Effect of Nicotine On Innate Antiviral Pathways and HCV Replication

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Shunhei Yamashina1, Tomokazu Mizui1, Kazuyoshi Kon1, Kenichi Ikejima1, Tsuneo Kitamura2, Yoshiyuki Takei3, Sumio Watanabe1
1. Dept of Gastroenterology, Juntendo university, Tokyo, Japan, 2. Dept of Gastroenterology, Juntendo University Urayasu hospital, Chiba, Japan, 3. Dept of Gastroenterology, Mie University, Mie, Japan

ABSTRACT in program book

Background: Previous clinical studies revealed that smoking, independent of alcohol, could aggravate the histological activity of chronic hepatitis C. Recently, it was shown that treatment with nicotine activates cellular mitogen-activated protein kinase (MAPK) including p38. The p38 signaling pathway is involved in the interferon (IFN)-mediated anti-HCV activity. Therefore, we investigated if treatment with nicotine interferes with the replication of HCV.

Methods: A cell line transfected with HCV replicon expressing selectable chimeric reporter protein of neomycin phosphotransferase and firefly luciferase (Huh7/Rep-Feo) were cultured in D-MEM media containing 10% FCS. Cells were incubated with nicotine (10-100 nM) and/or IFNa (10 U/ml) for 24 hours, and levels of HCV replication was analyzed by luciferase assay. SB-20358, wortmannin and LY294002 were used to inhibit MAPK and PI3K pathways. WST-1 assay was performed to evaluate the cytotoxic effects of nicotine, SB-20358, wortmannin and LY294002. The protein level of phospho-p38, phospho-Akt and phospho-STAT-1 in Huh7/Rep-Feo cells treated with nicotine was evaluated by western blot analysis.

Results: Incubation with nicotine at 100 nM for 24 hours suppressed the luciferase activity of Huh7/Rep-Feo cells to about 70% of control. This anti-viral effect of nicotine was blunted by addition of p-38 MAPK inhibitor SB-20358. On the other hand, treatment with 10 u/ml IFN-a for 24 hours reduced the replication of HCV replicon to about 50% of control; however, co-incubation of nicotine blunted the antiviral effect of IFN-a significantly. Addition of PI3K inhibitors (wortmannin, LY294002) attenuated this inhibitory effect of nicotine against IFN. Cell viability was not affected in the presence of nicotine, IFN, MAPK inhibitors. Treatment with nicotine enhanced the expression of phospho-p38, phospho-Akt and phospho-STAT1 in Huh7/Rep-Feo cells.

Conclusion: Nicotine inhibits the replication of HCV mediated by the activation of p38 MAPK pathway. In contrast, **when combined with IFN-a, nicotine disturbed the antiviral effects of IFN on HCV replication,** involving PI-3K/Akt signaling pathway. In conclusion, treatment with nicotine possesses the unique effects on innate cellular defense pathways through the activation of cellular MAPK.