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Investigation on antiproliferative effects of interferon-α, 5-fluorouracil and hydroxyurea on human Burkitt lymphoma and erythroblastic leukemia cell lines

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In this study, we investigated the growth inhibitory effects of IFN-α, 5-FU, HU and their combinations on Daudi (Human Burkitt lymphoma) and K562 (Human erythroblastic leukemia) cell lines. Sensitivity of two leukemia cell lines to IFN-α has shown large disparities. While Daudi cells were very sensitive to the antiproliferative effects of IFN-α, K562 cells were more resistant. After showing different degree of sensitivities of Daudi and K562 cells to IFN-α, we tested whether both cells showed similar responses against anticancer drugs such as 5-FU and HU. According to our results, Daudi cells were more resistant to the antiproliferative effects of 5-FU compared to K562. However, the initial antiproliferative effect of HU on K562 was less than that of Daudi cells. After showing the individual antiproliferative effects of IFN-α, 5-FU and HU, we investigated whether these cells can be over sensitized to combination treatment of 5-FU and HU along with IFN-α. To our results, on Daudi and K562 cells treated with IFN-α and 5-FU, the antiproliferative effect of IFN-α was diminished compared to the treatment with use of IFN-α alone in Daudi but not K562 cells. Treatment of the same cells with IFN-α and HU resulted in synergistic antiproliferative effect on K562 cells compared to the individual use of either drug. However, co-treatment of Daudi cells with IFN-α and HU diminished the antiproliferative effect of IFN-α compared to IFN-α use alone. Results presented here add new findings about the antiproliferative effects of IFN-α and its combinations with 5-FU and HU on human Burkitt lymphoma and human erythroblastic leukemia cells to medicine and science world. [Turk J Cancer 2002;32(1):12-19]

Key words: IFN-α , 5-FU, HU, leukemia, antiproliferative effect

Interferons (IFNs) are a family of multi-functional cytokines that were originally identified as the proteins responsible for the formation of cellular resistance to bacterial lipopolysaccharides (LPS) and viral infections (1). With studies following discovery of IFNs, it was shown that they also play a role in the control of cell growth, differentiation and regulation of the immune system. Interferons are divided in two groups, Type I (α and β) and Type II (γ). IFN-α, IFN-β and IFN-γ are released by lymphocytes, fibroblasts and mitogen-activated T-cells, respectively (2). There are 20 IFN-α subtypes, whereas IFN-β and -γ are encoded by only one gene. All of IFN-α genes and IFN-β gene are located on short arm of chromosome 9 and do not contain introns. The IFN-γ gene is located on the long arm of chromosome 12 and it contains three introns (3).

Interferons show their effects by binding to specific receptors on the cell surface. IFN-α and IFN-β bind to the same receptor and initiate the same kind of signal cascade. However, IFN-γ binds to a different receptor and initiates different signals (3,4). Binding of IFNs to specific cell surface receptors produce several physiologic responses such as inhibition of viral replication, induction of cell differentiation and inhibition of cell proliferation.

Cytokines, which can be synthesized by recombinant DNA technology today, have become an important therapeutic alternative in the treatment of malignant and infectious diseases (5). Statistical studies have shown that 90% of patients with hairy cell leukemia (HCL) give good response to therapy with recombinant IFN-α (6-8). Particularly, IFN-α shows an important antiproliferative effect on HCL cells (9-11). Thus, IFN-α has been widely used for treatment of this disease during the last ten years (10,12,13-15). IFN-α treatment on HCL cells reduces the expressions of some oncogenes (16), and activates NK cells (17,11). However, these findings do not explain the mechanism(s) of direct cytotoxic effect of IFN-α on HCL cells, because, IFN-γ can also activate NK cells or reduce expressions of oncogenes such as c-fos and c-myc, but IFN-γ has no cytotoxic effect on HCL cells (18). In this study, we have investigated the growth inhibitory effects of IFN-α, 5-FU (5-Fluorouracil), HU (Hydroxyurea) and their combinations on Daudi (Burkitt lymphoma) and K562 (Erythroblastic leukemia) cell lines. We found that Daudi and K562 cells have very different degree of sensitivity to antiproliferative effects of above drugs. Moreover, 5-FU and HU reduced the sensitivity of Daudi cells to antiproliferative effects of IFN-α.

