An Exochelin of *Mycobacterium tuberculosis* Reversibly Arrests Growth of Human Vascular Smooth Muscle Cells *in Vitro*

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Proliferation of vascular smooth muscle cells (VSMC) is characteristic of restenosis following balloon angioplasty. We show here that a low concentration of a novel iron chelator, desferri-exochelin 772SM, reversibly arrests the growth of human VSMC *in vitro*, specifically in G0/G1 and S phases. The lipophilic desferri-exochelin is effective more rapidly and at a 10-fold lower concentration than the nonlipophilic iron chelator deferoxamine. Treatment of growth-synchronized VSMC with the desferri-exochelin results in down-regulation of cyclin E/Cdk2 and cyclin A/Cdk2 activity but does not affect the cyclin D/Cdk4/retinoblastoma phosphorylation pathway. Both DNA replication and RNA transcription are inhibited in exochelin-treated cells, but protein synthesis is not. The ability of desferri-exochelin 772SM to reversibly block the growth of VSMC *in vitro* with no apparent cytotoxicity suggests that the exochelin may be useful as a therapeutic agent to limit restenosis in injured vessels.

Although vascular smooth muscle cells (VSMC) are normally quiescent, they enter the cell cycle when exposed to growth factors *in vitro* or following vascular injury *in vivo*. In animal models, proliferation and migration of VSMC begin soon after vascular injury occurs and culminate in formation of a neointima that encroaches on the interior space of the vessel (1). Such neointima formation is seen in a substantial number of patients following balloon angioplasty and may result in a narrowing or blockage (restenosis) of the vessel that requires further intervention (2). By preventing the immediate proliferation of VSMC following vascular injury, it may be possible to arrest neointima formation and restenosis. In this report, we describe the cell cycle-specific growth inhibitory effects of a novel iron chelator, desferri-exochelin 772SM, on serum- and growth factor-stimulated human VSMC *in vitro*.

Iron is required for a variety of cellular functions, including respiration, energy metabolism, and DNA synthesis. In addition, iron participates in redox reactions that generate free radicals, which may activate signaling pathways for cell proliferation. Treatment with iron chelators has previously been shown to cause growth arrest in several types of cells (3–5). However, the effect of chelation varied depending upon both the cell type and the chelator used (5–7).

The iron chelator deferoxamine (DFO) inhibited DNA synthesis in cultured rat VSMC (8) and blocked neointimal growth in a rat model of vascular injury (8). However, DFO enters cells only very slowly by pinocytosis (9) and causes hypotension when given in large doses *in vivo* (10). Therefore, DFO is of limited usefulness for determining mechanisms by which iron deprivation prevents cell cycle progression or for the clinical treatment of restenosis.

Exochelins, the secreted siderophores of *Mycobacterium tuberculosis*, are a family of high affinity iron chelators that are both water- and lipid-soluble, a property that allows them to enter cells rapidly and chelate specific intracellular iron pools (11–13). In our experiments, desferri-exochelin 772SM arrested the growth of human VSMC specifically in the G0/G1 and S phases. No cytotoxicity was seen after 72 h of exposure to the desferri-exochelin, and the cell cycle arrest was fully reversible. On the basis of these results, exochelins may prove useful experimentally for elucidating the role of iron in cell growth and clinically as drugs capable of inhibiting the formation of restenotic lesions following vascular injury.

MATERIALS AND METHODS

**Exochelin**—Synthetic desferri-exochelin 772SM (11) was provided by Keystone Biomedic, Inc. The exochelin was >98% pure and ~2% iron-saturated, and it was chemically and functionally indistinguishable from native desferri-exochelin 772SM isolated from the culture filtrate of *M. tuberculosis*.2 Desferri-exochelin 772SM has a molecular weight of 719, and was prepared as a 1.159 mM stock solution in 0.09% NaCl. Iron-loaded exochelin (ferri-exochelin) was prepared by solubilizing desferri-exochelin 772SM in 20% ethanol at 37 °C, diluting it in 0.1% trifluoroacetic acid, and saturating it with iron by the addition of a 10-fold excess of iron as ferric ammonium citrate. Ferri-exochelin 772SM was purified by using reverse phase high pressure liquid chromatography and eluting with a 0–50% acetonitrile gradient.

