

EFFECTS OF INTERFERON-ALFA-2a ON HUMAN HEPATOMA HepG2 CELLS

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ДЕЙСТВИЕ ИНТЕРФЕРОНА- α -2a НА КЛЕТКИ ЛИНИИ HepG2

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In the present work, the effect of interferon-alfa-2a on human hepatoma HepG2 cells has been studied. HepG2 cells were cultured with different concentrations of IFN- α for 7 days, and then apoptotic and proliferative indexes were evaluated. It has been shown that upon such incubation, hepatoma cells were arrested in G₀/G₁ phase; proliferative indexes were found to be decreased significantly and apoptosis rate was increased at IFN- α concentrations of 50 U/ml and 100 U/ml compared to the control (29% \pm 4.5% and 27% \pm 4.6% vs 44% \pm 3.2%, $p < 0.01$; and 34% \pm 9.6% and 43% \pm 22% vs 0.8% \pm 1.3%, $p < 0.01$ respectively). Colony counts per well decreased significantly at IFN- α concentrations of 100 U/ml compared to control. Clinical relevance of these findings remain to be established with further studies.

Key Words: α -interferon, hepatoma, apoptosis, proliferation.

Исследовано влияние интерферона- α -2a (IFN- α) на клетки линии HepG2, которые инкубировали с IFN- α в течение 7 дней, после чего определяли апоптотические и пролиферативные показатели. Установлено, что вследствие инкубации при концентрациях IFN- α 50 мкг/мл и 100 мкг/мл происходит остановка клеточного роста в фазе G₀/G₁, снижаются пролиферативные индексы (29% \pm 4,5% и 27% \pm 4,6% против 44% \pm 3,2%, $p < 0,01$) и повышаются апоптотические показатели (34% \pm 9,6% и 43% \pm 22% против 0,8% \pm 1,3%, $p < 0,01$ соответственно). При инкубации клеток линии HepG2 с IFN- α в концентрации 100 мкг/мл наблюдалось значительное уменьшение числа колоний.
Ключевые слова: интерферон- α , гепатома, апоптоз, пролиферация.

Interferon alfa (IFN- α) has been shown to have antiviral and various immunoregulatory activities including augmentation of cytotoxic activities of lymphocytes and macrophages, and induction of class I major histocompatibility complex antigens [1]. Additionally, experimental studies showed that IFN- α has antiproliferative and apoptotic effects in cancer cells [2–4]. IFN- α has been used in the treatment of hepatocellular carcinoma (HCC) with controversial results [5–7]. There are restricted *in vitro* data on effect of IFN- α on hepatoma cells. Recently, it was shown that IFN- α -2b induce apoptosis in the preneoplastic hepatocytes in rats as a result of a significant increase in the amount and translocation of Bax protein to the mitochondria [8]. Yano et al [9] showed that IFN- α express growth suppression effects by inducing inhibition of cell-cycle progression with or without apoptosis in different human hepatoma cell lines except HepG2 cell line. In another study, HepG2 cells had been shown to be unresponsive to type I and II interferons [10]. Here, we tried to find out effects of IFN- α -2a on human hepatoma HepG2 cells.

MATERIALS AND METHODS

Cell culture. Human hepatoma HepG2 cells were cultured in RPMI-1640 medium (Sigma, USA) contain-

ing 10% fetal bovine serum (FBS) (Biochrom KG, Germany), supplemented with penicilline (100 U/ml) and streptomycine (100 μ g/ml) at 37 °C in a humidified, 5% CO₂ atmosphere and 10⁵ cells were plated in 24-well plate with different concentrations of IFN- α (Roferon A, Roche, Switzerland) (0, 10, 50, 100 U/ml) and incubated for 3 days. On the end of 3rd day the culture medium renewal was performed with same doses of IFN- α and the cells were further incubated for 4 days.

Colony count. After the 7 days of incubation the cultures were fixed and the cells were stained with crystal violet. The number of colonies containing more than 50 cells were counted under inverted microscope at 20x magnification. The average of the colony count per well was accepted as clonogenic capacity.

Cell count and viability. On the end of the 7th day the cells were incubated for 20 min at 37 °C with 0.5 ml of phosphate buffered saline (PBS) containing 0.25% trypsin. After the inactivation of trypsin with 0.5 ml FBS, the cultured cells were suspended first by pipetting and then using syringes fitted with 27 G needle. Viability was assessed as viable cell number per μ l by using Trypan Blue stain (Sigma, USA) under microscope.

