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Dose-Related Effects of Repeated ETC-216 (Recombinant Apolipoprotein A-I$_{Milano}$/1-Palmitoyl-2-Oleoyl Phosphatidylcholine Complexes) Administrations on Rabbit Lipid-Rich Soft Plaques

In Vivo Assessment by Intravascular Ultrasound and Magnetic Resonance Imaging

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Objectives This study sought to evaluate in vivo the minimal dose of apolipoprotein (apo) A-I$_{Milano}$ phospholipid complex (recombinant apoA-I$_{Milano}$ and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes [ETC-216]) able to induce atherosclerosis regression in a rabbit model of lipid-rich plaques.

Background A single high dose of recombinant apoA-I$_{Milano}$ has promoted atherosclerosis regression in animal models. More recently, regression of atherosclerosis was achieved in coronary patients by repeated infusions of ETC-216.

Methods Thirty-six rabbits underwent perivascular injury at both carotid arteries, followed by a 1.5% cholesterol diet. After 90 days, rabbits were randomly divided into 6 groups and treated 5 times with vehicle or ETC-216 at 5, 10, 20, 40, or 150 mg/kg dose every 4 days. Carotid plaque changes were evaluated in vivo by intravascular ultrasound (IVUS) and magnetic resonance imaging (MRI), performed before and at the end of treatments. Magnetic resonance imaging scans were also recorded after administration of the second dose for rabbits infused with vehicle 40 or 150 mg/kg.

Results Atheroma volume in vehicle-treated rabbits increased dramatically between the first and the second IVUS analyses (+26.53%), whereas in ETC-216–treated animals, a reduced progression at the lower doses and a significant regression at the higher doses, up to −6.83%, was detected. Results obtained by MRI analysis correlated significantly with those at IVUS ($r = 0.706$; $p < 0.0001$). The MRI evaluations after the second infusion established that a significant regression was achieved with only 2 administrations of the highest dose.

Conclusions These results confirm the efficacy of ETC-216 for atherosclerosis treatment and provide guidance for dose selection and frequency to obtain a significant reduction of plaque volume. (J Am Coll Cardiol 2008;51:1098–103)

High-density lipoprotein (HDL) cholesterol levels are a potent inverse and independent epidemiologic risk factor for cardiovascular diseases (1,2), and HDL or its major protein, apolipoprotein (apo) A-I, are well-established antiatherosclerotic agents in animal models (3,4). Several approaches have been attempted in recent years to increase HDL cholesterol levels, including the direct administration of synthetic HDL or apoA-I, as a whole or as a peptide mimetic (3–5).

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Pre-clinical studies have previously shown that intravascular infusions of synthetic HDL containing a genetic variant of apoA-I, apoA-I$_{Milano}$, inhibit progression and induce rapid regression and remodeling of atherosclerosis

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(6–11). Importantly, Nissen et al. (12) showed that 5 weekly intravenous injections of synthetic HDL, constituted by recombinant apoA-I$_{Milano}$ and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes (ETC-216), induced atheroma regression in coronary patients. However, the 2 tested doses (15 and 45 mg/kg protein) induced about the same level of regression on human coronary vessels, leaving open the question of the minimal amount of ETC-216 required to achieve regression. Moreover, the optimal number of ETC-216 administrations needed to obtain a significant reduction in plaque size, an important practical question in terms of any future clinical use, was not addressed.

The primary objective of the current study was to establish the minimal effective dose of ETC-216 able to induce atherosclerosis regression in a rabbit model of lipid-rich soft plaques. The secondary objective was to determine the lowest number of administrations required to observe a significant plaque regression. These goals were achieved by in vivo assessment of plaque volume through intravascular ultrasound (IVUS) and magnetic resonance imaging (MRI) analyses. In previous studies by our group, both techniques have been applied successfully to quantitative analysis of arterial plaques in the same rabbit model (10,13,14).