**Cell Culture**—Human VSMC were isolated from saphenous veins discarded from surgical procedures or from aorta or iliac artery from unused transplant donor tissue (14). Primary VSMC cultures were maintained in Dulbecco’s modified Eagle’s medium/F-12 medium containing 2.5% heat-inactivated fetal bovine serum. 2.5% heat-inactivated human cord serum, penicillin (100 units/ml), and streptomycin (100 µg/ml) under 5% CO2 in air. All experiments were performed with second or third passage cells. The cells were quiesced by changing the medium to 0.05% human cord serum, 0.05% fetal bovine serum in Dulbecco’s modified Eagle’s medium/F-12 for 24 h, washing with PBS, and then changing to serum-free Dulbecco’s modified Eagle’s medium/F-12 medium for 48 h. Cells were released from quiescence by stimulation with 20 ng/ml recombinant hu-
man epidermal growth factor (EGF) (Research and Diagnostic Systems, Inc., Minneapolis, MN) and medium with 5% serum (2.5% fetal bovine serum, 2.5% human cord serum).

**Flow Cytometry**—The VSMC were harvested in 1 mM EDTA in PBS and then centrifuged at 200 \( \times g \) at 25 \(^\circ\)C for 10 min. The cell pellet was resuspended in PBS, and a sample was removed and stained with propidium iodide using the method of Krishan (15). Cell cycle analysis was performed using a Coulter Epics XL flow cytometer (Beckman-Coulter, Hialeah, FL). Data were collected for 10,000 events for each sample. The Modfit LT program (Verity Software House, Topsham, ME) was used for cell cycle modeling.

**Western Blotting**—The VSMC were harvested as above, pelleted, resuspended in lysis buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 0.1% Nonidet P-40, 1% glycerol, 1 mM dithiothreitol, 20 mM Na$_3$PO$_4$, and 1% protease inhibitor (Sigma)) and stored at –80 \(^\circ\)C. The cell suspensions were thawed on ice, and the cells were lysed by sonication. The sonicate was centrifuged to remove cell debris, and the supernatant was collected. Protein concentrations were determined for each lysate using the DC protein assay kit (Bio-Rad). Total protein samples (30 \( \mu \)g) were separated by electrophoresis through 10% SDS-polyacrylamide gels. The proteins were transferred to an Immobilon-P membrane (Millipore Corp., Bedford, MA) using a Mini Trans-Blot system (Bio-Rad). The membrane was stained with Ponceau Red to confirm equal loading and then blocked overnight at 4 \(^\circ\)C in PBS containing 5% nonfat dry milk. The appropriate primary antibody was added; the membrane was incubated for 2 h at room temperature, and then the secondary antibody was added for an additional 1-h incubation. The protein bands were detected by ECL+ Western blotting system (Amersham Pharmacia Biotech) and exposure of films (Amersham Pharmacia Biotech). Antibodies that were used include 14591A (anti-cyclin E) and 14001A (anti-Rb) from Pharmingen (San Diego, CA), and sc-753 (anti-cyclin D1), sc-751 (anti-cyclin A), and sc-528 (anti-p27) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-rabbit IgG-HRP (sc-2030) and anti-mouse IgG-HRP (sc-2031) (Santa Cruz Biotechnology) were used as secondary antibodies.

**Tritium Uptake**—VSMC were grown in six-well plates with 2 ml of medium/well. Tritiated thymidine ([methyl-$^3$H]thymidine), uridine ([5,6-$^3$H]uridine) and leucine ([4,5-$^3$H]leucine) (all from NEN Life Science Products) were each diluted in sterile PBS to a working concentration of 25 \( \mu \)Ci/ml. Eighty \( \mu \)l of the appropriate tritiated solution were added per well. After a 4-h incubation under standard cell culture conditions, the medium was aspirated, and the wells were washed twice with PBS. Each well was treated with 0.2 \( \mu \)l perchloric acid for 2–3 min and then washed again with PBS. One ml of 1% SDS, 0.1 \( \& \) NaOH was added per well, and the contents of each well were transferred to a large scintillation vial with 16 ml of Ecoscint H scintillation solution. The radioactivity in each sample was determined in a Beckman LS6500 scintillation counter.