Materials and Methods

Daudi (Human Burkitt Lymphoma, ATCC CLL 213) and K562 (Human Erythroblastic Leukemia, ATCC CLL 243) cell lines were gifts from Dr. Milton W. Taylor (Indiana University, Bloomington, INDIANA/USA).

Both cell lines were grown in RPMI-1640 medium supplemented with 10% FCS (Fetal Calf Serum) and 1% Antibiotic-antimycotic solution (Penicillin: 10.000 U/ml, Streptomycin: 10 mg/ml, Amphotericin B: 0.025 mg/ml) at 37^0C.

Daudi and K562 cells were preincubated at $1x10^5$ cells/ml for 6 hours in RPMI 1640 medium containing 10% FCS, then cells were treated with IFN-α, 5-FU, HU or with their combinations and harvested after 72 hours incubation at

37^0C and 5% CO_2. Cell viability was determined by trypan blue exclusion. Percent relative growth was determined according to the formula:

$$\text{Growth inhibition \%} = \frac{I_n - C_n}{C_n - C_o} \times 100$$

I_n : the number of treated cells at day *n*
C_n : the number of control cells at day *n*
C_o : the number of untreated control cells at day 0

RESULTS

Sensitivity of two leukemic cell lines to IFN-α has shown large disparities. While Daudi cells were very sensitive to the antiproliferative effects of IFN- α, K562 cells were more resistant. Viability of Daudi cells treated with 1 ng/ml IFN-α was only 17% at the end of 72 hours. incubation period, whereas the same amount of IFN-α was completely ineffective on K562 cells (data not shown). Therefore, we treated K562 cells with higher concentrations of IFN-α and found that even 1 μg/ml concentration of this IFN-α can kill 71% of K562 cells at the end of 72 hours incubation (Figure 1).

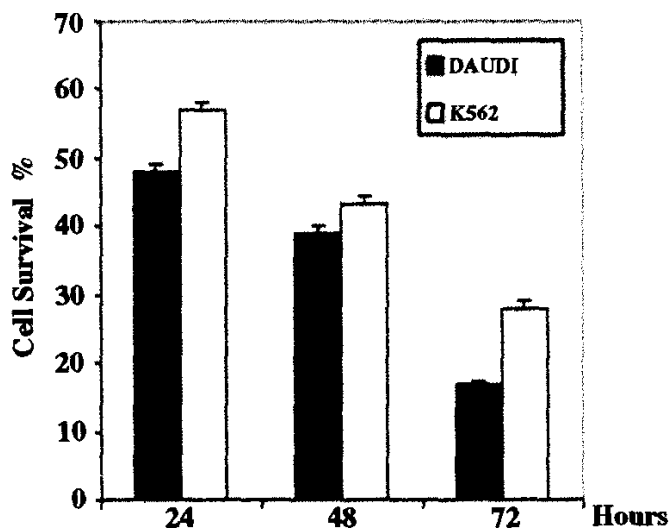

Fig 1. Daudi cells were treated with 1 ng/ml of IFN-α and K562 cells were treated with 1 μg/ml of IFN-α for 72 hours. Every 24 hours number of live cells were determined by trypan blue staining and growth inhibition was calculated. Results are the average of three independent experiments

After showing different degree of sensitivities of Daudi and K562 cells to IFN-α, we tested whether both cells showed similar responses against anticancer drugs. For this reason, we used the antiproliferative drugs, 5-FU and HU on these cell lines. Daudi and K562 cells were treated with 900 ng/ml concentration of 5-FU and 800 ng/ml for HU. When growth inhibition was measured at the end of the 72 hours incubation period, we found that cell viability was 42% for Daudi and 28% for K562 cells when treated with 5-FU (Figure 2). According to these results, Daudi cells seem to be more resistant to the antiproliferative effect of 5-FU compared to K562. However, the antiproliferative effects of HU looked similar in both cell lines after 72 hours incubation, even though the initial antiproliferative effect of HU on K562 was less than that of Daudi cells (Figure 3).