Methods

Experimental protocol. Lipid-rich plaque formation was induced in male New Zealand White rabbits (Harlan, Italy) as previously described (13). Procedures involving animals and their care were conducted in compliance with national and European Economic Community laws and policies.

At 90 days after surgery, pre-treatment right carotid artery scans were recorded by IVUS, followed by MRI scans of both the right and left carotid arteries. Rabbits were then randomized into 6 groups of 6 animals each and treated with vehicle (7.7% sucrose and 0.8% mannitol) or 5, 10, 20, 40, or 150 mg protein/kg body weight of ETC-216.

The ETC-216 was supplied by Esperion Therapeutics, Pfizer Inc. (Plymouth, Michigan), prepared in 7.7% sucrose and 0.8% mannitol, at a concentration of 15 mg/ml protein. The ETC-216 and vehicle were administered as an intrajugular infusion, at a constant rate of 1.0 ml/min every 4 days, for a total of 5 doses. No signs of toxicity by treatment were observed at each dose administered.

Before administration of the third dose, rabbits infused with vehicle (control), 40 mg/kg ETC-216, and 150 mg/kg ETC-216 underwent MRI analysis at both carotid arteries. One week after infusion of the fifth (last) dose, all rabbits underwent a scan of both carotid arteries by MRI, followed by an IVUS scan of the right carotid artery. Operators responsible for treatment administration were completely blinded with respect to the treatment.

IVUS imaging. The IVUS evaluations of the right carotid artery were performed using a mechanical IVUS system (Galaxy 2, Boston Scientific, Fremont, California) as described (13). Two blinded independent observers performed the IVUS analyses. The origin of the right carotid artery from the aortic arch was used as the anatomical landmark to co-register pre- and post-treatment scans. Atheroma area was calculated as the external elastic membrane area minus luminal area. Plaque area was measured on all slices covering the lesion. The sum of the areas was multiplied by the slice thickness value (0.5 mm) to obtain plaque volume. The point of maximal plaque formation was considered the cross-section with the maximal plaque area.

Abbreviations and Acronyms

apo = apolipoprotein
ETC-216 = recombinant apolipoprotein A-I$_{Milano}$ and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes
HDL = high-density lipoprotein
IVUS = intravascular ultrasound
MRI = magnetic resonance imaging

