Integrin $\alpha_V\beta_3$ as a Target for Blocking HIV-1 Tat-Induced Endothelial Cell Activation In Vitro and Angiogenesis In Vivo

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Objective—The transactivating factor (Tat) of HIV-1 binds to $\alpha_v\beta_3$ integrin present on endothelial cells contributing to neovascularization. Here, we investigated the biological consequences of Tat/$\alpha_v\beta_3$ interaction and the antagonist effect of an Arg-Gly-Asp (RGD)-based peptidomimetic.

Methods and Results—Binding of Tat to endothelial $\alpha_v\beta_3$ triggers focal adhesion kinase and nuclear factor-κB activation, leading to endothelial cell proliferation, membrane ruffling, and motility in vitro and neovascularization in vivo. The RGD-peptidomimetic SCH221153 inhibits Tat/$\alpha_v\beta_3$ interaction in a solid phase binding assay and endothelial cell adhesion to immobilized Tat with a potency higher than that of RGD-containing peptides. Accordingly, SCH221153 inhibits Tat/$\alpha_v\beta_3$-dependent focal adhesion kinase and nuclear factor-κB activation, proliferation, membrane ruffling, and motility in endothelial cells. Finally, SCH221153 inhibits the angiogenic response triggered by Tat in the chick-embryo chorioallantoic membrane without affecting physiological vascularization. SCH221153 exerts these inhibitory effects without affecting the interaction of Tat with endothelial heparan sulfate proteoglycans or with the vascular endothelial growth factor receptor-2/kinase domain–containing receptor. In all the assays the negative control SCH216687 was ineffective.

Conclusion—These data provide new insights on the mechanism of endothelial cell activation by Tat and point to RGD peptidomimetics as prototypes for the development of novel Tat antagonists. **(*Arterioscler Thromb Vasc Biol.* 2005;25:2315-2320.)**

Key Words: HIV-1 ■ Tat ■ endothelium ■ integrin ■ angiogenesis

Tat protein, the main transactivating factor of HIV-1,[1] is released by HIV-1–infected cells[2] targeting different types of uninfected cells and causing a variety of biological effects related to distinct AIDS-associated pathologies. In AIDS patients, Tat contributes to tumorigenesis,[2] to the pathogenesis of dementia,[3] to the immune system suppression (by interfering with the function of different cells of immunity),[2] and to heart disease and atherosclerosis.[4]

The involvement of endothelial cells (ECs) in Tat-dependent pathologies is manifold.[5] By inducing neovascularization, Tat contributes to tumor progression and Kaposi sarcoma (KS) development.[2] Also, Tat increases endothelial permeability and alters the expression of endothelial leukocyte receptors, leading to the extravasation of HIV-1+ monocytes and HIV-1 dissemination. These effects, in turn, contribute to the pathogenesis of lymphomas, AIDS-dementia,[5] cardiovascular diseases, and atherosclerosis.[6] A decrease in endothelium-dependent vasorelaxation and endothelial nitric oxide synthase expression may also play a role in endothelial dysfunctions mediated by Tat.[7]

On these bases, Tat is considered a main target for the development of anti-AIDS therapies, and different anti-Tat strategies have been described, including gene therapies, vaccines, and Tat antagonists.[8]

Tat accumulates in the extracellular matrix as an immobilized protein,[9] and substrate-immobilized Tat interacts with $\alpha_v\beta_3$ integrin of ECs promoting their adhesion and triggering a complex signal transduction pathway that leads to activation of Tat-adherent ECs.[10] Accordingly, $\alpha_v\beta_3$ interaction is required for the chemotactic and mitogenic activity of Tat in vitro and for its angiogenic activity in vivo.[11] The Arg-Gly-Asp (RGD) tripeptide present in position 78 to 80 in Tat protein is required for $\alpha_v\beta_3$ interaction and EC adhesion.[11] Accordingly, RGD-containing peptides inhibit cell adhesion to immobilized Tat.[12]

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Nonpeptidic analogs (peptidomimetics) of the RGD motif endowed with integrin-antagonist capacity block different pathological processes including angiogenesis[13] and endothelial dysfunction that may lead to atherogenesis.[4] Here, we demonstrate that the RGD-peptidomimetic SCH221153, a selective $\alpha_v\beta_3$ antagonist,[13] blocks $\alpha_v\beta_3$/Tat interaction with high potency and specificity, thus preventing Tat-mediated EC activation in vitro and angiogenesis in vivo.

