Blockade of the Intermediate-Conductance Calcium-Activated Potassium Channel as a New Therapeutic Strategy for Restenosis

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**Background**—Angioplasty stimulates proliferation and migration of vascular smooth muscle cells (VSMC), leading to neointimal thickening and vascular restenosis. In a rat model of balloon catheter injury (BCI), we investigated whether alterations in expression of Ca$^{2+}$-activated K$^+$ channels (K$_{Ca}$) contribute to intimal hyperplasia and vascular restenosis.

**Methods and Results**—Function and expression of K$_{Ca}$ in mature medial and neointimal VSMC were characterized in situ by combined single-cell RT-PCR and patch-clamp analysis. Mature medial VSMC exclusively expressed large-conductance K$_{Ca}$ (BK$_{Ca}$) channels. Two weeks after BCI, expression of BK$_{Ca}$ was significantly reduced in neointimal VSMC, whereas expression of intermediate-conductance K$_{Ca}$ (IKCa1) channels was upregulated. In the aortic VSMC cell line, A7r5 epidermal growth factor (EGF) induced IKCa1 upregulation and EGF-stimulated proliferation was suppressed by the selective IKCa1 blocker TRAM-34. Daily in vivo administration of TRAM-34 to rats significantly reduced intimal hyperplasia by $\approx$40% at 1, 2, and 6 weeks after BCI. Two weeks of treatment with the related compound clotrimazole was equally effective. Reduction of intimal hyperplasia was accompanied by decreased neointimal cell content, with no change in the rate of apoptosis or collagen content.

**Conclusions**—The switch toward IKCa1 expression may promote excessive neointimal VSMC proliferation. Blockade of IKCa1 could therefore represent a new therapeutic strategy to prevent restenosis after angioplasty. (*Circulation. 2003; 108:1119-1125.*)

**Key Words:** angioptasy • restenosis • ion channels

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Percutaneous balloon angioplasty, an intervention to relieve arterial stenosis and improve blood flow, is complicated by vascular restenosis within weeks as the result of proliferation of vascular smooth muscle cells (VSMC) and consequent narrowing of the vessel lumen. Complex interactions between numerous growth-stimulating molecules have been proposed to promote migration and proliferation of VSMC, leading to neointima formation. Proliferating VSMC are characterized by alterations in functional plasticity as they switch from a contractile phenotype to a dedifferentiated phenotype.

Ca$^{2+}$-activated K$^+$ channels (K$_{Ca}$) are important regulators of VSMC function. Mature VSMC predominantly express the calcium-activated large-conductance channel (BK$_{Ca}$) or maxi K$,^4$ a product of the Slo gene, which plays a pivotal role in VSMC relaxation by dampening depolarization-dependent activation of Ca$^{2+}$ channels and Ca$^{2+}$ influx through membrane hyperpolarization. In contrast to the vasodilatory function of BK$_{Ca}$, the role of other K$_{Ca}$ channels in VSMC is incompletely understood. The intermediate-conductance K$_{Ca}$ channel encoded by the IKCa1 gene (also known as IK1, hSK4, KCa4, and K$_{Ca}$3.1 as per the new IUPHAR nomenclature: http://www.iuphar.org/compendium2.htm) has been proposed to be an important regulator of cell proliferation. In lymphocytes and fibroblasts, upregulation of IKCa1 expression is an essential step in mitogenesis.~6,9~

In the present study, we tested the hypothesis that a reorganization of K$_{Ca}$ channel expression pattern after angioplasty promotes neointimal cell proliferation. After balloon catheter injury (BCI) to rat carotid artery (CA), neointimal VSMC switched K$_{Ca}$ gene expression from Slo to IKCa1, representing a change from a K$_{Ca}$ subtype mediating vasodilation to a K$_{Ca}$ subtype promoting cell proliferation. Blockade of IKCa1 by the antimycotic clotrimazole (CLT) and its selective derivative TRAM-34 resulted in inhibition of epidermal growth factor (EGF)-stimulated VSMC proliferation in vitro and in reduced neointima formation in vivo.