After showing the individual antiproliferative effects of IFN-α, 5-FU and HU, we wanted to determine whether these cells can be oversensitized to combination treatment of 5-FU and HU along with IFN-α. When we co-treated Daudi and K562 cells with IFN-α and 5-FU, we found that the antiproliferative effect of IFN-α was diminished compared to the treatment with use of IFN-α alone in Daudi but not in K562 cells (Figure 4). Treatment of the same cells with IFN-α and HU resulted in synergistic antiproliferative effect on K562 cells compared to the individual use of either drug. However, co-treatment of Daudi cells with IFN-α and HU diminished the antiproliferative effect of IFN-α compared to IFN-α use alone (Figure 5).

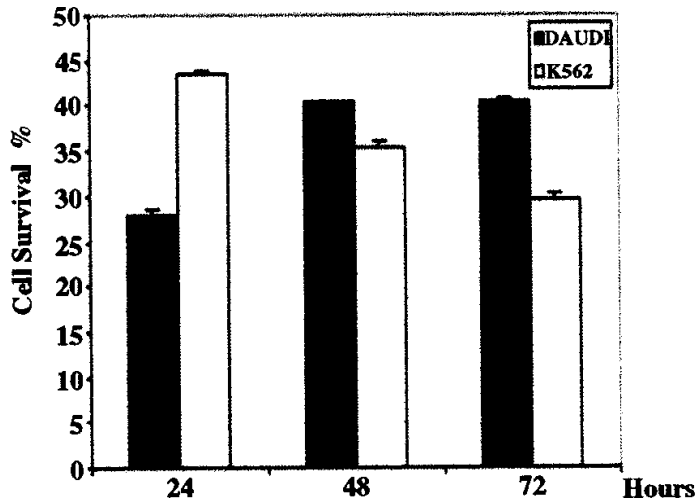

Fig 2. Daudi and K562 cells were treated with 900 ng/ml of 5-FU for 3 days. Every 24 hours number of live cells were determined by trypan blue staining and growth inhibition was calculated. Results are the average of three independent experiments

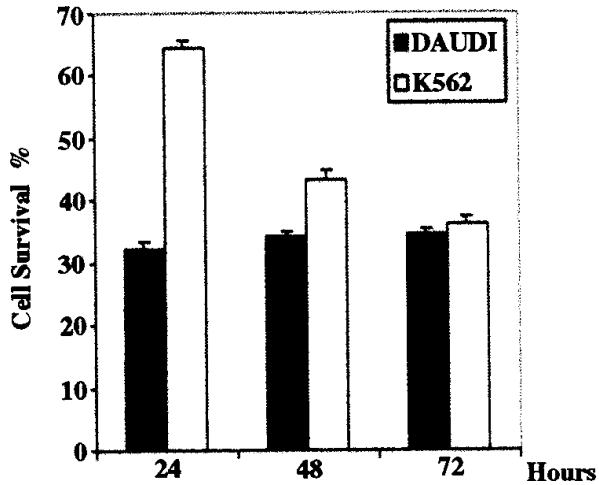

Fig 3. Daudi and K562 cells were treated with 800 ng/ml of HU for 3 days. Every 24 hours numbers of live cells were determined by trypan blue staining and growth inhibition was calculated. Results are the average of three independent experiments