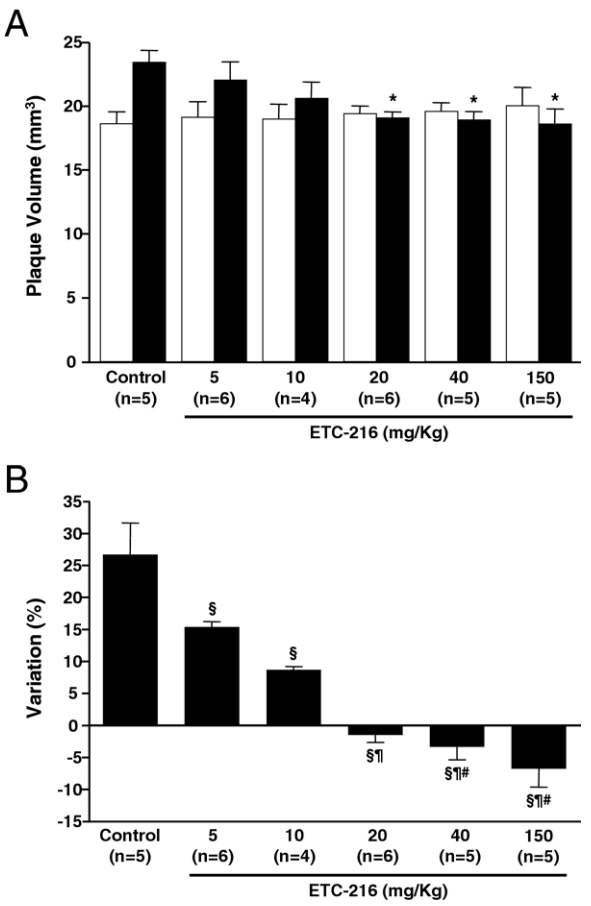

Figure 1 **Absolute Values and Percent Changes Evaluated by IVUS**

Absolute values **(A)** and percent change **(B)** of total atheroma volume evaluated by IVUS at the right carotid arteries in rabbits treated with vehicle (control) or different doses of ETC-216. Data are expressed as mean ± SEM. **(A) Open bars** represent pre-treatment; **solid bars** represent post-treatment. *$p < 0.05$ versus control post-treatment. **(B)** §$p < 0.05$ versus control; ¶$p < 0.0005$ versus ETC-216 at 5 mg/kg; #$p < 0.05$ versus ETC-216 at 10 mg/kg. ETC-216 = recombinant apolipoprotein A-I$_{Milano}$ and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes; IVUS = intravascular ultrasound.

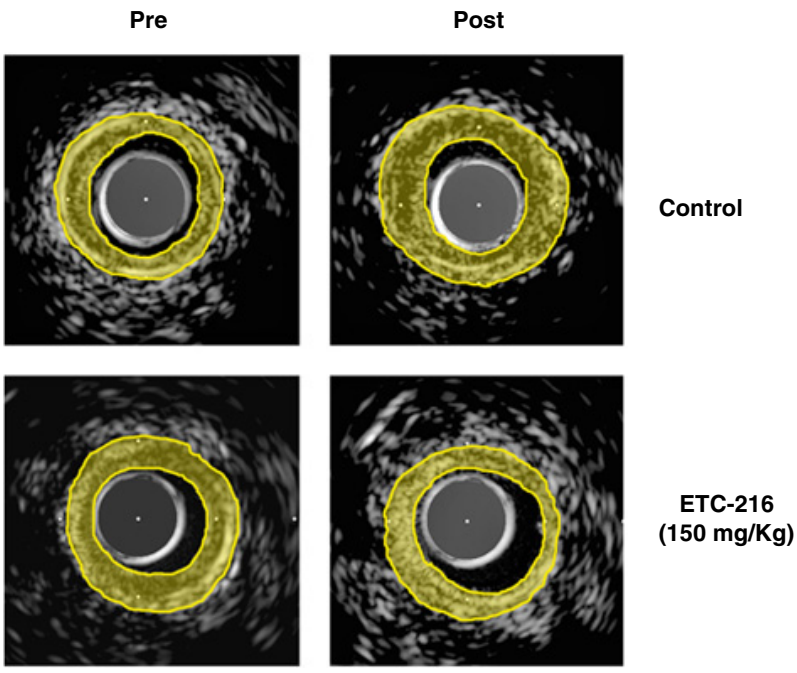

Figure 2 **Examples of IVUS Images of Atherosclerotic Plaques Acquired Before and After Treatment With Vehicle (Control) or 150 mg/kg ETC-216**

Plaque areas are identified in **yellow**. A progression is visible in the control animal, whereas a regression is observed in the ETC-216–treated rabbit. Abbreviations as in Figure 1.

MRI. An MRI scan was performed in all animals on a 2-T magnetic resonance (MR) imager (SMIS, Surrey, England) with a quadrature surface coil placed on the rabbit's neck used as a receiver and a larger coil for transmitting (RAPID-Biomedical, Rimpar, Germany).

The MR images of both carotid arteries were acquired before and after administration of the paramagnetic contrast agent B22956/1 (Bracco Imaging SpA, Turin, Italy) at a dose of 0.075 mmol/kg (intravenously in the ear) to localize stenoses and to enhance the lesions. The MR angiography was obtained with a 3-dimensional gradient-echo (repetition time [TR]/echo time [TE] = 14/2 ms, flip = 40°, voxel = 312 × 312 × 833 μm^3). For arterial wall lesion imaging, axial T1-weighted and T2-weighted spin echocardiographic images were acquired (TR/TE = 1,800/40 ms for T2, TR/TE = 600/15 ms for T1, pixel = 258 μm, slice thickness = 1.8 mm).