**Kinase Assays**—Kinase assays were based on the methods of Matsushime et al. (16). Cells were harvested by scraping into immunoprecipitation buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2.5 mM dithiothreitol, 0.1% Tween 20, 10\% phenylmethylsulfonyl fluoride, 100 units/ml aprotinin, 10 mM \( \beta \)-glycerophosphate, 1 mM Na$_3$F, and 0.1 mM sodium orthovanadate) and snap-frozen in liquid nitrogen. Extra lysates were dislodged by scraping, incubated in the wells for 20 min at 4 \(^\circ\)C, and then transferred to a microcentrifuge tube. The lysate was clarified by centrifuging for 30 min at 26,000 \( \times g \) at 4 \(^\circ\)C. Tritiated thymidine uptake was measured as an indicator of cellular proliferation. Each value is the mean ± S.D. for triplicate samples.

Sigma unless otherwise indicated.

**$^{32}$P, Labeling and Immunoprecipitation**—VSMC grown in six-well plates were washed with prewarmed labeling medium (Dulbecco’s modified Eagle’s medium, no phosphate; Life Technologies, Inc.), the medium was removed, and then 0.5 ml of medium was added per well. $^{32}$PO$_4$ in HCl (ICN, Costa Mesa, CA) was added to a final concentration of 2 \( \mu \)Ci/ml, and the cells were incubated for 2 h under standard cell culture conditions. The cells were washed with PBS, and 0.3 ml of lysis buffer (1% (v/v) Nonidet P-40, 0.15 mM Na$_3$PO$_4$, 0.2 mM sodium vanadate, 100 units/ml aprotinin) was added to each well. The cells were dislodged by scraping, incubated in the wells for 20 min at 4 \(^\circ\)C, and then transferred to a microcentrifuge tube. The lysate was clarified by centrifuging for 30 min at 26,000 \( \times g \) at 4 \(^\circ\)C. For immunoprecipitation, protein A PLUS-agarose beads (Santa Cruz Biotechnology) were preabsorbed with saturating amounts of rabbit polyclonal B-myb antibody (Santa Cruz sc-724). Fifty \( \mu \)l of precoated beads were added to each sample and incubated for 2 h at 4 \(^\circ\)C with mixing. The immunoprecipitated proteins on beads were washed four times with lysis buffer and then resuspended in protein sample buffer. The samples were boiled for 5 min, the beads were spun out, and the supernatants were electrophoresed through a 10% polyacrylamide gel. After drying down the gel, phosphorylated proteins were visualized by autoradiography.

**RESULTS**

Experiments were performed with a lipophilic exochelin, desferri-exochelin 772SM (13). To determine the effect of the exochelin on growth of VSMC, we treated actively growing cells with various concentrations of desferri-exochelin 772SM (closed circles), iron-loaded exochelin (open circles), or deferoxamine (squares). Tritiated thymidine uptake was measured as an indicator of cellular proliferation. Each value is the mean ± S.D. for triplicate samples.

![Graph showing dose response of VSMC to iron chelators](image)

**FIG. 1. Dose response of VSMC to iron chelators.** Actively growing VSMC were treated for 6 h (a) or 24 h (b) with various concentrations of desferri-exochelin 772SM (closed circles), iron-loaded exochelin (open circles), or deferoxamine (squares). Tritiated thymidine uptake was measured as an indicator of cellular proliferation. Each value is the mean ± S.D. for triplicate samples.

Sigma unless otherwise indicated.
into DNA during S phase provides an indirect measure of cell growth and replication. In VSMC treated with desferri-exochelin 772SM for 6 or 24 h, [methyl-3H]thymidine uptake was inhibited in a dose-dependent manner (Fig. 1). In contrast, cell growth was not attenuated in VSMC treated with iron-loaded exochelin 772SM (Fig. 1a). Therefore, inhibition of VSMC growth by the desferri-exochelin is probably due entirely to its iron chelating capacity. VSMC treated with the nonlipophilic iron chelator deferoxamine (DFO) for 6 h exhibited little inhibition of [methyl-3H]thymidine uptake (Fig. 1a). Although treatment for 24 h with DFO caused a dose-dependent inhibition of [methyl-3H]thymidine uptake in the VSMC, a 10-fold higher concentration of DFO was needed to achieve a level of inhibition comparable with that of the desferri-exochelin (Fig. 1b). Since cells exposed to desferri-exochelin 772SM for 72 h retained the ability to exclude trypan blue (not shown), the exochelin was not cytotoxic.