Methods

Reagents

The RGD-peptidomimetic SCH221153 and its control analog SCH216687[13] were from Schering-Plough Research Institute. Linear GRGDSPK and GRADSPK peptides were from Neosystem Laboratoire (Strasbourg). Cyclo(-Arg-Gly-Asp-D-Phe-Val) peptide (cRGDFV) and cyclo(-Arg-Ala-Asp-D-Phe-Val) peptide (cRADFV) were from Bachem (Beidendorf). Synthetic biotinylated Tat was from Tecnogen. Bovine fibronectin (FN) was from Sigma (St. Louis, MO). The goat anti-rabbit biotinylated antibodies and Alexa Fluor 488 streptavidin were from Molecular Probes (Eugene, OR). Anti-β_3 antiserum was provided by G. Tarone, University of Turin, Italy. Anti-$\alpha_v\beta_3$ monoclonal LM609 antibody was from Chemicon International. Human integrin $\alpha_v\beta_3$, glutathione-*S*-transferase (GST)-Tat, and GST-Tat fused to the green fluorescent protein were purified as described.[14,15] The GST moiety does not interfere with the transactivating, angiogenic, and cell-adhesive activities of Tat[10] (and references therein).

Cell-Free $\alpha_v\beta_3$ Integrin/GST-Tat Interaction and Cell Adhesion Assay

The assays were performed as described.[14,16]

Cell Cultures

Transformed fetal-bovine aortic endothelial GM7373 cells (obtained from the National Institute of General Medical Sciences [Institute for Medical Research, Camden, NJ]) were cultured in Eagle minimal essential medium containing 10% fetal calf serum (FCS) (Gibco, Grand Island, NY), vitamins, essential and nonessential amino acids. Human umbilical vein ECs (HUVECs; obtained from Biowhittaker, Walkersville, MA) were cultured in endothelial growth medium-2 medium (Biowhittaker). Porcine aortic ECs transfected with vascular endothelial growth factor receptor-2/kinase–domain containing receptor (KDR) (KDR/PAECs)[16] were cultured in Ham F12 medium (Gibco) with 10% FCS and 2% glutamine. HL3T1 cells are derived from HeLa cells and contain integrated copies of the pL3CAT plasmid in which the *chloramphenicol acetyltransferase* (*CAT*) bacterial gene is driven by HIV-1 long terminal repeat.[15] They were cultured in Dulbecco modified Eagle medium containing 10% FCS (Gibco).

FAK and NF-κB Activation Assays

GM7373 cells in 24-well culture tissue plates were made quiescent by 24-hour serum starvation and then incubated at 37°C for 30 minutes with GST-Tat (3 nmol/L) in the absence or in the presence of the peptidomimetics (0.3 μmol/L). For focal adhesion kinase (FAK) activation assay, cells were lysed by a 5 minutes incubation at 90°C in reducing SDS-PAGE sample buffer and analyzed by Western blotting using an anti–phospho-Tyr_{397}-FAK antibody (BioSource International). The autoradiography was digitized, and the integrated densities of the bands corresponding to phosphorylated FAK were evaluated with the Image Pro-Plus analysis system (Media Cybernetics). Data were expressed as fold increase of FAK phosphorylation in respect to untreated control cells. For nuclear factor-κB (NF-κB) activation assay, the amount of activated NF-κB p50 subunit in cell extracts was determined using the TransAM NF-κB p50 Assay Kit (Active Motif).

Tat Internalization and HIV-1 Long Terminal Repeat Transactivation Assays

The assays were performed as described.[17] For further details, see online Methods at http://atvb.ahajournals.org.