The MR images from different time points were co-registered by using the spinal cord structure as anatomical reference and analyzed by 2 blinded observers. The MRI plaque area was measured on both carotid arteries and on all slices covering the lesion. The sum of areas was multiplied by the slice thickness value (1.8 mm) to obtain plaque volume. For each carotid artery, plaque volumes were calculated as the mean value from those obtained with the different contrast images (T2, T1 before contrast agent, T1 after contrast agent).

Statistical analysis. Data are expressed as mean ± SEM. Group differences were tested for statistical significance by analysis of variance for repeated measurements with 1 grouping factor or 1-way analysis of variance, followed by the Tukey post-hoc test; a value of $p < 0.05$ was considered statistically significant. The Pearson correlation coefficient was calculated for interobserver and intraobserver variability

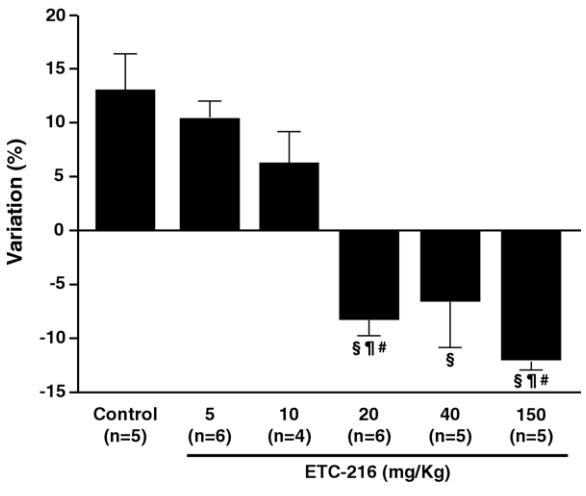

Figure 3 **Percent Change in Plaque Area at the Maximum Plaque Burden Evaluated by IVUS**

Rabbits were treated with vehicle (control) or ETC-216. Data are expressed as mean ± SEM. §$p < 0.001$ versus control; ¶$p < 0.005$ versus ETC-216 at 5 mg/kg; #$p < 0.05$ versus ETC-216 at 10 mg/kg. Abbreviations as in Figure 1.

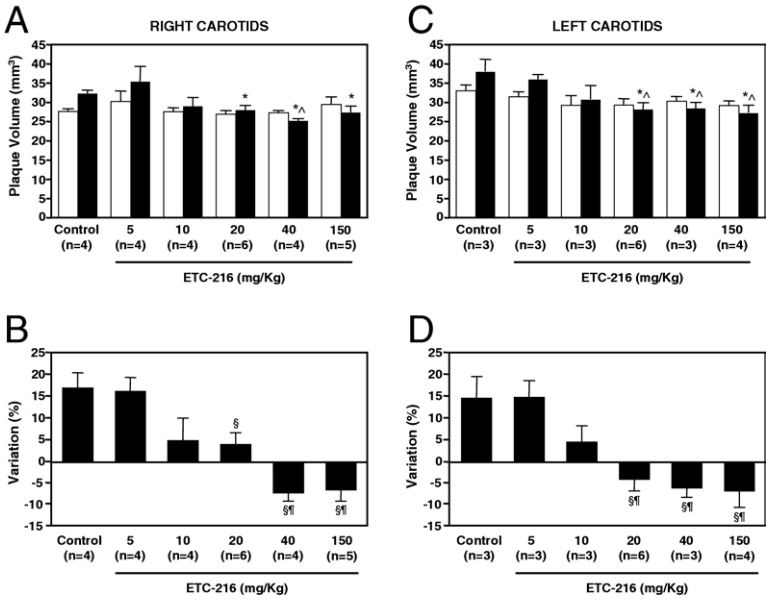

Figure 4 **Absolute Values and Percent Changes Evaluated by MRI**

Absolute values **(A and C)** and percent change **(B and D)** of total atheroma volume evaluated by MRI in rabbits treated five times with vehicle (control) or different doses of ETC-216. Data are expressed as mean ± SEM. **(A and C) Open bars** represent pre-treatment; **solid bars** represent post-treatment. *p < 0.05 versus control post-treatment; ^p < 0.05 versus ETC-216 at 5 mg/kg. **(B and D)** §p < 0.05 versus control; ¶p < 0.05 versus ETC-216 at 5 mg/kg. MRI = magnetic resonance imaging; other abbreviations as in Figure 1.

and for statistical comparison of plaque volumes obtained by IVUS, MRI, and histology.