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Methods

Balloon Catheter Injury and Treatment Protocols
Under the aegis of a protocol approved by the local Animal Care and Use Committee, Sprague-Dawley rats (weight, 350 to 450 g) were subjected to BCI of the left CA by use of a 2F Fogarty embolectomy catheter (Baxter Scientific). Rats were killed 2 weeks (n = 5) after BCI, and left and right CA were excised. Separate groups (each n = 7) were treated with CLT (120 mg/kg per day) for 1, 2, and 6 weeks after BCI. Another group (n = 7) was treated with CLT (120 mg/kg per day) for 2 weeks. TRAM-34 and CLT serum levels and TRAM-34 concentrations in liver and subcutaneous fat were quantitatively determined by bioassay.

Neointimal thickening was determined in paraffin-embedded and differential nonserial cross sections stained with hematoxylin and eosin to visualize nuclei and cytoplasm, or with Sirius red to detect collagen. Histomorphological analysis was done with the use of a computerized analysis system (Scion Image) in a blinded manner.

Mature and Neointimal VSMC and Patch-Clamp Experiments
Isolation of mature and neointimal VSMC, whole-cell patch-clamp experiments in situ, and data analysis were performed as described previously.

Reverse Transcription and Single-Cell RT-PCR
Reverse transcription of mRNA from single-cell samples and “multiplex” single-cell RT-PCR were performed as described previously. Primer pairs for small KCa (rSK-1, rKCa1), and endothelial nitric oxide synthase (ENOS) as endothelial cell markers are stated elsewhere. First and “nested” primer pairs for rSlo and myosin heavy chain (rMyHC) as VSMC markers spanned intronic sequences, and identity of PCR products was verified by sequencing. Primer, rSlo: F5′-GGAGCATTAGGGATGTGTT-3′; R5′-GGGATGGAGTGAGAGAGA-3′; nested: F5′-TTTTCCGGCT-G AGAGATGCC-3′; R5′-TTTTCCGGCT-G AGAGATGCC-3′; rat glyceraldehyde-3-phosphate dehydrogenase (rGAPDH); F5′-CGCAACAGT-CAAAGCTGAG-3′; R5′-CACCATGACCTCTTTCCAGGCA-3′; (GenBank accession: AB170801). Each 25-μL PCR reaction consisted of 500 nmol forward and reverse primer, 150 nmol probe, 3 μL cDNA, and 1× TaqMan Universal Master Mix (Perkin-Elmer ABI). PCR parameters were 50°C×2 minutes, 95°C×10 minutes, and 50 cycles at 95°C×15 seconds, 60°C×1 minute.

Threshold cycles (Ct) were calculated by means of TaqMan software (ABI, User Bulletin No. 2). Real-time RT-PCR signals for rKCa1 and rMyHC were standardized to rGAPDH by use of the equation CtX–CtCONTROL=ΔCt. The equation, ΔCtX=ΔCtX−ΔCtCONT, was used to determine changes in expression, where the ΔCtX−value (EFG-stimulated) was subtracted from the control ΔCtCONT−value (w/o=without stimulus) of the same experiment. Fold increases in expression were calculated by the equation 2ΔCt=−fold change.

Statistical Analysis
Data are given as mean±SEM. If appropriate, the Wilcoxon rank sum test or χ2 analyses were used to assess differences between groups. Values of P<0.05 were considered significant.

Results

Alterations in KCa Functional Expression in Neointimal VSMC After BCI
To measure functional KCa channel expression, we performed whole-cell patch-clamp experiments on freshly isolated mature VSMC and neointimal VSMC in situ after BCI. Mature VSMC (n = 14) from normal CA exhibited an outward Ca2+ activated and voltage-dependent KCa current with characteristics of the cloned BKCa channel. The outward KCa current was small at negative membrane potentials, increased steeply at positive membrane potentials, and was blocked by the selective BKCa inhibitor iberiotoxin (IbTX) (Figure 1A, left panel), with a potency (KD 1120 Circulation September 2, 2003

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nmol/L) blocked these currents with potencies similar to the cloned IKCa1 channel. The currents were not affected by TRAM-7 (1 μmol/L), an inactive analog of TRAM-34, or by the SK blocker APA (1 μmol/L) or the Kc blocker 4-aminopyridine (2 mmol/L) (not shown). When normalized for membrane capacitance (Figure 1D), the mean KCa current in neointimal VSMC was significantly increased at 40 and 0 mV and reduced at +100 mV, compared with mature VSMC, reflecting the shift from BKCa expression in mature VSMC to a mixture of IKCa and BKCa in neointimal cells.