KDR-Binding and Phosphorylation Assays

KDR-binding assay was performed as described.[16] For further details, see online Methods at http://atvb.ahajournals.org. For KDR phosphorylation assay, subconfluent cultures of KDR/PAECs were made quiescent by 16-hour serum starvation. Then, cells were preincubated for 30 minutes at 37°C with medium containing SCH221153 or SCH216687 (30 nmol/L) and then incubated for 15 minutes with GST-Tat (1.2 nmol/L). Cells were washed and lysed in Hepes buffer pH 8, 150 mmol/L NaCl, 1% Triton X-100, protease, and phosphatase inhibitors (70 nmol/L pepstatin, 100 nmol/L leupeptin, 1.5 μmol/L aprotinin, and 1 mmol/L sodium orthovanadate). Aliquots (25 μg) of protein were separated by SDS-PAGE (7%) and probed with anti–phospho-tyrosine antibody (Santa Cruz Biotechnology).

Evaluation of the Mitogenic Activity of Immobilized GST-Tat

GM7373 cells were allowed to adhere onto 96-well plates coated with different proteins. Then, cells were washed with 2.0 mmol/L EDTA/PBS, trypsinized, and counted in a Burker chamber (T_0) or incubated for 72 hours in fresh medium containing 0.4% FCS in the absence or in the presence of the peptidomimetics before counting.

EC-Monolayer Wound Healing and Membrane-Ruffle Formation Assay

GM7373 cell monolayers adherent to 3.5 cm-polystyrene nontissue culture plates coated with different substrata were wounded with a rubber policeman and incubated at 37°C with medium containing 0.4% FCS in the absence or in the presence of the peptidomimetics. Then, the cells at the edge of the wound were photographed, and those showing membrane ruffles were counted under an inverted microscope (Olympus 1×51) connected to a Camedia C-4040 digital camera (Olympus Biosystem). For wound-healing assay, wounded monolayers were incubated for 48 hours at 37°C and photographed under the inverted microscope. The extent of wound repair was evaluated by measuring the area of the wound by computerized image analysis using the Image Pro-Plus analysis system. In some experiments, wounded monolayers of GM7373 cells adherent to GST-Tat were fixed in 3% paraformaldehyde, 2% sucrose, permeabilized with 0.2% Triton-X100, and saturated with 3% BSA (all in PBS). Then cells were incubated for 1 hour at room temperature with anti–phospho-Tyr_{397}-FAK antibody (8 nmol/L plus 3% BSA in PBS), washed and incubated for 45 minutes with goat anti-rabbit biotinylated antibodies (1:500 in PBS), then washed and incubated for 30 minutes with Alexa Fluor 488 streptavidin. Cells were photographed under an Axioplan 2 microscope equipped for epifluorescence (Carl Zeiss).

Chick-Embryo Chorioallantoic Membrane Assay

The assay was performed as described.[16]

Statistical Analysis

Results are expressed as mean±SEM of 3 to 6 separate experiments. Student *t* test was used for statistical analysis.

Results

Effect of the RGD-Peptidomimetic SCH221153 on Tat/$\alpha_v\beta_3$ Interaction

As shown in Figure 1A, $\alpha_v\beta_3$ binds to immobilized GST-Tat. The interaction is specific because $\alpha_v\beta_3$ does not bind to immobilized BSA nor to GST protein.[10] Preincubation of $\alpha_v\beta_3$ with 1.0 mmol/L SCH221153 prevents its interaction