Results

Treatment effect evaluated by IVUS. In 5 of 36 animals, the quality of IVUS images recorded at the end of treatment did not allow reliable measurements of the plaque area; therefore, the results described below refer to 31 rabbits. In line with previous studies by our group (10,13), the inter-observer variability in plaque volume measurements was 0.887 ($p < 0.0001$) and the intraobserver variability was 0.946 ($p < 0.001$). Figure 1 shows absolute plaque volumes and percent variations that occurred during the treatment period, and examples are shown in Figure 2. Pre-treatment plaque volumes were not different among the 6 groups (Fig. 1A). Total atheroma volume in the vehicle (control) group

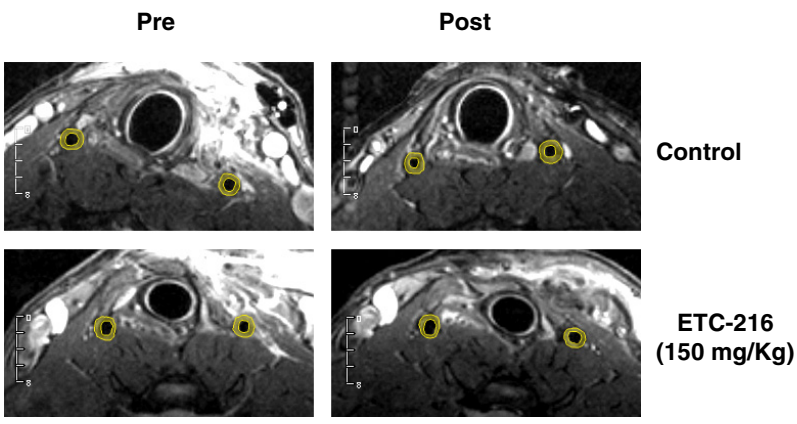

Figure 5 **Examples of T2-Weighted Magnetic Resonance Images of Carotid Arteries Acquired at the Same Level Before and After Treatments With Vehicle (Control) or 150 mg/kg of ETC-216**

Plaque areas are identified in **yellow**. A marked progression is visible in the control rabbit, whereas a regression is observed in the ETC-216-treated animal. Abbreviations as in Figure 1.

increased dramatically in the time between the first and the second IVUS evaluation (Fig. 1). In contrast, ETC-216 at the 5- and 10-mg/kg doses reduced atheroma progression. At the 3 highest doses, a progressive and significant regression of plaque volume was observed, ranging from −1.5 ± 1.17% with 20 mg/kg to −6.83 ± 2.86% with 150 mg/kg administration.

Percent changes by treatments were also evaluated at the maximum plaque burden. A similar, marked plaque regression was observed with the administration of the 3 highest doses of ETC-216 (Fig. 3).