**Alterations in BKCa and IKCa1 mRNA Expression in Neointimal VSMC After BCI Correlate With Changes in Functional Expression**

We used “multiplex” single-cell RT-PCR to determine whether the changes in functional BKCa and IKCa expression after BCI correlated with alterations in mRNA levels for the Slo and IKCa1 genes, respectively. The VSMC marker MyHC was detected in all mature VSMC (34/34) and in all neointimal VSMC (18/18) 2 weeks after BCI. Endothelial cell–specific eNOS expression was not detected in any of the cell samples, demonstrating that our VSMC samples are not contaminated with endothelial cells. None of the negative controls (n=24) yielded any PCR products.

Mature VSMC that functionally express BKCa and not IKCa channels (Figure 1A) contained substantial quantities of Slo mRNA (87%; 54/62) and no IKCa1 mRNA (0/27; Figure 2). Two weeks after BCI, the Kc gene expression pattern in neointimal VSMC was altered (Figure 2), in keeping with the changes observed in the amplitude of BKCa and IKCa currents in these cells (Figure 1B and 1D). Slo transcripts were detected less frequently (24/67; 36%) and IKCa1 transcripts more frequently (42/67; 63%) than in mature VSMC (χ² analysis; P<0.01 and P<0.001, respectively). Transcripts of SK1-SK3 genes were not detected in mature or neointimal VSMC (data not shown). These results indicate that changes in Slo and IKCa1 mRNA levels after BCI contribute to the changes in BKCa and IKCa functional expression in VSMC.

**EGF-Induced Upregulation of IKCa1 Expression and Proliferation of VSMC**

The switch from BKCa expression in mature VSMC to a mixture of IKCa and BKCa in neointimal cells after BCI may reflect a change from a contractile to a proliferating phenotype. To test this hypothesis, we examined IKCa function and IKCa1 mRNA expression in the aortic VSMC cell line A7r5 after mitogenic stimulation with EGF. Forty-eight hours after stimulation, the amplitude of the K⁺ current increased 3-fold
compared with untreated cells (Figure 3A and Table 1, P < 0.01). Parallel RT-PCR studies revealed a 6-fold increase in \( \text{IKCa1} \) mRNA levels as early as 2 hours after EGF stimulation and a 3-fold increase after 48 hours (Table 1). Pharmacological studies confirmed that the \( \text{K}^+ \)/\( \text{Ca}^2+ \) currents in EGF-treated cells were indeed \( \text{IKCa1} \) (Figure 3B). The currents were not affected by the \( \text{BKCa} \) inhibitor IbTX (100 nmol/L) but were blocked by TRAM-34 (K \( \text{D} \) 8 \( \mu \)mol/L), ChTX (K \( \text{D} \) 6 \( \mu \)mol/L), and CLT (K \( \text{D} \) 30 \( \mu \)mol/L) with potencies similar to \( \text{IKCa1} \). Thus, the channel expression pattern in EGF-stimulated A7r5 cells resembles that seen in proliferating neointima in vivo.

To test whether the enhanced \( \text{IKCa} \) expression in VSMC might have functional consequences, we examined whether the \( \text{IKCa1} \) inhibitors TRAM-34 and CLT could suppress EGF-stimulated mitogenesis of A7r5 cells. The cell count increased 145 ± 8% 48 hours after EGF stimulation but only 109 ± 3% in unstimulated A7r5 cells (P < 0.001). TRAM-34 (IC \( \text{50} \) 8 \( \mu \)mol/L) and CLT (IC \( \text{50} \) 14 \( \mu \)mol/L) suppressed EGF-stimulated proliferation in a dose-dependent fashion, reducing mitogenesis to baseline levels at 100 nmol/L (Figure 3C). The inactive triarylmethane TRAM-7 (1 \( \mu \)mol/L) did not suppress proliferation, indicating that the suppressive effect of TRAM-34 and CLT are not the result of nonspecific toxicity. The \( \text{BKCa} \) blocker IbTX (100 nmol/L) also had no effect on proliferation (Figure 3C). These results suggest that upregulation of \( \text{IKCa1} \) channel expression is required for EGF-induced VSMC proliferation, as has been reported in lymphocyte activation and fibroblast mitogenesis.6–8,18

