Application of Serial In Vivo Magnetic Resonance Imaging to Evaluate the Efficacy of Endothelin Receptor Antagonist SB 217242 in the Rat Carotid Artery Model of Neointima Formation

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Background—Alleviating vascular restenosis after percutaneous transluminal angioplasty remains a formidable challenge. Although multiple factors have been implicated in the pathophysiology of this vascular remodeling disorder, only limited therapeutic success has been achieved. Endothelin (ET)-1 has recently been implicated in the pathogenesis of neointimal growth. We report the in vivo efficacy of SB 217242, a nonpeptide dual ETA/ETB receptor antagonist with high oral bioavailability, in the rat carotid artery balloon angioplasty model.

Methods and Results—The lumen volumes of carotid arteries were estimated serially with magnetic resonance imaging (MRI) at baseline and at day 7 and day 14 after balloon catheter–induced denudation of the carotid arterial wall in the rat. Histomorphometric analysis was performed at day 14 after surgery to quantitate intimal hyperplasia. Statistical analysis was performed with ANOVA followed by post hoc Newman-Keuls multiple comparison test. In comparison to vehicle-treated animals, a 20% protection (P<0.05) from reduction was shown in the estimated lumen volume with long-term administration of SB 217242 (15 mg/kg BID PO). Histologic analyses indicated a 42% decrease (P<0.05) in neointimal growth. The MRI lumen volumes had a significant correlation with the corresponding histologic indices.

Conclusions—Serial MRI provides the opportunity to assess the progression of vascular lumen volume in vivo after balloon angioplasty. MRI measurements can, in conjunction with in vitro histologic measurements, contribute to the understanding of the actions of pharmacologic agents in experimental models of neointima formation. With the use of serial MRI and histologic measurements, it is demonstrated that protection from both lumen volume reduction and neointima formation is obtained in this model by use of a potent, nonpeptide dual ETA/ETB receptor antagonist, SB 217242. Furthermore, this study provides additional support to the implication of ET-1 in the pathophysiology of neointima formation. (Circulation. 1998;97:2252-2258.)

Key Words: magnetic resonance imaging ■ angioplasty ■ restenosis ■ endothelin

The clinical success rate of percutaneous transluminal coronary angioplasty is limited by a high incidence of neointimal formation and vascular restenosis.1 Neurohormonal factors, such as angiotensin II, along with several growth factors such as platelet-derived growth factors and basic fibroblast growth factor, have been implicated in the process of vascular restenosis.2–4 Increased levels of ET-1 have been observed in the human coronary sinus after percutaneous transluminal coronary angioplasty and therefore it has been suggested that ET-1 may also be involved in the pathogenesis of vascular restenosis.6 Along with its role in regulating vascular tone,7 it is known that ET-1 is a mitogen in smooth muscle and can synergize with other mitogens such as platelet-derived growth factor.8,9 These effects have been shown to be inhibited by ET,–selective receptor antagonists.9–11 Evidence of elevated mRNA levels of endothelin-converting enzyme have been reported recently in the rat vascular injury model12,13 and in human coronary atherosclerosis.14 Elevated plasma ET-1 levels have also been reported in patients with symptomatic atherosclerosis.15 Exogenous ET-1 has been shown recently to promote neointimal growth after balloon angioplasty–induced vascular injury in the rat in vivo16 and the attenuation of the neointimal growth obtained by using an endothelin receptor antagonist, SB 209670, has also been described.15

MRI is a noninvasive, high-resolution spatial mapping technique with excellent anatomic contrast that has found extensive applications in basic research and clinical radiolo-
MRI allows noninvasive serial monitoring of the same experimental animal at multiple time points without any significant perturbation of the native in vivo tissue environment. The application of this technology is maturing within biologic research environments, and several pharmacologic applications in preclinical animal models have recently been reported. In the present study the ability of SB 217242, a new potent ET₂ antagonist with high bioavailability, to protect in vivo lumen volume was evaluated serially with high-resolution MRI while the efficacy of the compound to ameliorate neointimal growth was analyzed with standard postmortem histologic staining. The MRI data were obtained to provide relevant information on lumen cross-sectional area of the arteries at different stages of neointimal formation in vivo. MRI was incorporated in this study with two goals in mind: first, to develop a volume index for in vivo lumen patency that would enable reliable examination of test agents in experimental animal models with respect to this valuable information; second, to demonstrate the feasibility of developing an in vivo time profile of the balloon catheter–induced injury in this experimental model of restenosis. To our knowledge, this is the first in vivo study in this experimental model in which serial changes in the arterial lumen volume are reported in conjunction with a comparison to long-term pharmacologic evaluation.