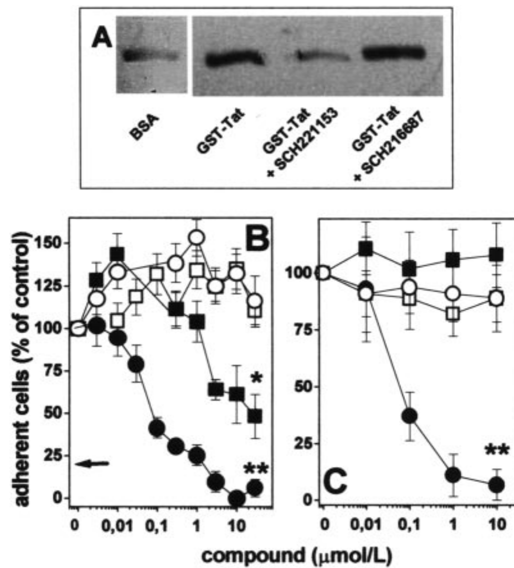

Figure 1. Effect of SCH221153 on Tat/$\alpha_v\beta_3$ interaction and EC adhesion. A, $\alpha_v\beta_3$ was incubated onto plastic-coated BSA or GST-Tat in the absence or in the presence of SCH221153 or SCH216687. Then, plastic-bound proteins were extracted and analyzed by Western blotting with anti-β_3 antibodies. B, GM7373 cells were allowed to adhere to plastic-immobilized GST-Tat (black symbols) or FN (white symbols) in the presence of SCH221153 (circles) or SCH216687 (squares). Then, the number of adherent cells was evaluated and expressed as percentage of cells adherent to GST-Tat or to FN in the absence of competitors. Arrow points to the value of cell adhesion to immobilized GST-Tat in the presence of anti–$\alpha_v\beta_3$-neutralizing monoclonal antibody LM609 (700 nmol/L). C, HUVECs were allowed to adhere to GST-Tat or FN in the absence or in the presence of SCH221153 or of SCH216687 (symbols as in B). Then, the number of adherent cells was evaluated. $^*P<0.001$, $^{**}P<10^{-4}$, Student *t* test.

with GST-Tat whereas preincubation with the negative control SCH216687 is ineffective (Figure 1A). It should be pointed out that recent observations using BIACORE technology have shown that Tat/$\alpha_v\beta_3$ interaction occurs with an affinity similar to that of different physiological $\alpha_v\beta_3$ ligands (dissociation constant (K_d) being equal to 32 nM, 27 nM, and 64 nM for Tat, fibrinogen, and vitronectin, respectively).[10] These data underline the efficacy of SCH221153 as an inhibitor of Tat/$\alpha_v\beta_3$ interaction.

GM7373 cells adhere to GST-Tat–coated plastic in a $\alpha_v\beta_3$-dependent manner, as demonstrated by the inhibition exerted by anti-$\alpha_v\beta_3$ monoclonal antibody LM609, here used as a positive control (Figure 1B). Accordingly, SCH221153 inhibits GM7373 cell adhesion to immobilized GST-Tat in a dose-dependent manner, whereas the negative control SCH216687 exerts only a limited inhibition with a potency ≈200× lower than that of SCH221153 (Figure 1B). In keeping with its selective $\alpha_v\beta_3$-antagonist activity, SCH221153 does not affect GM7373 cell adhesion to the $\alpha_5\beta_1$ ligand FN (Figure 1B). SCH221153 inhibits also the adhesion of HUVECs to GST-Tat without affecting their adhesion to FN (Figure 1C). Again, SCH216687 was ineffective in preventing HUVECs adhesion to immobilized Tat, further supporting the specificity of the Tat-antagonist effect of SCH221153.

SCH221153 inhibits GM7373 cell adhesion to immobilized GST-Tat with a potency that is 200× and 25× higher than that showed by the linear peptide GRGDSPK and by the cyclic peptide cRGDFV, respectively. Also, SCH221153 is 170× more potent than its negative control SCH216687, whereas GRGDSPK and cRGDFV peptides are only 4 to 6× more potent than their corresponding negative controls (see Table I, available online at http://atvb.ahajournals.org).