**Figure 2.** Multiplex single-cell RT-PCR analysis of single mature and VSMC. Ethidium bromide–stained gels of RT-PCR products from representative mature VSMC and neointimal VSMC at week 2 after BCI. rSlo and rIKCa1 (upper panel) and myosin heavy chain rMyHC (lower panel). Columns show quantitative analysis of rSlo and rIKCa1 expression in mature VSMC (rats, n = 9) and neointimal VSMC (rats, n = 5). **P < 0.01, Wilcoxon rank sum test.

**Figure 3.** EGF upregulates \( \text{IKCa1} \) expression and induces proliferation of the VSMC line, A7r5. A, Representative \( \text{Ca}^2+ \)/activated \( \text{K}^+ \) currents in unstimulated (w/o) and EGF-stimulated cells (48 hours). B, Blockade of \( \text{IKCa} \) currents in A7r5 cells by 100 nmol/L TRAM-34 but not by 100 nmol/L IbTX. C, Dose-dependent inhibition of EGF-induced proliferation (percent cell proliferation after 48 hours) by TRAM-34 (n = 4 to 10, •) and CLT (n = 6 to 8, ○) but not by inactive TRAM-7 (1 \( \mu \)mol/L; n = 3, ▲) or by IbTX (100 nmol/L; n = 7; Δ). Dashed line indicates baseline proliferation in A7r5 cells in the absence of EGF.

**TRAM-34 and CLT Suppress BCI-Induced Intimal Hyperplasia In Vivo**

\( \text{IKCa1} \) upregulation in proliferating neointimal VSMC and the effectiveness of \( \text{IKCa1} \) blockers in suppressing EGF-induced proliferation of A7r5 VSMC suggest that in vivo \( \text{IKCa1} \) blockade might reduce intimal hyperplasia in CA of rats after BCI. We tested this idea by administration of CLT and TRAM-34 after BCI. Data from these experiments are summarized in Table 2, and representative cross sections of CA are shown in Figure 4.

An initial 2-week trial with CLT (120 mg/kg per day) yielded encouraging results, but the CLT-treated rats gained less weight than the vehicle-treated group, and hepatomegaly developed as the result of reported CLT liver toxicity mediated by inhibition of P450-dependent enzymes. We therefore switched to the selective \( \text{IKCa1} \) inhibitor TRAM-34 (120 mg/kg...
per day), which has no effect on P450-dependent enzymes and does not cause overt acute toxicity after intravenous administration. Although neointima formation progressively increased from week 1 to week 6 after BCI in the vehicle-treated group, the area of the neointimal cell layer in the TRAM-34–treated group was significantly smaller at week 1 (−64%; \(P<0.01\)), week 2 (−35%; \(P<0.01\)), and week 6 (−43%; \(P<0.01\)) after BCI (Figure 4 and Table 2). Two weeks’ treatment with CLT also resulted in a pronounced reduction of neointimal formation (−50%; \(P<0.001\), Figure 4 and Table 2). The area of the medial smooth muscle cell layer was not different between rats treated with TRAM-34, CLT, or vehicle. The ratio of neointimal/media areas (N/M) was not different between rats treated with TRAM-34, CLT, or vehicle.

TABLE 1. Mitogenic Regulation of rIKCa1 Expression and Function in A7r5 Cells

<table>
<thead>
<tr>
<th>Cell Treatment</th>
<th>n</th>
<th>rGAPDH (Ct)</th>
<th>rIKCa1 (ΔCt)</th>
<th>rMyHC (ΔCt)</th>
<th>Cell Area</th>
<th>n (Cells)</th>
<th>I_{Ca} (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>w/o</td>
<td>16</td>
<td>22.6±0.5</td>
<td>12.4±0.4</td>
<td>9.2±0.3</td>
<td>w/o</td>
<td>14</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>EGF (2-h)</td>
<td>17</td>
<td>22.7±0.6</td>
<td>9.6±0.4†</td>
<td>2.8 (−6-fold)</td>
<td>EGF</td>
<td>16</td>
<td>3.4±0.6†</td>
</tr>
<tr>
<td>EGF (48-h)</td>
<td>11</td>
<td>21.7±0.8</td>
<td>10.8±0.4†</td>
<td>1.6 (−3-fold)</td>
<td></td>
<td></td>
<td>9.0±0.4</td>
</tr>
</tbody>
</table>