Methods

Study Design and Surgery Protocol

Male Sprague-Dawley rats (350 to 400 g) were prescanned in an MRI system (see details below) before undergoing balloon catheter–induced denudation of the endothelium in the carotid arteries. Animals in the first group were pretreated with SB 217242 (15 mg/kg) diluted in sterile water, administered by gavage BID) starting 3 days before surgery and were maintained on the same dosage regimen for the duration of the study. A second group, which served as a control group, received identical treatment but was administered only vehicle. For angioplasty, all animals were anesthetized with ketamine (10 mg/kg) and xylazine (50 mg/kg), and the distal left common carotid and the external carotid arteries were exposed through a midline incision in the neck. A sterile 2F Fogarty arterial embolectomy catheter (model 12 to 060 to 2F, Baxter Healthcare) was introduced through the external carotid artery and guided through the common carotid artery up to the aortic arch. The balloon was then distended sufficiently with saline to generate slight resistance, and withdrawn back to the site of insertion. This procedure was performed a total of two times. The catheter was subsequently removed and the external carotid ligated with 4.0 silk suture without occluding flow to the occipital artery. All animals were allowed food and water ad libitum after surgery. A shunt group, in which only a midline incision was performed, was also studied along with the angioplasty-injured animals to study normal variations within intact animals. All experiments were performed in accordance with the Guidelines of the Animal Care and Use Committee, SmithKline Beecham Pharmaceuticals, and the American Association for Laboratory Animal Care.

MRI Evaluation

The animals were monitored by MRI on day 7 and on day 14 after injury to evaluate in vivo lumen cross-sectional area. Each day the animals were lightly anesthetized with a mixture of 1% to 1.5% isoflurane (Abbott Laboratory) and 0.8 to 1.0 L/min of O₂ during the MRI procedure. MRI was performed on a 4.7 T/40 cm Bruker imaging spectrometer (Billerica) with a 15-cm self-shielded gradient coil insert. A nine-strut half-birdcage radiofrequency resonator was used for both transmission and reception of the radiofrequency signal. A spin-echo sequence with two-dimensional Fourier encoding was used for imaging with a repetition time (TR) of 4 seconds and an echo time (TE) of 23 ms. To generate appropriate contrast between the lumen area and the surrounding tissue, gradients of 1 g/cm with a duration of 6.5 ms were used on both sides of the refocusing 180 degree radiofrequency pulse to dephase the signal from flow-induced motion. A matrix size of 256×256 was chosen over a field of view of 3×3 cm, providing an in-plane resolution of 120×120 μm. Eighteen contiguous slices (1.5 mm thick) were collected daily for each animal. All images were cardiac gated with the trigger for data acquisition set to 40 ms from the QRS complex. Motion artifacts from respiration did not significantly degrade the image quality and therefore simultaneous respiratory gating was not required for this study. Data acquisition time was ~35 minutes for each animal.

The index for quantitative monitoring of the vascular response to injury was defined as the volume measured by adding the lumen cross-sectional area from contiguous slices along a fixed section of the carotid artery. The neointima formation in the rat model being fairly uniform over the length of the artery over which histologic analysis is performed justified developing a consistently defined volumetric index to reflect the global status of the lesion. Seven contiguous slices, starting from the third slice proximal to the carotid bifurcation, were used for generating the volume index. This index offered the advantage of evaluating the lesion globally and was statistically more robust because random errors from tracing averaged out over the seven slices used for the calculation and minor positioning variations during serial measurements did not contribute to significant errors. A mean cross-sectional area, using these seven slices, was also calculated for each group at each time point.