To obtain a profile of the normal progression of VSMC through the cell cycle, we synchronized VSMC by quiescing them for 3 days, resulting in 80–95% of the cells accumulating in G0/G1 as determined by flow cytometry (FACS). The quiesced cells were stimulated with medium containing 5% serum and 20 ng/ml EGF, and samples were taken every 2 h. By FACS analysis, the VSMC entered S phase 16–18 h after stimulation and entered G2/M 22–24 h after stimulation (not shown).

To determine whether desferri-exochelin 772SM arrests cell growth at a particular point in the cell cycle, we added 50 μM desferri-exochelin to cultures of VSMC at specific time points during or after release from quiescence and analyzed samples by FACS. When the desferri-exochelin was added to quiesced VSMC simultaneously with EGF and serum, the cells remained arrested in G0/G1, while the control cells traversed the cell cycle (Fig. 2a). Similarly, when desferri-exochelin was added to cells that were mostly in S phase (18 h), the cells were arrested in S phase, while the control cells progressed to G2/M (Fig. 2b). However, when desferri-exochelin was added to cells that were mostly in G2/M (27 h), the cells progressed through G2/M and into G1 in the same manner as the control cells (Fig. 2c). Therefore, desferri-exochelin 772SM specifically blocks VSMC progression through the G0/G1 and S phases but not through G2/M.

To assess the reversibility of the effect of the desferri-exochelin, we treated quiescent VSMC with desferri-exochelin 772SM for 24 h and then washed the cells and incubated them in fresh medium containing serum and EGF but no exochelin. The cells resumed normal progression through the cell cycle within 12 h, as determined by FACS (Fig. 3). Therefore, the cell cycle arrest caused by the desferri-exochelin is reversible.

Uptakes of [methyl-3H]thymidine, [5,6-3H]uridine, and L-[4,5-3H]leucine were measured to evaluate the effect of desferri-exochelin on DNA replication, RNA transcription, and protein synthesis, respectively. Quiesced VSMC were stimulated with EGF and serum for 6 h to obtain a synchronous G1 cell population or for 20 h to obtain a synchronous S phase.
population, as confirmed by FACS. Desferri-exochelin 772SM or vehicle (saline) was added to the synchronized cells for 2 h, and then the appropriate tritiated compound was added for an additional 4 h. There was, as expected, no uptake of [methyl-\textsuperscript{3}H]thymidine in the control or treated cells in G\textsubscript{0}/G\textsubscript{1}. The high uptake of [methyl-\textsuperscript{3}H]thymidine seen in control cells in S phase was almost totally blocked by treatment with desferri-exochelin (Fig. 4a). Desferri-exochelin treatment markedly reduced uptake of [5,6-\textsuperscript{3}H]uridine in both of the synchronized cell populations (Fig. 4b). In contrast, uptake of L-[4,5-\textsuperscript{3}H]leucine was similar in control and exochelin-treated VSMC in both populations (Fig. 4c). Therefore, desferri-exochelin treatment of VSMC inhibits DNA replication in S phase and RNA transcription in both G\textsubscript{0}/G\textsubscript{1} and S phases but does not inhibit protein synthesis within this time frame.

Progression through the cell cycle is controlled primarily by the cyclins and cyclin-dependent kinases (Cdks) (17). Although the protein levels of cyclins vary through the cell cycle, the protein levels of Cdks are relatively constant. Kinase activity of the Cdks is regulated by site-specific phosphorylation and by their association with cyclins or cell cycle inhibitors such as p21 and p27. In G\textsubscript{1}, cyclin D associates with Cdk4 and Cdk6 to form active kinases that hyperphosphorylate the retinoblastoma protein (Rb). In late G\textsubscript{1}/early S phase, cyclin E forms an active complex with Cdk2, which further phosphorylates Rb. As cells progress through S phase, cyclin E is replaced by cyclin A in the active Cdk2 complex. As cells enter M phase, the Cdk2 complex is degraded, and cyclin B and Cdc2 associate to form the active kinase.