Treatment effect evaluated by MRI. In some animals, MR images were not analyzable because of intense breathing or chewing movements; therefore, results refer to 22 animals for the left carotid artery and 27 rabbits for the right carotid artery. In line with previous results by our group (14), the interobserver variability in plaque volume measurements was 0.776 ($p < 0.0001$) and the intraobserver variability was 0.933 ($p < 0.0005$). Absolute plaque volume and percent variations were evaluated in the 6 groups before and at the end of treatments; results are shown in Figure 4. Analyses of right and left carotid arteries showed a similar trend and were consistent with the results obtained by IVUS. Pre-treatment plaque volumes again did not differ among groups (Figs. 4A and 4C). Total atheroma volume in controls strongly increased during the treatment period (Figs. 4 and 5). Although not significant, the 10-mg/kg ETC-216 treatment resulted in a reduced progression of plaque volume. Significantly reduced progression (right carotid artery) or regression (left carotid artery) were detected at the 20-mg/kg dose, whereas a clear and comparable regression was measured at 40 and 150 mg/kg ETC-216 (−7.29 ± 1.79% and −6.56 ± 2.68% at the right carotid artery and −6.04 ± 2.10% and −6.76 ± 4.41% at the left carotid artery, respectively) (Figs. 4 and 5).

Changes of plaque volume after 2 administrations were evaluated by MRI to determine timing of treatment efficacy. Results were similar for the right and left carotid arteries (Fig. 6). In the control group, a progressive increase in plaque volume was detected. At the 40-mg/kg dose, a small, nonsignificant plaque regression was seen after 2 administrations, whereas just 2 infusions of 150 mg/kg ETC-216 were associated with significant plaque regression.

Comparison between IVUS and MRI measurements. A correlation between IVUS and MRI plaque volume changes by treatment was assessed at the right carotid artery (Fig. 7). A highly significant linear regression was obtained ($r = 0.706$; $p < 0.0001$).

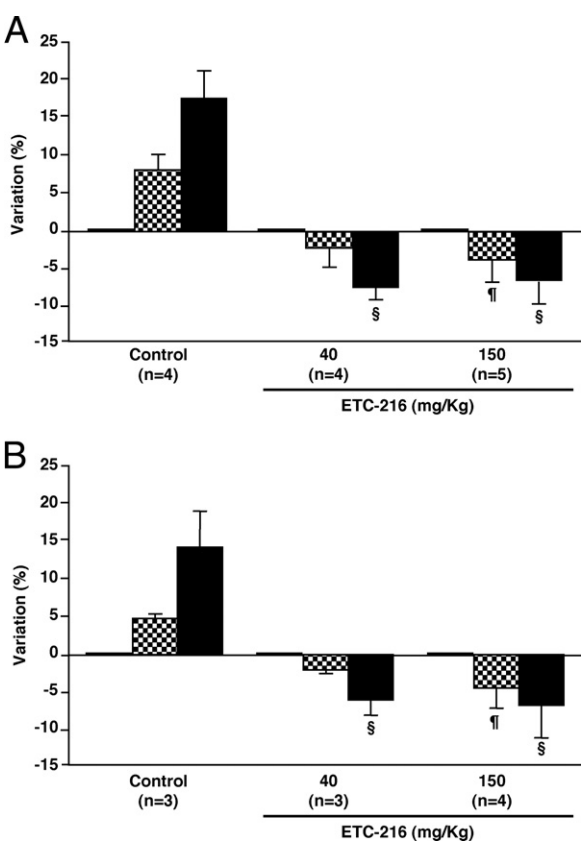

Figure 6 Percent Change of Total Atheroma Volume at MRI After 2 and 5 Administrations

Percent change of total atheroma volume at right **(A)** and left **(B)** carotid arteries evaluated by MRI after 2 and 5 administrations of vehicle or ETC-216 (40 mg/kg and 150 mg/kg). **Shaded bars** represent effects of 2 administrations; **solid bars** represent effects of 5 administrations. Data are expressed as mean ± SEM. ¶$p < 0.05$ versus control after 2 administrations; §$p < 0.05$ versus control after 5 administrations. Abbreviations as in Figures 1 and 4.

Discussion

The major finding of this study is that multiple intravenous administrations of 5 different doses of ETC-216 in a rabbit model of carotid soft lipid-rich plaque resulted in a dose-related effect on plaque volume, ranging from a lower progression to a marked regression. As in previous studies (8–10), apoA-I$_{Milano}$ administrations affected plaque size mainly by lowering plaque lipid accumulation (data not shown).