Histologic Evaluation

After the MRI evaluation, the common carotid arteries were isolated from each animal on day 14 after surgery and neointima formation was quantified after in situ perfusion fixation (100 mmHg) with 10% (wt/vol) phosphate-buffered formalin. Four slices (5 μm in thickness), one each from four contiguous sections of the common carotid, were used for histologic morphometry. For histologic staining, a standard hematoxylin and eosin stain was used and quantitative morphologic measurements were performed with a Bioscan Optimus cell imaging system. For analysis of treatment efficacy, only the average neointimal and medial areas obtained with all four sections of each artery were used.

Data Analysis and Statistics

Data analyses were performed with the ANALYZE (CN Software) package on an SGI UNIX workstation. All indices calculated from the images were expressed as mean±SEM. At each time point a one-way ANOVA was used to evaluate differences between treatment groups; a repeated-measures ANOVA was used to evaluate differences with time within each treatment group. In both cases, the ANOVA was followed by a post hoc Newman-Keuls test for multiple comparisons of means. A value of P<0.05 was accepted as statistically significant. Although all statistical analyses were performed with the estimated volumes, they are equally valid for the mean cross-sectional areas because in each case the mean area is related to the overall volume by a constant (1/7).

Results

Figure 1, A and B, shows typical single-slice magnetic resonance images used in the study for estimating lumen volumes. Measurement of lumen areas from seven such
contiguous slices generated the lumen volume index that served as a quantitative measure for the lumen patency in vivo. Figure 2A shows the volumes for the contralateral unballooned artery; that for the ballooned artery is shown in Figure 2B. The Table shows a summary of the changes in the mean lumen cross-sectional area for each group.

A general trend of compensatory vascular change leading to increased lumen volumes is observed with time in the
contralateral unballooned arteries for both the vehicle and the drug groups, with statistically significant differences occurring at day 14 with respect to baseline (P<0.01 for both the vehicle and the drug groups). The volume of the ballooned artery progressively decreased in both the groups (Figure 2B). The estimated lumen volume of the ballooned artery was significantly lower on day 14 with respect to baseline for both groups (P<0.001 for vehicle and P<0.05 for drug-treated group). Although no significant difference was obtained at day 7 (with respect to baseline) for the ballooned artery in either group, the lumen volume at this time was significantly larger than the same at day 14 (P<0.01 for vehicle; P<0.05 for the drug-treated group), alluding to the fact that significant vasoresponse of the ballooned arteries occurs some time between day 7 and day 14. The larger probability value obtained for the drug-treated group in each of these tests indicates a protective effect provided by the drug. No significant differences were detected by ANOVA for either artery at any time point in the sham group, indicating the absence of any trends to suspect any systematic error propagation in the data from the development of the animals or instrumental variabilities.

In comparisons between treatments, no significant difference was detected between the contralateral unballooned artery of any group at any time point. For the ballooned artery, no significant difference was detected at baseline and at day 7 between the three groups. On day 14 after surgery, however, the drug-treated group had a significantly larger volume than that of the vehicle-treated group (P<0.05). Expressed as a percentage, on day 14 the lumen volume of the ballooned artery was 57.4% of its baseline presurgery value. The corresponding number for the drug-treated group was 77.9%. Therefore, with volume used as an index of lumen patency, a 20% protection from a reduction in vivo lumen volume was provided by long-term administration of SB 217242.

Representative low-power micrographs of the left carotid artery from the vehicle-treated and the drug-treated groups are shown in Figure 3. The mean I/M ratio calculated from the histologic morphometry is shown in Figure 4. The average I/M ratios were statistically identical for the contralateral vessel in the drug-treated, vehicle-treated, and the sham groups, respectively. The contralateral vessels between the drug-treated, control, and the sham groups were morphologically identical; all devoid of neointimal growth and normal in appearance. For the ballooned artery, the control group (vehicle-treated) had a significantly larger I/M ratio (P<0.05) than the drug-treated group. Because the medial area remained identical in all the groups, the data indicate that a 42% decrease in the formation of neoIntima is obtained by the long-term oral administration of SB 217242.