To assess the effect of the exochelin on the cell cycle regulatory proteins, we stimulated quiescent VSMC with serum and EGF, with or without desferri-exochelin 772SM, and analyzed samples taken at specific time points. The degree of Rb phosphorylation and the level of cyclin proteins and kinase inhibitors were measured by Western blot analysis. The activities of the Cdks were measured using immunoprecipitation kinase assays. When the desferri-exochelin was added to quiescent cells simultaneously with EGF and serum, there were no significant differences between exochelin-treated and untreated cells in the levels of cyclin D, p27, or Cdk4 kinase activity (Fig. 5).

In contrast, the levels of cyclin E and cyclin A in exochelin-treated cells were reduced compared with control cells (Fig. 6). By densitometric analysis of the Western blots, the cyclin E protein level was reduced by approximately 40% in the exochelin-treated cells at both the 15- and 18-h time points. By 21 h after stimulation, the control cells were entering S phase, and the cyclin E level was decreasing, while cyclin A was increasing. At this point, the exochelin-treated cells had 50% less cyclin A than the untreated cells. The reduced protein levels of cyclins E and A were accompanied by a 50% reduction in Cdk2 kinase activity. In both control and exochelin-treated cells, the Rb protein was progressively phosphorylated starting 8 h after stimulation. However, by densitometry, the percentage of Rb that was hyperphosphorylated after the 15-h time point was decreased in exochelin-treated cells compared with untreated cells. Therefore, the desferri-exochelin does not affect the cyclin D/Cdk4/Rb pathway, but does down-regulate cyclin E/Cdk2 and cyclin A/Cdk2 activity and Rb phosphorylation. In addition, there was a 50–85% reduction in the total amount of Rb protein present in the exochelin-treated cells, which is consistent with a G\textsubscript{1} arrest (18).

When desferri-exochelin 772SM was added after the cells had reached S phase, the exochelin did not cause a decrease in the amount of cyclin E or cyclin A protein, and Rb was fully phosphorylated, as compared with untreated cells. However,
FIG. 5. Effect of desferri-exochelin on cell cycle regulatory proteins in G1. 50 μM desferri-exochelin 772SM (+) or vehicle (−) was added to quiescent cells (0 h) simultaneously with serum and EGF. At specific time points following treatment, cells were harvested, and the protein levels of cyclin D and p27 were determined by Western blotting. Cdk4 kinase activity was measured by immunoprecipitation and kinase assay.

FIG. 6. Down-regulation of cyclin E, cyclin A, and Cdk2 by desferri-exochelin. 50 μM desferri-exochelin 772SM (+) or vehicle (−) was added to quiescent VSMC (0 h) simultaneously with serum and EGF. Cells were harvested at specific time points following treatment, and the protein levels of cyclin E and cyclin A were determined by Western blotting. Cdk2 kinase activity was measured by immunoprecipitation and kinase assay. The protein level and degree of phosphorylation of Rb were determined by Western blotting.

Cdk2 activity was inhibited by at least 55% (Fig. 7). One important S phase substrate of cyclin A/Cdk2 is B-myb. To evaluate the effect of exochelin treatment on the phosphorylation of B-myb in S phase, quiescent cells were stimulated with EGF and serum for 16 h, and then 50 μM exochelin or vehicle was added and cells were harvested at 18, 21, and 24 h, with each sample labeled with 32P for 2 h before harvesting. By immunoprecipitation of B-myb from these samples and quantification by densitometry, we found that the phosphorylation of B-myb was consistently reduced by 45–50% in the exochelin-treated cells as compared with untreated cells (not shown).

DISCUSSION

The proliferation of VSMC following vascular injury is central to neointima formation and restenosis. Vascular injury during balloon angioplasty induces increased expression of growth factors and cytokines that stimulate the proliferation of VSMC. The mRNA levels of the proto-oncogenes c-fos and c-jun are elevated within 15 min of balloon injury in rat aortas and are translated into functional proteins within 2 h (19). These early proteins may act as transcription factors for growth factor genes that promote VSMC growth following vascular injury. Replication of VSMC in the media wall within 24–72 h after injury may represent a shift in cellular phenotype that determines subsequent migration, inflammation, and matrix remodeling that result in restenosis (20).