Tat binds also the vascular endothelial growth factor receptor-2/KDR[18] and heparan sulfate proteoglycans (HSPGs).[19] KDR/PAECs and HL3T1 cells were used to study the effect of SCH221153 on Tat interaction with KDR and HSPGs, respectively.[16] At variance with suramin (here used as a positive control),[16] SCH221153 does not affect the binding of biotinylated Tat nor Tat-dependent KDR autophosphorylation in KDR-overexpressing PAECs (KDR/PAECs) (see Figure I, available online at http://atvb.ahajournals.org). Also, at variance with the HSPG-antagonist heparin,[17] SCH221153 affects neither HSPG-dependent GST-Tat–green fluorescent protein internalization nor Tat-dependent HIV-long terminal repeat-transactivation in HL3T1 cells (see Figure II, available online at http://atvb.ahajournals.org). Nevertheless, SCH221153 retains the capacity to inhibit the adhesion of both KDR/PAECs and HL3T1 cells to immobilized GST-Tat without affecting their interaction with FN (see Figures I and II).

In conclusion, SCH221153 specifically inhibits the interaction of Tat with $\alpha_v\beta_3$ without affecting its interaction with KDR or HSPG receptors.

Effect of SCH221153 on Signal Transduction Triggered by Tat/$\alpha_v\beta_3$ Interaction

In ECs, integrin engagement by physiological ligands activates FAK and NF-κB that, in turn, mediate EC migration and proliferation in vitro and neovascularization in vivo.[20] On the other hand, Tat triggers FAK[21] and NF-κB[22] activation in different cell types. Accordingly, free GST-Tat induces FAK phosphorylation and NF-κB activation in GM7373 cells (Figure 2). Again, these responses are inhibited by SCH221153 but not by SCH216687, demonstrating that $\alpha_v\beta_3$ engagement is required for the activation of these 2 second messengers by Tat in ECs.

Effect of SCH221153 on $\alpha_v\beta_3$/Tat-Dependent Endothelial Cell Activation In Vitro

Integrin-dependent EC adhesion and signal transduction can be considered the first steps of the process that, through EC migration and proliferation, leads to neovascularization.[23] Accordingly, immobilized GST-Tat induces a significant increase in the proliferation rate of adherent GM7373 cells when compared with FN- or Poly-L-Lysin–adherent cells (Figure 3A). SCH221153 inhibits cell proliferation of GST-Tat–adherent cells whereas SCH216687 is ineffective. Also, SCH221153 has no effect on the limited proliferation observed in FN-adherent cells (Figure 3B). It must be pointed out that both compounds were added to cells only after their adhesion and spreading onto the substrate had occurred; this prevented cell rounding or complete detachment of ECs.[10]

Cell-membrane ruffling precedes the migration of EC body[24] and is considered a morphological phenotype of

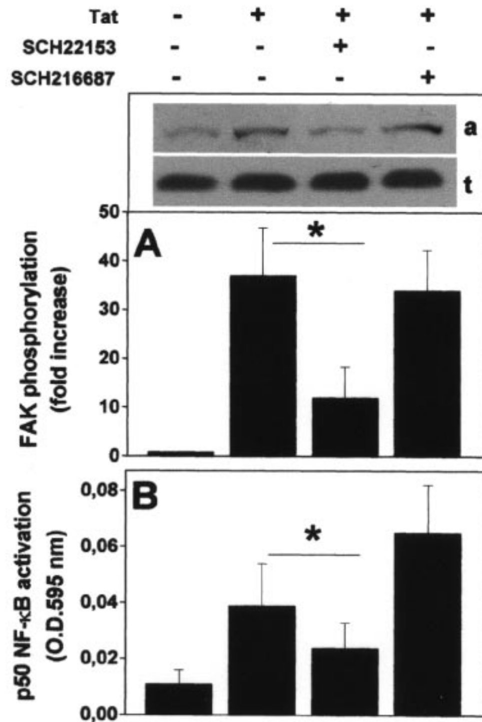

Figure 2. Effect of SCH221153 on signal transduction triggered by Tat/$\alpha_v\beta_3$ interaction. Serum-starved GM7373 cells were treated with or without GST-Tat (3 nmol/L), SCH221153, or SCH216687 (both at 0.3 mmol/L). Then, FAK phosphorylation (A) or NF-κB activation (B) were evaluated. In A, a indicates phosphorylated protein; t, total protein. *$P<0.001$, Student t test.