In an attempt to further elucidate the MRI data, a two-dimensional template to simultaneously portray MRI data versus histologic I/M ratio for each animal in the study was examined. After evaluation with histology, it was noted that

| Changes in Arterial Mean Cross-Sectional Area (mm²) in Each Treatment Group as a Function of Time |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Treatment Group                     | Right | Left  | Right | Left  | Right | Left  |
| Vehicle-treated                        | 1.22±0.07 | 1.25±0.08 | 1.27±0.07 | 1.23±0.08 | 1.51±0.07 | 0.72±0.09 |
| Drug-treated                           | 1.27±0.07 | 1.29±0.06 | 1.43±0.13 | 1.22±0.09 | 1.55±0.08 | 1.00±0.10 |
| Sham                                  | 1.43±0.09 | 1.37±0.07 | 1.56±0.17 | 1.51±0.18 | 1.49±0.17 | 1.46±0.17 |
the sections on the common carotid ≈ 3 mm from the bifurcation (external and internal) and stretching for 4.5 mm distally developed the maximal neointimal growth. Lumen areas from three contiguous magnetic resonance slices over the corresponding section were added to estimate the in vivo lumen volume of the vessel over that specific section. The MRI volume so obtained was plotted against the corresponding average I/M ratio for the same animal. This procedure was repeated for all 28 animals used (Figure 5). The global means, the only information normally used for routine analysis in such studies, are also plotted in the same graph. Statistical analysis showed the global mean for the drug-treated group to be significantly larger \( (P < 0.05) \) than the vehicle-treated group with respect to the MRI volume index, whereas the opposite was true with respect to the histologic I/M ratio index.

**Discussion**

The in vivo blood vessel lumen patency, particularly in clinical studies, is a decisive factor in evaluating drug candidates and determining clinical outcomes of therapy. Therefore, an early evaluation of the efficacy and potency of compounds with respect to vascular patency in preclinical experimental models of restenosis may be important. A previously reported study with ACE inhibitors in rats concurred in the assessment of lumen patency with the angiographically assessed lumen patency in the corresponding clinical study, confirming the importance of such measurements in preclinical assessment of potential clinical candidates. With the recent application of such high-resolution, noninvasive spatial imaging techniques such as MRI to small animal models, the measurement of in vivo cross-sectional area of small conduit arteries has become possible. In this context, the relationship between the in vitro histopathologic index (I/M ratio) and the in vivo MRI index (lumen volume) required investigation.

It is interesting to observe that in a previous study with ACE inhibitors, although significant reductions in neointimal area (as measured by postmortem histology) were reported, no significant protection from reduction of lumen patency could be demonstrated by these compounds in vivo (as measured by MRI). Accordingly, in the current study, there was a difference in the protection provided by the drug as measured by the in vivo and the in vitro indices. Functional vasomotor attributes of the vascular smooth muscle cells have been implicated for this discrepancy. In addition to the presence or absence of functional variables such as altered blood flow, response to shear stress caused by injury or local blood flow, vasoactive hormones, vascular tone, and so forth, a purely geometric reason for such a difference may be attributed to the physical dimensions of the carotid lumen cross-sectional area and the neointimal surface area around its rim. The latter being smaller, for a given difference in the neointimal area between the vehicle-treated and the drug-treated groups, the change (expressed as a percentage of the
corresponding vehicle-treated value) in the neointimal area would always be larger than the corresponding change in the cross-sectional lumen area. Moreover, compensatory vessel enlargement also may lead to differences between the changes observed in vessel cross-sectional measurements and neointimal growth. Indeed, for humans, it has been shown previously that the lumen cross-sectional area loss has a much higher degree of correlation with the response of external elastic membrane than the intimal hyperplasia, suggesting the importance of adaptive arterial wall remodeling in restenosis. A significant correlation between the two indices (lumen volume and I/M ratio), as plotted in Figure 5, exists in the current data \( r = -0.67; P < 0.05 \) for the vehicle-treated group and \( r = -0.62; P < 0.05 \) for the drug-treated group. Although some tissue preparation parameters (eg, tissue shrinkage) may randomly affect the histologic measurements, this correlation suggests the degree of agreement to be expected between the two indices. Given the complexities of in vivo measurements, a higher degree of correlation of the MRI data with a morphologic ex vivo measurement is not expected. In comparisons of global means in Figure 5, it is also apparent that the difference between the sham group and the vehicle group is more pronounced on the histologic axis than the corresponding difference on the MRI axis. This illustrates the value of MRI by demonstrating that a large difference in the I/M ratio in this experimental model does not imply a concomitant difference in the in vivo lumen volumes. Therefore, in this model it may be possible for a pharmacologic agent to achieve a significant reduction in neointimal growth without a significant difference in preserving the lumen volume of the arteries in vivo.