Apoptosis and proliferation. The Coulter DNA-prep reagent system (Miami FL, USA) was used to stain the DNA of the cultured cells with propidium iodide (PI) for the quantitative measurement of cellular DNA content by flow cytometry. The reagents were used in conjunction with the Coulter DNA Prep workstation (USA). Flow-cytometry was performed on Beckman Coulter

Received: April 30, 2003.

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Abbreviations used: HCC — hepatocellular carcinoma.

Epics XL–MCL (USA). Data were analysed for apoptosis and cell cycle using the Multicycle Software (Phoenix Flow Systems, USA) [11]. Apoptosis ratio of cultured HepG2 cells were measured as percentage of hipodiploidic peak. The proliferation ratio of cultured cells was assessed by using the formula shown below [12]:

$$\text{Proliferative index (\%)} = \frac{100 \times \text{Cell number in mitosis} + \text{Cell number in S-phase}}{\text{Total cell number}}$$

Statistical analysis. Data were presented as mean \pm standard error. The results were analysed by using Kruskal Wallis test and $p < 0.05$ was accepted as statistically significant.

RESULTS

Colony assay. Colony counts per well are demonstrated in Table. Although at low concentrations of IFN- α the colony count decreased compared to control, statistically significant decrease was observed at IFN- α concentration 100 U/mL compared to control (35 ± 4.7 vs 49 ± 2.9 , $p < 0.01$).

Table. The effects of Interferon- α -2a on HepG2 cells

Treatment	Colony assay per well	Viable cell count, per mm ³	Apoptosis, %	Proliferative index %
Control	49 \pm 2.9*	638 \pm 140	0.8 \pm 1.3 *	44 \pm 3.2 *
IFN 10 U/ml	48 \pm 6.2	643 \pm 246	19 \pm 8.2 *	43 \pm 5.6
IFN 50 U/ml	45 \pm 5.0	570 \pm 168	34 \pm 9.6*	29 \pm 4.5 *
IFN 100 U/ml	35 \pm 4.7*	457 \pm 106	43 \pm 22*	27 \pm 4.6 *

* $p < 0.01$, values are expressed as mean \pm standard deviation ($X \pm SD$).

Viability. Viability assay (see Table) showed that although viable cell count decreased along with increasing concentrations of IFN- α , it did not reach statistically significant degrees.

Proliferative index and apoptosis. We have shown that upon IFN- α incubation, the cells were arrested in G₀/G₁ phase; proliferative indexes (see Table) were found to be decreased significantly at IFN- α concentrations of 50 and 100 U/mL compared to control ($29\% \pm 4.5\%$ and $27\% \pm 4.6\%$ vs $44\% \pm 3.2\%$, $p < 0.01$).

By flow cytometry, it was shown that apoptosis rate increased upon incubation of cells with increasing doses of IFN- α compared to control ($19\% \pm 8.2\%$, $34\% \pm 9.6\%$ and $43\% \pm 22\%$ vs $0.8\% \pm 1.3\%$, $p < 0.01$) (see Table).

DISCUSSION

The application of IFN- α on the treatment of patients with HCC is extensively being studied along with *in vitro* studies of IFN- α action on hepatoma cells [9, 10, 13–15]. IFN- α is thought to act either by activation of the immune system, or direct suppression of cancer cells growth [9, 14]. The maximal serum IFN- α concentration was found to be 23–53 U/ml [15, 16]. Using mentioned concentrations, Yano et al [9] didn't observe apoptotic or antiproliferative effects of IFN- α on hepatoma cells *in vitro*. Melen et al [17] has demonstrated that hepatoma cells possess relatively low basal expression levels of IFN signaling molecules STAT1, STAT2 and p48, but their expression was strongly upregulated by IFN- α and they suggest that low sensitivity of hepatoma cells to IFN- α could be due to lack of the IFN-specific signaling components. In consistent with these findings, in the

present study incubation of hepatoma cells with high concentrations of IFN- α led to the significant inhibition of HepG2 cell growth and moreover, their apoptosis. Our findings are contradictory to the data of Tnani et al [10] who demonstrated that HepG2 cells do not exhibit significant antiproliferative responses to either type I or II interferons; maybe, methodology of our works are different from those of [10].

In the clinical trials IFN- α has been used at different doses with minimal or no response rates in HCC [6, 7]. The most successful results were reported by Lai et al [5] who used high doses and observed tumor regression greater than 50% in 31.4% of patients.

In conclusion, our data indicate that IFN- α induces apoptosis and have antiproliferative effect on hepatoma cells and these effects were more pronounced at higher IFN- α concentrations. Whether this observation has clinical relevance, it remains to be established in further studies.

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