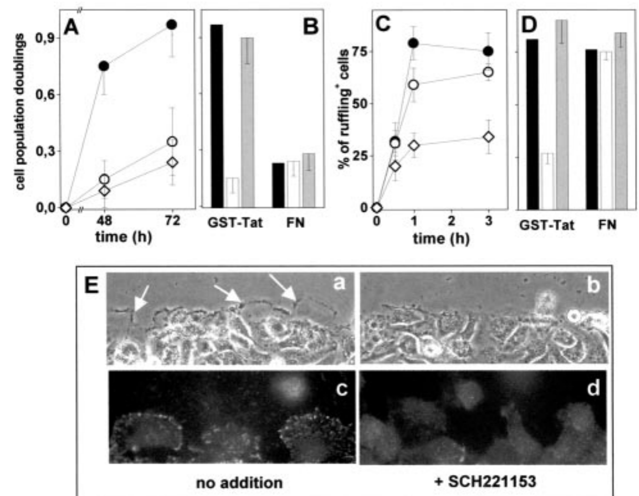

Figure 3. Effect of SCH221153 on Tat-dependent proliferation, membrane ruffling, and FAK phosphorylation. GM7373 cells adherent to immobilized GST-Tat (●), FN (○), or PL (◇) were incubated for the indicated period of time (A). Alternatively, GST-Tat– or FN-adherent GM7373 cells were incubated for 72 hours in the absence (black bars) or in the presence of SCH221153 (white bars) or of SCH216687 (gray bars) (0.3 μmol/L) (B). Then, cells were counted. Data are expressed as cell population doublings. Monolayers of GM7373 cells adherent to the different proteins (symbols as in A) were wounded and incubated for the indicated periods of time (C). Alternatively, wounded monolayers of GM7373 cells adherent to GST-Tat or to FN were incubated for 3 hours in the absence or in the presence of peptidomimetics (symbols and doses as in B) (D). Then, membrane ruffling was evaluated. E, Photographs of the edge of a wounded monolayer of GST-Tat–adherent GM7373 cells in the absence (no addition) or in the presence of SCH221153. a and b, phase contrast photographs (200×). Arrows point to the most prominent ruffles. c and d, cells immunostained with anti–phospho-Tyr_{397}-FAK antibody and photographed under an epifluorescence microscope (630×).

motile cells.[25] Wounded monolayers of GM7373 cells adherent to GST-Tat rapidly form membrane ruffles at their leading edge. Cell-membrane ruffling is observed also in FN-adherent cells and, to a much lesser extent, in PL-adherent cells (Figure 3C). SCH221153 hampers membrane ruffling in GST-Tat–adherent cells (Figure 3D). Specificity of the effect is shown by the inability of SCH216687 to affect this process and by the lack of effect of SCH221153 on membrane ruffling in FN-adherent GM7373 cells (Figure 3D). Also, SCH221153 prevents the localization of phosphorylated FAK at the leading edge of migrating cells after wounding of GST-Tat–adherent monolayer (Figure 3E).

The ability of immobilized GST-Tat to stimulate proliferation and motility in adherent GM7373 cells results in an increase in the capacity of a mechanically wounded cell monolayer to cover the denuded area when compared with FN- or PL-adherent cells (Figure 4). SCH221153, added to cell cultures after wounding, inhibits the capacity of GST-Tat–adherent cells to heal the wound, with no effect on the slower repair observed in FN-adherent cells (Figure 4B and 4C). Under the same experimental conditions SCH216687 does not alter wound repair in GST-Tat– or FN-adherent cells (Figure 4B).

Effect of SCH221153 on Tat-Mediated Neovascularization

As shown in Figure 5, chick-embryo chorioallantoic membranes (CAM) implanted with gelatin sponges that were loaded with GST-Tat show numerous allantoic vessels converging toward the implant. SCH221153 causes a significant inhibition of this activity, whereas SCH216687 is ineffective. Both compounds do not alter the unstimulated physiological vascularization of the chick-embryo CAM. No vascular reactions were detectable around the sponge in the specimens treated with vehicle alone.

