Reverse Regulation of Endothelial Cells and Myointimal Hyperplasia on Cell Proliferation by a Heatshock Protein-Coinducer After Hypoxia

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Background and Purpose—Myointimal hyperplasia (MIH) cells are related to permanent upregulated proliferation as tumor-like cells. The aim of this study is to assess whether treatment of cells after hypoxia by Iroxanadine heat-shock protein (HSP-coinducer) predicts recovery through cell proliferation.

Methods—Vascular smooth muscle cells (VSMC) and brain capillary endothelial cells (HBEC) were isolated from human origin and MIH-cells from early carotid restenosis after surgery. Cell proliferation was quantified by bromuridine (BrdU) incorporation after hypoxia/reoxygenation. HSP72 and cyclin-dependent kinase (CDKN1A) mRNA expression was assessed by reverse transcription-polymerase chain reaction (PCR) and cell cycle distribution by flow cytometry (FACS) analysis.

Results—After hypoxia/reoxygenation, the proliferation of MIH-cells increased, whereas endothelial cells decreased (MIH: 0.266±0.016 versus 0.336±0.024; P<0.05; HBEC: 1.249±0.10 versus 0.878±0.11; P<0.05). Whereas augmented proliferation of MIH-cells was reduced (40% to 45%) by HSP-coinducer, diminished HBEC proliferation increased (46.2%). Stress-activated-protein-kinase (SAPK)p38-dependent cell cycle redistribution was generated by an increase in HSP72 and CDKN1A mRNA levels in MIH-cells.

Conclusions—The 2 key players of early restenosis (MIH, EC) were oppositely regulated and correspondingly after treatment by HSP-coinducer reverse recovered. Drug candidate may have therapeutic potential in (re)restenosis. (Stroke. 2008;39:1022-1024.)

Key Words: carotid stenosis ■ endothelium ■ heatshock-protein ■ proliferation ■ smooth muscle cells
Acc. No.: U13369), amplified cyclin dependent kinase (CDKN1A)/WAF-1 (forward and reverse Acc. No.: L25610) and HSP72 cDNA (forward and reverse Acc. No.: L12723) specific primer pairs were designed. The amplified reverse transcription-polymerase chain reaction (PCR) products were visualized and demonstrated on figure. Statistical analyses were performed by 1-way ANOVA and significance calculated by Student \( t \) test.

**Results**

The proliferation rate of nonpathological cells: VSMC (n=4) and HBECs (n=6) decreased (21.2%, \( P<0.049 \), and 30.7%, \( P<0.009 \)) after hypoxia/reoxygenation. Conversely, the activity of cultured MIH (n=7) cells increased (31.4%, \( P<0.001 \); Figure 1a). Using HSP-coinducer at normoxia, the upregulated proliferation rate of MIH-cells reduced (22.8 \( \pm \) 1.1 \( P<0.048 \)) similarly after hypoxia/reoxygenation (0.1 \( \mu \)mol/L to 1 \( \mu \)mol/L Iroxanadine 37.1 \( \pm \) 2.5\% \( P<0.007 \), 34.3 \( \pm \) 3.0\% \( P<0.009 \); Figure 1b). Contrarily, the downstreamed proliferation rate of HBECs was upregulated while the test drug did not elicit any effect on VSMC (Figure 1c).

In MIH cell population the subpopulation of G0/G1 phase cells represented the highest percentage, but no change was observed after HSP-coinduction. The number of S phases of cells downstreamed (17.8 \( \pm \) 0.7/12.3 \( \pm \) 1.0\%; \( P<0.008 \)) and G2/M increased (10.2 \( \pm \) 1.1/17.0 \( \pm \) 0.9\%; \( P<0.0078 \); Figure 2a, b). An opposite effect appeared by SAPK p38 inhibitor (SB203580 1 \( \mu \)mol/L). The number of S phase cells increased (17.0 \( \pm \) 0.9 /21.2 \( \pm \) 1.0\%; \( P<0.046 \)) except for G2/M subpopulation (8.2 \( \pm \) 0.8 /9.8 \( \pm \) 1.0\%; Figure 2a). The HSP-coinducer (1 \( \mu \)mol/L) counteracted the effect of SB203580 (1 \( \mu \)mol/L) and increased the cell ratio of G2/M phase and decreased the upregulated cell ratio of S phase (Figure 2b). Thus, the HSP-coinducer induced p38-dependent cell cycle redistribution on cell subpopulations.

In VSMC after hypoxia/reoxygenation, HSP72 and CDKN1A mRNA levels did not change (data not depicted). In HBEC treated with HSP-coinducer (for 5 days) after hypoxia/reoxygenation both the HSP72 mRNA (4.9-fold) and the CDKN1A mRNA (4.7-fold) decreased. In MIH a slight upregulation of HSP72 (2.6- to 3.0-fold) and CDKN1A mRNA level (1.5- to 1.9-fold) was measured. The HSP-coinducer treatment reversed the effect of hypoxia/reoxygenation (Figure 3).

**Figure 1.** Proliferation of HBEC, VSMC and MIH-cells by incorporated BrdU. Proliferation (after 1 h hypoxia, 24 h reoxygenation) was measured by ELISA, calculated (mean ± SEM). Difference of significance are *\( P<0.05 \), **\( P<0.01 \) and ***\( P<0.005 \) by Student \( t \) test. a, Proliferation rations of 3 cells are compared on normoxia (dark bars); on hypoxia (open bars) and Hypox−Hypoxia; SD=Serum Deprivation; b, treated MIH cell proliferation on normoxia and on hypoxia/reoxygenation. Control cells (dark bars), treated by HSP-coinducer: 0.1 \( \mu \)mol/L (open lines), 1.0 \( \mu \)mol/L (ruled bars) and 10 \( \mu \)mol/L (dotted bars); c, proliferation of HBEC and control VSMC after hypoxia/reoxygenation are depicted at normoxia (dark bars) and after hypoxia/reoxygenation (open bars). The HSP-coinducer treatment is demonstrated as shown above.
Discussion

A definitive therapy on restenosis and in-stent stenosis, especially targeting VSMC as well as endothelial cell dysfunction, is warranted.4

Cultured MIH-cells with hypoxia/reoxygenation are used as model of restenosis to test drug actions. In between MIH-cells and endothelial cells reverse regulation was observed on cell proliferation particularly after hypoxia. The proliferation of nonpathological cells (VSMC and EC) decreased while pathological MIH-cells greatly increased. This novel observation explains different mechanisms in early restenosis.

Drug-induced HSPs may have therapeutic effects under different pathological conditions.1 After treatment by HSP-coinducer, the augmented proliferation of MIH-cells decreased but the proliferation of deregulated EC cells was enhanced. Simultaneously, the expression of HSP72 and CDKN1A are upregulated in agreement with clinical reports where compromised endothelial cell proliferation and upregulated HSP72 expression was described in VSMC after transluminal balloon angioplasty.5

MIH-cells are characterized by augmented proliferation, similar to tumor cells. Induced by heat or stress proteins, tumor suppressor p53 inhibited cell growth by enhanced expression of CDKN1A/p21.6 While not in HBEC and VSMCs, CDKN1A/p21 upregulation was documented in the MIH cell culture after HSP-induction.

As we documented, MIH and endothelial cells, the 2 key players of early restenosis, were oppositely regulated. Iroxanadine, a HSP-coinducer, may prevent restenosis by its regulatory action.

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Disclosures

None.

References

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