Anti-proliferation effect of 3-amino-2-imino-3,4-dihydro-2H-1,3-benzothiazin-4-one (BJ-601) in human vascular endothelial cells: G0/G1 p21-associated cell cycle arrest.

李文森

Yu CH;Wu J;Su YF;Ho PY;Liang YC;Sheu MT;Lee WS

Abstract

T The aim of this study was to examine the anti-proliferation effect of 3-amino-2-imino-3,4-dihydro-2H-1,3-benzothiazin-4-one (BJ-601) on human vascular endothelial cells and its possible molecular mechanism underlying. Our data showed that BJ-601 at a range of concentrations (0–40 mM) dose- and time-dependently decreased cell number in cultured human dermal microvascular endothelial cells (HDMVECs), but not human fibroblasts. The BJ-601-induced growth inhibition in HDMVECs was reversible. [3H]thymidine incorporation demonstrated that BJ-601 arrested the HDMVECs at the G0/G1 phase of the cell cycle. Western blot analysis revealed that BJ-601 (0–40 mM) dose-dependently increased the levels of the protein p21, but not of p27, p53, cyclins A, D1, D3 and E, cyclindependent kinase 2 (CDK2), and CDK4 in HDMVECs. Immunoprecipitation showed that the formation of the CDK2–p21 complex, but not CDK2–p27, CDK4–p21 and CDK4–p27 complexes, was increased in the BJ-601-treated HDMVECs. Kinase assay further demonstrated that CDK2, but not CDK4, kinase activity was decreased in a dose-dependent manner in the BJ-601-treated HDMVECs. Pretreatment of HDMVECs with a p21 antisense oligonucleotide, which blocked the expression of p21 protein, reversed the BJ-601- induced inhibition of [3H]thymidine incorporation into HDMVECs. Moreover, cotreatment of the endothelial cells with protein kinase C (PKC) inhibitor, staurosporine, prevented the BJ-601-induced decrease of [3H]thymidine incorporation into HDMVECs. Administration of BJ-601 dose-dependently inhibited capillary-like tube formation of HDMVECs in Matrigel. In conclusion, these data suggest that BJ- 601 inhibits HDMVECs proliferation by increasing the level of p21 protein, which in turn inhibits CDK2 kinase activity, and finally causes retardation of the cell cycle at the G0/G1 phase.