Discussion

$\alpha_v\beta_3$/Tat interaction plays an important role in the activation of ECs that, in turn, contributes to the arise of AIDS-associated pathologies.[26] On the other hand, nonpeptidic analogs of the cell adhesion motif RGD endowed with integrin-antagonist capacity have been successfully used to block different angiogenesis-related processes.[13] Here, we demonstrate that the RGD-peptidomimetic compound SCH221153 inhibits Tat/$\alpha_v\beta_3$ interaction and the consequent EC activation in vitro and neovascularization in vivo.

Tat can be present as a free molecule or associated with the extracellular matrix.[9] Consistently, $\alpha_v\beta_3$ is expressed both at the basal and luminal aspects of endothelium.[27] Here, we observed that free Tat triggers an $\alpha_v\beta_3$-dependent activation of FAK and NF-κB in ECs and that immobilized Tat induces EC adhesion, proliferation, and the acquisition of a motile phe-

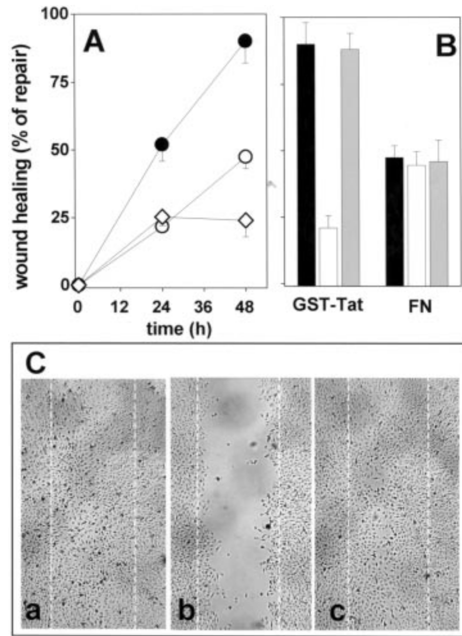

Figure 4. Effect of SCH221153 on Tat-dependent repair of a mechanically wounded EC monolayer. A, GM7373 cells adherent to GST-Tat (●), FN (○), or PL (◇) were wounded and incubated for the indicated periods of time. B, Wounded monolayers of GM7373 cells adherent to GST-Tat or to FN were incubated for 48 hours in the absence (black bars) or in the presence of SCH221153 (white bars) or of SCH216687 (gray bars) (0.3 μmol/L). Then, the area of the wound was evaluated. Data are expressed as percentage of repair in respect to the denuded area measured at the beginning of the experiment. C, Photographs (50×) of wounded monolayers of Tat-adherent GM7373 cells after 48 hours in the absence (no addition) or in the presence of SCH221153 or of SCH216687. White dotted lines mark the edge of the wound at the beginning of the experiment.

notype; this contributes to the faster rate of healing of a Tat-adherent wounded EC monolayer. Notably, SCH221153 retains its Tat-antagonist capacity when the transactivating factor is presented to ECs either in its free or substratum-immobilized form.

Several experimental evidences point to the specificity of the Tat/$\alpha_v\beta_3$ antagonist activity of SCH221153: (1) the control compound SCH216687 was poorly effective in all the assays; (2) SCH221153 inhibits new blood vessel formation triggered by Tat in the chick-embryo CAM without affecting basal vascularization; (3) SCH221153 inhibits EC adhesion, membrane ruffling and wound healing in cells adherent to immobilized Tat but not immobilized FN. These observations are in keeping with the high specificity of SCH221153 for $\alpha_v\beta_3$ when compared with different integrin receptors.[13]