The in vivo time profile of the injury indicates the lack of significant lumen narrowing on day 7 after surgery and suggests that there is an accelerated loss of lumen patency between day 7 and day 14 after surgery. On day 3 after surgery, the lumen cross-sectional area is also preserved (data not shown). This trend of preservation of lumen volume in the first week after surgery, the lumen cross-sectional area is also preserved (data not shown). This trend of preservation of lumen volume in the first week after surgery and a delayed loss of the same is in accord with a previous study. Some ex vivo evidence exists that agrees with this observation by alluding to the possible influence of functional vasoconstriction of the arteries at the day 14 time point but not at the day 7 time point. Of the numerous intricate biochemical and pathologic events that are triggered by balloon catheter-induced denudation of the endothelial layer some events such as structural growth of the neointima, upregulation of ET\(_A\) and ET\(_B\) receptors, and exposure of subendothelial ET\(_A\) receptor sites to increased local ET-1 may favor a functional vasoconstrictory response at the day 7 or earlier time points. However, the presence of simultaneous hyporeactivity to spasmogens, expression of inducible nitric oxide synthase in medial layers, and so forth may act as counterbalancing mechanisms to compensate the lumen caliber during the first week after angioplasty. Beyond day 7 however, the structural growth of the intimal layer is accelerated and can present an overwhelming diffusional barrier to nitric oxide synthase–mediated pathways. This, when taken in combination with the absence of a functional endothelium and the lack of endothelium-mediated relaxation mechanisms (eg, acetylcholine or nitric oxide–mediated relaxation), the return to normalcy of the vessels in terms of their vasoresponse to spasmogens like ET-1 may explain a significant lumen caliber loss at the day 14 time point. Clearly, given the complexities of the in vivo environment, more detailed experiments would be necessary to identify a dominant mechanism for the lumen caliber loss between day 7 and day 14.

On day 14 after injury, long-term treatment with SB 217242 significantly inhibited lumen narrowing (20%) and significantly attenuated the neointima formation (42%). A similar degree of protection from neointimal growth in this model was demonstrated by histologic measurements for SB 209670. Taken together, the data indicate that the inhibition of ET-mediated signal transduction pathways would lead to \( \approx 40\% \) to \( \approx 50\% \) reduction of neointimal growth and provide \( \approx 20\% \) protection from lumen narrowing in vivo. A more complete therapy may require interference with neurohormonal or biochemical pathways common to more than one of many pharmacologically distinct chemotactic factors and mitogen.

The detection limit on spatial resolution for MRI is dictated by numerous parameters, some of which are signal-to-noise ratio, intrinsic signal decay rates, limited in vivo data acquisition times, and the static magnetic field strength of the magnet. For the present studies, the in-plane resolution was sufficient to delineate the lumen cross-sectional area with reasonable accuracy but not the arterial wall thickness. Although surgically implanted radiofrequency coil technology has been implemented recently for imaging the wall thickness, the absence of well-characterized contrast mechanisms between the wall and the surrounding muscle limits quantitation with higher precision. Therefore further developments in MRI instrumentation and contrast parameters may be necessary to pursue arterial wall measurements in vivo in this experimental angioplasty model.

In summary, this study demonstrates the utility of MRI in long-term monitoring of lesions and evaluating the efficacy of pharmacologic agents in this experimental model of neointima formation. Furthermore, the data presented here demonstrate that the use of SB 217242, a highly potent dual ET\(_A\)/ET\(_B\) receptor antagonist, can provide beneficial effects in terms of both attenuation of neointima proliferations and vascular patency in vivo in this animal model.

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**References**

Endothelin Antagonist and Restenosis


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