PEGIFN alpha-2a and 5-fluorouracil suppresses proliferation of human hepatocellular carcinoma in P53-mediated apoptotic response

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Background and Aims: The tumor suppressor p53, activated in response to DNA damage, induces cell cycle arrest or apoptosis through transcriptional activation of its target genes. Thus, p53 has a central role in tumor suppression. On the other hand, interferon-α is used for the treatment of some forms of human cancer but the molecular basis for treatment is poorly understood. We hypothesized that pegylated interferon alpha-2a (PEG-IFN) and 5-fluorouracil (5-FU) reduce human hepatocellular carcinoma (HepG2) cell lines, in which p53 is functionally active) proliferation in nude mice.

Methods and Results: Cultured HepG2 cells (106 cells/mice) were subcutaneously injected to 5-weeks male BALB/c nude mice. Seven days later when the diameter of the tumor reached approximately 5-10 mm, the mice were randomly divided into 6 groups. Group 1: control group; Group 2: 5-FU (10 mg/kg/d), five times a week; Group 3: 5-FU (20 mg/kg/d); Group 4: PEG-IFN (1.5 mg/kg once a week); Group 5: PEG-IFN (15 mg/kg); Group 6: 5-FU (10 mg/kg/d) + PEG-IFN (1.5 mg/kg). The term of the treatment is 7 weeks. Group 3 nude mice died for 3 weeks. Microphotographs did not show any differences among the groups. The tumor volumes of Groups 2, 4 and 6 were significantly smaller than those of Groups 1 and 5. A significant difference of the incidence of apoptosis in TUNEL-stained sections was obtained between Group 6 and Groups 2 and 4. Furthermore, the p53 and the p53 phosphorylation levels were extracted and detected with anti-p53 antibody and anti-p53-phospho-Serine46 antibody using immunoprecipitation assay, respectively. The p53 phosphorylation levels of Groups 4 and 6 were significantly increased compared to those of Groups 1, 2 and 5. The p53 phosphorylation levels of Group 6 were significantly higher than those of other groups.

Conclusion: We found in vivo effect of hepatocellular carcinoma apoptosis by the link between the p53 and the pegylated interferon system. The pegylated interferon may provide an effective new strategy for antitumor therapy.

INVOLVEMENT OF POLY(ADP-RIBOSE) POLYMERASE-1 (PARP-1) IN THE DEVELOPMENT AND TREATMENT OF HEPATOCELLULAR CARCINOMA

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Background: PARP-1 is a nuclear nick sensor which favors DNA repair and is also involved in the regulation of gene transcription and inflammatory response as a co-activator of the transcription factor NF-κB. NF-κB has also been implicated in hepatocellular carcinoma (HCC). Aim: To evaluate the effect of the genetic deletion or inhibition of PARP-1 on the development of and treatment of HCC.

Methods: In vivo model: HCC has been induced in mice with a single injection of N,N-diethylnitrosamine (DEN). As PARP inhibitors we have used 3,4-dihydro-5-[4-(1-piperidinyl)-butoxy]-1H-(2H)-isoquinolinone (DPQ) and 4-amino-1,8-naphthalimide (ANI). Mice were sacrificed after either 12 weeks (wild type: 6 control, 7 DEN and 7 DEN-DPQ) or after 9 months: PARP-1 knockout (7 DEN and 3 control) and wild type (7 DEN and 3 control). In vitro model: we have studied the effect as potentiators of chemotherapy (doxorubicin) of PARP inhibitors (ANI and DPQ) on human HCC cell line (HepG2) and compared to non transformed human hepatocytes (WRL-68). The activation of NF-κB has been measured with EMSA; cell viability was evaluated with con methyl-thiazolyl-tetrazolium (MTT).

Results: After 12 weeks of treatment macroscopic and microscopic analysis did not show any evidence of HCC development, however, while NF-κB activation was evident in DEN-only treated mice (251.4±62.02), mice treated with DEN plus DPQ (178.5±49.4) had a significantly reduced activation of NF-κB (p < 0.05). In mice sacrificed after 9 months a lower number of tumors and a reduced tumor size has been found in PARP-1 knockout mice. In tumor cell lines a potentiation of the effect of doxorubicin was observed when used in combination with the PARP inhibitor ANI. At concentrations 1 and 5 mg/ml doxorubicin (doxorubicin 1 μg/ml 86.7±10.73 and doxorubicin 1 μg/ml + ANI 10 mM 60.8±20.7, p < 0.05) doxorubicin 5 mg/ml 70.3±19.09 and doxorubicin 5 mg/ml + ANI 10 mM 45.8±15.7, p < 0.05. We have also found that inhibition of PARP was able to induce non apoptotic cell death in HepG2 cells affecting much less to normal hepatocytes.

Conclusion: PARP inhibitors in combination with doxorubicin or by themselves might protect the liver against HCC development and could be considered as a therapeutic alternative.

THE HEPATOPROTECTIVE ROLE OF KETANSERIN IN ACUTE CADMIUM LIVER INJURY

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Background and Aims: Exposure to toxic metals and pollutants is a major environmental problem. Cadmium is a metal causing acute hepatic injury but the mechanism of this phenomenon is poorly understood. Ketanserin is a serotonin 5-HT2 receptor antagonist which has been introduced for the treatment of arterial hypertention and vasospastic disorders. In this study, the protective role of 5-HT2 receptor blocker with ketanserin against cadmium-induced acute hepatotoxicity was investigated.

Methods: Male Wistar rats were injected with a dose of cadmium (6.5 mg CdCl2/kg bodyweight, intraperitoneally). Normal saline (group I) or ketanserin (3 mg/kg bodyweight, group II) were injected 2 h prior and 4 h after cadmium intoxication and rats were killed at 0, 6, 12, 24, 48 and 60 h. Hematoxylin-eosin-stained liver sections were histologically assessed for necrosis, apoptosis, peliosis, mitoses and inflammatory infiltration. Apoptosis was also quantified by the TUNEL assay for hepatocytes and nonparenchymal liver cells.

Results: In group I rats, both necrosis and hepatocyte apoptosis showed a biphasic elevation at 12 and 48 h, whereas ketanserin administration (group II) greatly reduced them at all time points examined. Nonparenchymal cell apoptosis peaked at 48 h (group I), whereas in ketanserin treated rats (group II) the apoptotic index was greatly reduced. Macroscopic and microscopic peliosis in group I rats peaked at 48 h and 24 h, respectively. Macroscopic peliosis was totally reversed by ketanserin in group II, whereas microscopic peliosis was greatly reduced.

Conclusions: 5-HT2 receptor blockade induced by ketanserin exerts an intense hepatoprotective effect against acute cadmium-induced hepatotoxicity.