Intravascular ultrasound and MRI have emerged as the preferred imaging modalities to evaluate the impact of pharmacological therapies on arterial plaque progression/regression (10,12,15,16). Both IVUS and MRI have been applied by our research group to characterize the same rabbit model used in the present study (13,14) and to investigate the efficacy of acute administrations of synthetic HDL containing apoA-I$_{Milano}$ in promoting plaque regression (10). As expected on the basis of previous findings (14), data obtained in this study by the 2 methods were comparable and significantly correlated, and were highly correlated with histology (IVUS vs. histology: $r = 0.845$, $p < 0.001$; MRI vs. histology: $r = 0.775$, $p < 0.005$).

The present study was designed to identify the minimal effective dose of ETC-216 capable to determine a significant plaque regression, with the same treatment strategy as

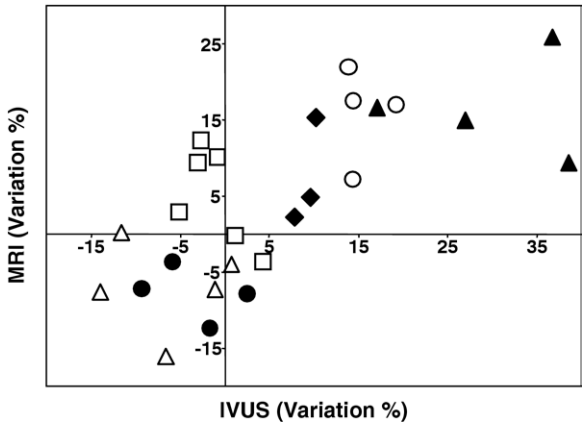

Figure 7 **Correlation Between IVUS and MRI Plaque Volume Percent Change at the RCAs**

Solid triangles = control; **open circles** = 5 mg/kg ETC-216; **solid diamonds** = 10 mg/kg ETC-216; **open squares** = 20 mg/kg ETC-216; **solid circles** = 40 mg/kg ETC-216; **open triangles** = 150 mg/kg ETC-216 ($r = 0.706$; $p < 0.0001$). RCA = right carotid artery; other abbreviations as in Figures 1 and 4.

applied to coronary patients (12). The 2 highest doses given to rabbits (40 and 150 mg/kg) correspond to the doses administered to patients (15 and 45 mg/kg), keeping in account pharmacokinetic differences between rabbits and humans (17). To identify a possible minimal effective dose, 3 lower doses of ETC-216 were also included in the present study. The results obtained by IVUS and MRI were comparable, with no differences being observed in the response of left and right carotid arteries except for the dose of 20 mg/kg, which was associated with regression (Figs. 1 and 4) or reduced progression (Fig. 4), altogether indicating stabilization of plaque volume. Interestingly, the 2 highest doses (40 and 150 mg/kg) were associated with regression of plaque size, with no greater regression occurring at the higher dose, similar to results obtained in the clinical study (12). From these results it can be concluded that, in this animal model, the minimal dose of ETC-216 required to obtain a plaque regression with 5 treatments is between 20 and 40 mg/kg. It could be speculated that for humans this value may correspond to 8 to 15 mg/kg (17).

Another goal of the study was to evaluate the possibility that a significant regression of plaque volume could be achieved after just 2 administrations of the 2 highest doses. Although no significant effects were determined by 2 infusions of the 40-mg/kg dose, a significant regression was observed after 2 administrations of 150 mg/kg ETC-216. These results are in line with a very recent study showing a significant plaque regression after 2 administrations of 75 mg/kg ETC-216 (18).

In conclusion, the present results indicate the remarkable efficacy of 5 administrations of ETC-216 in reducing progression or inducing regression of atheroma, and suggest the feasibility of reducing the number of administrations to achieve a significant plaque regression in humans. In addition, the excellent correlation between IVUS and MRI data indicates the possibility of a future wide range application of this noninvasive methodology to the monitoring of the efficacy of pharmacological treatments.

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