Real-time RT-PCR analysis of rat intermediate-conductance Ca^{2+}-activated K⁺ channel (rIKCa1) and rat myosin heavy chain (rMyHC) expression (left) and Ik_Ca currents at 0 mV (right) in rat VSMC cell line A7r5 after EGF stimulation for 48 hours. \(ΔCt−ΔCt_{\text{vehicle}}\). \(2^{ΔΔCt}\) indicates fold increase in expression; w/o, no stimulation. 
†\(P<0.01\), ‡\(P<0.001\) vs w/o; Wilcoxon rank sum test.

TABLE 2. Effect of TRAM-34 and CLT on Intimal Hyperplasia After BCI

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>Neointimal Area, mm²</th>
<th>Medial Area, mm²</th>
<th>Wall Area Bounded by EEL, mm²</th>
<th>N/M</th>
<th>Residual Lumen Area, mm²</th>
<th>rL/Ct</th>
<th>Nuclei Count, Cell No.</th>
<th>Rate of Apoptosis, %</th>
<th>Collagen Content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>4</td>
<td>0.011±0.001</td>
<td>0.084±0.005</td>
<td>0.095±0.007</td>
<td>0.13±0.01</td>
<td>0.22±0.01</td>
<td>0.93±0.04</td>
<td>159±17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>11</td>
<td>0.097±0.006</td>
<td>0.099±0.003</td>
<td>0.195±0.009</td>
<td>1.05±0.06</td>
<td>0.16±0.01</td>
<td>0.64±0.05</td>
<td>984±82</td>
<td>0.9±0.6</td>
<td>11±2</td>
</tr>
<tr>
<td>6 wk after BCI</td>
<td>5</td>
<td>0.169±0.008</td>
<td>0.091±0.008</td>
<td>0.260±0.011</td>
<td>1.92±0.22</td>
<td>0.13±0.01</td>
<td>0.50±0.04</td>
<td>1525±79</td>
<td>&lt;&lt;1</td>
<td>19±3</td>
</tr>
<tr>
<td>TRAM-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1 wk</td>
<td>4</td>
<td>0.004±0.001*</td>
<td>0.084±0.004</td>
<td>0.088±0.004</td>
<td>0.05±0.01†</td>
<td>0.21±0.02</td>
<td>0.96±0.05</td>
<td>47±16*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>6</td>
<td>0.063±0.005†</td>
<td>0.095±0.002</td>
<td>0.158±0.006†</td>
<td>0.66±0.05†</td>
<td>0.21±0.02*</td>
<td>0.91±0.06†</td>
<td>601±361</td>
<td>1.0±0.6</td>
<td>15±2</td>
</tr>
<tr>
<td>6 wk after BCI</td>
<td>5</td>
<td>0.096±0.018†</td>
<td>0.082±0.006</td>
<td>0.179±0.022†</td>
<td>1.15±0.18†</td>
<td>0.18±0.01†</td>
<td>0.81±0.06†</td>
<td>612±97†</td>
<td>&lt;&lt;1</td>
<td>11±4</td>
</tr>
<tr>
<td>CLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk after BCI</td>
<td>7</td>
<td>0.049±0.010†</td>
<td>0.102±0.004</td>
<td>0.151±0.009†</td>
<td>0.49±0.10†</td>
<td>0.24±0.02†</td>
<td>0.82±0.05*</td>
<td>407±82†</td>
<td>0.6±0.5</td>
<td>13±1</td>
</tr>
</tbody>
</table>

EEL indicates external elastic lamina; N/M, ratio of neointimal/media areas; and rL/Ct, ratio of residual/contralateral lumens.
*\(P<0.05\), †\(P<0.01\), ‡\(P<0.001\) vs vehicle, Wilcoxon rank sum test.
versus 3.5 for CLT) accumulates in these tissues. Thus, after subcutaneous administration in peanut oil, TRAM-34 was slowly released into the blood stream, resulting in serum levels sufficient to suppress proliferation (in vitro: IC50 8 nmol/L) over a 24- to 48-hour period.