Since the critical events that initiate VSMC proliferation occur within the first few hours after vascular injury (1), a pharmacological agent that can effectively block the immediate proliferation of VSMC may inhibit neointima formation. Antagonists to growth factors have been tested as antiproliferative drugs but without significant success in vivo. Recent studies have focused on the cell cycle machinery itself as a target for antiproliferative agents. Increased levels and activation of cell cycle proteins during lesion formation have been demonstrated (21). The importance of Cdkks in cell cycle regulation has generated interest in finding chemical inhibitors of the Cdkks. Several strategies have targeted individual gene products with antisense oligonucleotides (22–24) or ribozymes (25) in an effort to block signaling pathways. However, the discovery of numerous members of the Cdk and cyclin families suggests that cell cycle regulation is much more complex than a single linear chain of events, with many converging and redundant pathways. Therefore, blocking individual signaling pathways may not effectively prevent cell cycle progression.

Iron chelation may provide a broader approach to inhibiting VSMC growth. Iron is an essential element found in all mammalian cells. It is present in the structure of many enzymes and proteins that regulate energy metabolism, respiration and DNA synthesis. In addition, iron participates in redox reactions that generate free radicals. Since H2O2 is involved in the induction of c-fos and c-myc and can directly increase DNA synthesis in VSMC (26), a change in the intracellular redox potential may activate signaling pathways for cell proliferation. Iron chelation may inhibit VSMC growth by blocking redox-modulated signaling pathways as well as by inhibiting the activity of various critical enzymes.

In these studies, we used a unique lipophilic chelator, desferri-exochelin 772SM, which has a very high affinity for iron and is able to block redox reactions (13). In synchronized human VSMC, desferri-exochelin 772SM arrested growth in both G0/G1 and S phases. This growth inhibition was reversible, and there was no cytotoxicity observed after 72 h of treatment. In contrast, the lipid-insoluble iron chelator deferoxamine was able to block growth of VSMC only after a much longer incubation time and at a 10-fold higher concentration. The greater efficacy of desferri-exochelin 772SM is probably due to its lipophilicity. A correlation between lipophilicity and antiproliferative activity has been shown previously for other iron chelators (27). The lipophilic property of the desferri-exochelin allows it to enter cells rapidly and may allow it to preferentially chelate iron from critical intracellular sites that are not accessible to lipid-insoluble chelators (13). By chelating iron from pools other than cytosolic free iron, desferri-exochelin may avoid up-regulating transferrin receptors that import iron or inducing release of iron from intracellular ferritin.

Treatment with iron chelators in several types of cells has previously been shown to cause cell cycle arrest in either G0 or S phase (3–5). However, the effect of chelation varied depending upon both the cell type and the chelator used (5–7). Some iron chelators produced an irreversible block in cell growth, while others were cytotoxic (6, 28). Conflicting results were
described NPAT protein, which promotes S phase entry is a second mechanism by which exochelin affects Cdk2 activity. The addition of desferri-exochelin to cells that have already entered S phase does not reduce the amount of cyclin E or Rb phosphorylation. However, the kinase activity of Cdk2 is still inhibited, suggesting that there is a second mechanism by which exochelin affects Cdk2 activity, perhaps by altering the phosphorylation or dephosphorylation state of the Cdk2 kinase. The activity of the S phase transcription factor B-myb is dependent on phosphorylation by cyclin A/Cdk2 (32) and has been implicated in regulation of cell cycle progression. The reduced phosphorylation of B-myb by Cdk2 may contribute to the S phase arrest induced by the desferri-exochelin.

One of the major cellular requirements for iron in late G1 and S phase is due to the increased activity of the iron-containing enzyme ribonucleotide reductase, which catalyzes the rate-limiting step of nucleotide biosynthesis (33). The cell cycle arrest in S phase and the lack of DNA synthesis in VSMC after treatment with desferri-exochelin 772SM probably result at least in part from inhibition of ribonucleotide reductase. Blocking DNA synthesis through inactivation of ribonucleotide reductase may activate a checkpoint mechanism that disrupts the Cdk2 pathway to prevent further progression through S phase.

Iron chelation by desferri-exochelin arrests the growth of VSMC in both G0/G1 and S phases. By interfering with more than one cell cycle regulatory pathway, desferri-exochelin provides an approach to inhibiting cell growth that is broader and may be more effective than a strategy that targets individual cell cycle proteins. The ability of desferri-exochelin 772SM to reversibly block the growth of VSMC in vitro with no apparent cytotoxicity suggests that exochelin may be useful as a therapeutic agent to prevent or inhibit postangioplasty restenosis.

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