-130830 on plasma lipids

RS-130830 treatment for 8 or 12 weeks had no effect on total plasma cholesterol concentration when compared to controls. There was also no effect on LDL-cholesterol, VLDL-cholesterol, or HDL-cholesterol concentration. Treatment of male mice with RS-130830 for 8 weeks caused an 89% increase in plasma triglyceride concentration (\( p < 0.05 \)), but there was no corresponding effect in female mice treated for 12 weeks. These data are summarised in Table 1.

#### 3.3. Effect of RS-130830 on plaque stability

MMP inhibitor treatment had no effect on the incidence of acute plaque rupture in any of the treated animals compared to their control groups in these studies. There was also no effect on the incidence of buried fibrous caps at any time-point. These data are summarised in Table 2.

#### 3.4. Effect of RS-130830 on vessel morphometry

These data are summarised in Table 3. Plaque cross-sectional area was 76% larger in RS-130830-treated animals at 8 weeks (\( p = 0.004 \)) and 33% larger at 36 weeks (\( p < 0.05 \)). A difference in lumen area was only observed after 12 weeks of RS-130830 treatment (39% increase, \( p < 0.01 \), and a change in medial area was only seen when RS-130830 was given for 8 weeks (15% increase, \( p = 0.015 \)). None of the morphometric parameters differed between animals receiving delayed RS-130830 treatment and untreated controls (Table 3).

![Fig. 4. Survival curves for apolipoprotein E knockout mice fed a high-fat diet for up to 36 weeks, and treated with RS-130830 in the diet (40 mg/kg of bodyweight/day). Panel A: Animals treated with RS-130830 throughout the study, compared with untreated controls. Panel B: Animals fed high-fat diet for 16 weeks before receiving RS-130830, compared with untreated controls. There was no significant change in survival in either study.](https://academic.oup.com/cardiovascres/article-abstract/71/3/586/339177)
3.5. Effect of RS-130830 on plaque composition

These data are summarised in Table 4, and representative sections are shown in Figs. 2 and 3. Mean lesion smooth muscle cell content was reduced by 57% in RS-130830 treated animals at 12 weeks (p=0.002). No differences in macrophage content were observed between groups at any time point. No change in fibrillar collagen content was seen at any time point. The plaque lipid content of animals receiving RS-130830 was increased by 81% at 12 weeks (p=0.004), and increased by 41% at 16 weeks (p<0.05).

3.6. Effect of RS-130830 on plaque cell apoptosis

As shown in Table 4, RS-130830 treatment did not cause any significant change in the number of ISEL-positive cells in the plaques of male apoE knockout mice fed high-fat diet for 8 weeks.

3.7. Effect of RS-130830 on mortality

Treatment with RS-130830 had no effect on the incidence of sudden death. The survival curves are shown in Fig. 4.

4. Discussion

These studies show that administration of a broad-spectrum MMP inhibitor, RS-130830, to apoE knockout mice has no beneficial effect on either brachiocephalic atherosclerotic plaque development or stability. On the contrary, treated animals had larger plaques which, at early time points, had a more unstable phenotype. When treatment was administered to mice with existing plaques there were no effects on plaque size or phenotype. Plasma analysis confirmed that levels of RS-130830 well in excess of 50 nmol/L were routinely achieved, and thus were in the concentration range where a broad range of MMPs is inhibited by direct coordination with the catalytic site [13,14]. At the chosen dosage level, the minimum plasma concentration was approximately 20 times greater than the IC50 value for RS-130830 against MMP-14, for which it has the weakest inhibitory activity amongst the MMPs tested.

There was no effect of RS-130830 treatment on plasma total cholesterol or VLDL-, LDL- or HDL-cholesterol. However, in male mice treated for 8 weeks there was a significant 89% increase in plasma triglycerides (Table 1). Female mice treated for 12 weeks showed no such change. Interestingly, male apoE/MMP-12 double knockout mice also show an increase in plasma triglyceride concentration [11], raising the possibility that MMPs are involved in triglyceride metabolism. Indeed, the conversion of pre-adipocytes to adipocytes appears to be dependent on proteinases including MMPs [15], and MMP inhibitors reduce triglyceride accumulation in these cells. However, plaque development and severity were reduced in apoE/ MMP-12 double knockout mice [11] whereas in the current study these were increased, suggesting that the effects of RS-130830 on plaques were not the indirect consequence of altered plasma triglyceride levels.