To understand the mechanism by which TRAM-34 and CLT reduced neointima formation, we investigated cell proliferation, apoptosis, and extracellular matrix (collagen) content. The neointimal nuclei count, a measure of cell proliferation, was reduced by 70% (P<0.05) after 1 week, by 39% (P<0.01) after 2 weeks, and by 61% (P<0.001) after 6 weeks of TRAM-34 treatment.

A similar reduction (−59%, P<0.001) in the neointimal nuclei count was observed in the CLT-treated group at 2 weeks after BCI. However, the collagen content and the rate of apoptosis (percentage of apoptotic nuclei) in the neointima was not different in TRAM-34–treated and CLT-treated rats compared with vehicle-treated control animals (Table 2 and Figure 5A and 5B). Taken together, our results demonstrate that in vivo IKCa1 blockade reduces neointima formation and vessel narrowing through inhibition of VSMC proliferation.

**Discussion**

Using the rat balloon catheter injury model, we demonstrate that neointimal formation after angioplasty is associated with a switch in Kca channel expression from exclusive BKCa expression in mature contractile VSMC to downregulated BKCa and upregulated IKCa expression in proliferating neointimal VSMC. A similar upregulated IKCa channel expression pattern was observed in proliferating aortic VSMC A7r5 cells after stimulation with EGF, and selective IKCa1 blockade suppressed A7r5 mitogenesis in vitro, suggesting that IKCa1 channels play an important role in VSMC proliferation. Consistent with this idea, in vivo blockade of IKCa1 channels reduced BCI-triggered neointimal formation and vessel re-narrowing, which suggests a novel therapeutic strategy for the prevention of restenosis after angioplasty.

Neointimal proliferation and IKCa upregulation after BCI is mediated by numerous mitogenic factors. Using the aortic VSMC line A7r5 as a model system, we demonstrated that mitogenic doses of EGF augment IKCa1 RNA and IKCa1 current amplitude. Uregulated IKCa1 expression has been similarly reported to contribute to the proliferation of mitogen-stimulated fibroblasts8 and human T lymphocytes.6,7,14,18 Enhanced IKCa1 expression might therefore be a functional characteristic of proliferating and dedifferentiated cells.8,15

IKCa channels may promote VSMC mitogenesis by enhancing the electrochemical driving force for Ca2+ influx through membrane hyperpolarization and thus sustain a high intracellular Ca2+ concentration required for gene transcription, as has been reported in lymphocytes and fibroblasts.5,7,14 IKCa1 may play a more important role than BKCa in shaping Ca2+ signals of proliferating VSMC because its higher Ca2+ affinity3,4,6,7,11,13–16 would result in channel opening and membrane hyperpolarization in response to subtle increases in the intracellular Ca2+ concentration. Induction of IKCa1 expression might thus be a required step for neointimal VSMC proliferation after BCI. Consistent with such a role, IKCa1 blockade by CLT and the specific inhibitor TRAM-34 suppressed the proliferation of cultured VSMC. IKCa1 blockers may therefore have therapeutic value for preventing neointimal proliferation and restenosis after BCI.
In a rat model of BCI, in vivo administration of CLT significantly reduced neointimal thickening, but the trial was discontinued after 2 weeks because of reduced weight gain and the development of severe hepatomegaly, presumably because of liver toxicity caused by blockade of cytochrome P450–dependent enzymes. A subsequent trial with TRAM-34, an IKCa1 selective inhibitor that does not block cytochrome-P450 enzymes or exhibit acute toxicity, significantly reduced neointimal hyperplasia and vessel narrowing without causing visible signs of organ damage. The therapeutic effect of TRAM-34 was due to inhibition of neointimal cell proliferation and not due to increased apoptosis or decreased matrix formation. In conclusion, targeting IKCa1 channels in proliferating VSMC with TRAM-34 might have therapeutic utility in the prevention of restenosis after angioplasty and for the treatment of other cardiovascular disorders characterized by abnormal VSMC proliferation.

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