In KDR/PAECs, SCH221153 does not affect the interaction of Tat with KDR. Relevant to this point, the occupancy and activation of KDR play a pivotal role in mediating the angiogenic activity of Tat.[18] The observation that SCH221153 inhibits Tat-mediated angiogenesis in the chick-embryo CAM without affecting Tat/KDR interaction and autophosphorylation suggests that the binding of Tat to both $\alpha_v\beta_3$ and KDR receptors are required to stimulate neovascularization, the single interaction with either 1 of the 2 receptors being insufficient to support a full angiogenic response. This is in keeping with the existing cross-talk between integrins and tyrosine kinase receptors in neovascularization triggered by angiogenic growth factors.[28]

Accordingly, both $\alpha_v\beta_3$[29] and KDR[30] mediate the biological effects of Tat in KS, pointing to the possibility of multitarget therapies aimed at blocking the 2 receptors simultaneously. Relevant to this point, a humanized anti-$\alpha_v\beta_3$ antibody selectively targets human KS in nude mice,[31] and the KDR inhibitor SU5416 exerts therapeutic benefits in patients with AIDS-associated KS.[32] It is also worth noting that Tat synergizes with fibroblast growth factor-2 in the pathogenesis of KS.[33] The observation that SCH221153 inhibits also $\alpha_v\beta_3$/fibroblast growth factor-2 interaction and consequent EC activation[13] suggests that RDG peptidomimetics may exert multitarget effects in KS.

Besides angiogenesis, the implication of ECs in AIDS-associated pathologies is manifold. Thus, RGD peptidomimetics may inhibit also Tat-induced endothelial permeability and related dissemination of HIV-1 and HIV-1+ monocytes, possibly preventing the rise of lymphomas and of AIDS-dementia. Also, they may prevent Tat-dependent vasorelaxation and endothelial nitric oxide synthase downregulation, with possible benefits for AIDS-associated cardiovascular diseases. Related to this point, smooth muscle cells, well known effectors of atherogenesis, adhere to immobilized Tat via $\alpha_v\beta_3$,[34] suggesting that RGD peptidomimetics may exert Tat-antagonist activity also on this cell type.

A number of integrin antagonists have been developed, including cyclic RGD-containing peptides and anti-$\alpha_v\beta_3$ monoclonal antibodies. Here, we found that the Tat-antagonist potency of SCH221153 is higher than that of cyclic and linear RGD-containing peptides. Also, small-molecular weight RGD mimetics with oral bioavailability, like SCH221153, may have a number of advantages over peptide-based or antibody-based approaches. Indeed, nonpeptide

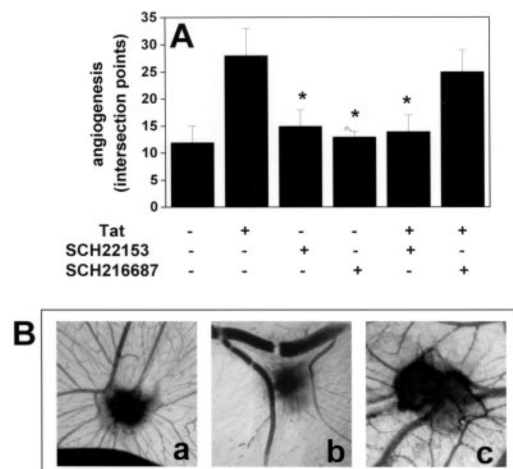

Figure 5. Effect of SCH221153 on Tat-induced angiogenesis in the chick-embryo CAM. A, Chick-embryo CAMs were implanted with gelatin sponges adsorbed with vehicle or GST-Tat (12 pmoles) in the absence or in the presence of SCH221153 and SCH216687 (7 nmoles). At day 12 the angiogenic response was scored. B, Chick-embryo CAMs implanted with gelatin sponges containing GST-Tat in the absence (no addition) or in the presence of SCH221153 and SCH216687 (original magnification 5×).

RGD-analogs can be designed or modified to increase their oral bioavailability[35] and their transepithelial transport.[36] Taken together, our data suggest the possibility to use RGD peptidomimetics for the treatment of $\alpha_v\beta_3$-dependent pathologies associated with AIDS.

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