It has been postulated that MMP activity within lesions is detrimental and may trigger plaque disruption [2,3,16]. However, recent studies using apoE knockout mice in which MMP-3, -7, -9 or -12 has also been deleted have failed to support this idea, though it must be stressed that the data have been rather contradictory. For instance, deletion of MMP-3 had no effect on aortic atherosclerosis [17] but increased lesion size in the brachiocephalic artery [11]. Deletion of MMP-9 reduced the aortic atherosclerotic burden [18] but increased the size and instability of lesions in the brachiocephalic artery [11]. Deletion of MMP-12 had no beneficial effect on aortic lesion size [17,18] but decreased lesion size in the brachiocephalic artery [11]. To further cloud the issue of the involvement of MMPs in plaque development and disruption, studies investigating TIMP-1 participation have reported that either deletion or over-expression reduces atherosclerosis in apoE knockout mice [19,20]. Taken together, these data suggest that the effects a broad-spectrum MMP inhibitor may have on atherosclerosis in apoE knockout mice are not predictable from an inspection of its inhibitory profile.

In fact, after 8 or 36 weeks treatment with RS-130830, lesions were significantly larger than those in control mice. Inhibition of MMPs would be expected to result in decreased matrix degradation, thus increasing the deposition and content of matrix proteins within the atherosclerotic plaque and increasing its bulk. However, no significant difference in the lesion fractional content of collagen was observed between RS-130830-treated and control animals, so what could account for the increased lesion size in treated mice? There was no significant change in the ratio of macrophages to smooth muscle cells in atherosclerotic plaques from RS-130830-treated animals. It has been demonstrated that monocytes express a small number of proteases and that MMPs are not required for their migration into tissues, consistent with these data [21]. There were, however, increases in the plaque lipid content in animals treated for 12 or 26 weeks, and this may explain the difference in lesion sizes between treated and control animals. No difference was observed in the number of apoptotic cells in lesions between RS-130830-treated and control animals and the rates of apoptosis were low (Table 4), suggesting that cell necrosis may be a more important cause of cell death, resulting in extracellular lipid accumulation.

There was a reduction in the smooth muscle cell content of plaques from treated animals at several time points, which is similar to findings in a pulmonary artery organ culture model where broad-spectrum MMP inhibi-
tion suppressed smooth muscle cell proliferation and increased the rate of apoptosis [22]. Proteolysis of matrix proteins can release growth factors (e.g. TGF-β and FGF-2) in an active form, potentially promoting smooth muscle cell growth [23–26]. An MMP inhibitor might block these processes and thus inhibit smooth muscle cell proliferation.

Numerous studies employing endogenous [19,27,28] or synthetic [29,30] MMP inhibitors, or gene deletion approaches [20,31–34], have shown that inhibition of MMP activity restricts smooth muscle cell migration. Thus an MMP inhibitor with good activity against MMP-9, such as RS-130830, could retard the migration of medial smooth muscle cells into the atherosclerotic plaque. If this is true, it implies that smooth muscle cell migration has little impact on atherosclerotic plaque growth or destabilisation. In conditions of mechanical injury, smooth muscle cell migration appears to be important for initiation of lesion formation in vessels that do not normally have smooth muscle cells present in the intima, but any effect of inhibiting migration is overwhelmed by later processes [29]. On the basis of the lack of effect of RS-130830 in the current studies, this may also be true of atherogenesis and early lesion development in the mouse.

Despite reductions in the smooth muscle cell content and increases in the lipid content of plaques from RS-130830-treated animals, there was no increase in the number of acute plaque ruptures or the number of buried fibrous caps. The significant changes in the various markers of plaque instability were scattered across the time course, and at no time point were all of them adversely modulated. This may account for the lack of any change in actual plaque stability.

In summary, MMPs have previously been shown to have a range of roles in plaque expansion and destabilisation, with some suppressing growth and supporting stability and others fostering expansion and causing rupture. The current studies show that a broad-spectrum synthetic MMP inhibitor is generally deleterious in terms of plaque growth and markers of plaque instability (though there was no significant change in the incidence of plaque rupture). This suggests that indiscriminate inhibitors of MMPs are unlikely to provide benefit in terms of clinical cardiovascular disease, and that more specific agents are needed. Recent data with MMP knockout mice suggest that a specific inhibitor of MMP-12 may be particularly useful in this regard [11].

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