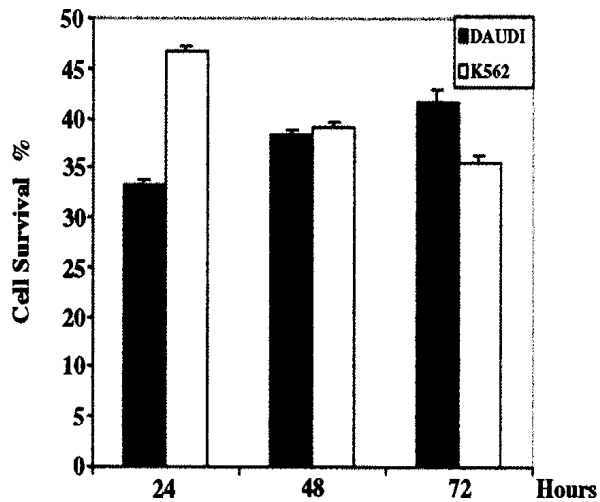

Fig 4. Daudi and K562 cells were treated with 900 ng/ml of 5-FU and with 1 ng/ml of IFN-α for 3 days. Every 24 hours number of live cells were determined by trypan blue staining and growth inhibition was calculated. Results are the average of three independent experiments

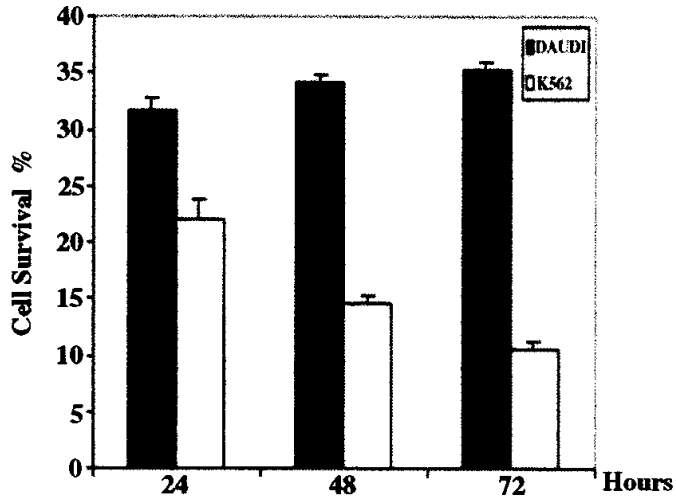

Fig 5. Daudi and K562 cells were treated with 800 ng/ml of HU and with 1 ng/ml of IFN-α for 3 days. Every 24 hours number of live cells were determined by trypan blue staining and growth inhibition was calculated. Results are the average of three independent experiments

Discussion

In this study, we have investigated the growth inhibitory effects of IFN-α, 5-FU, HU and their combinations on Daudi and K562 cells. Sensitivity of these cells to IFN-α has shown robust differences. While the Daudi cells seem to be very sensitive to antiproliferative action of this interferon, K562 cells were found to be much more resistant. Even though, we previously published the growth inhibitory effect of IFN-α on Daudi and Eskol cells (11), the main goal of this research was to test whether the use of IFN-α along with 5-FU and HU would increase the antiproliferative effects. What we found interesting, however, was the unexpected protective effects of 5-FU and HU when they were used in combination with IFN-α. Even though, 1 ng/ml concentration of IFN-α killed nearly 85% of Daudi cells after 72 hours incubation, addition of 5-FU and HU along with IFN-α nearly doubled the number of live cells after 3 days of incubation under the same growth conditions. We could not find similar observations in literature, that's why, we speculate that 5-FU and HU might be affecting pathway(s) necessary for IFN-α to show antiproliferative effects on Daudi cells. One possible explanation for this could be at the level of binding of IFN-α to its cognate receptor, 5-FU and HU might alter the structure of receptor such that IFN-α can no longer initiate a signal, or signal transducers like JAKs, STATs or downstream effectors are affected by both so that they can not function properly. An alternative is that 5-FU and HU might activate signals

which can override the antiproliferative effects of IFN-α. The observations we made here seem to be cell type specific, because, combination of 5-FU and IFN-α had no effect on antiproliferative function of IFN-α while combination of HU and IFN-α showed synergistic antiproliferative effect on K562 cells.

Previously, antiproliferative or cytotoxic effects of IFN-α have been shown by many different groups on different malignancies. For example, Maeda et al (19) reported growth inhibitor effects of IFN-α on RPMI 4788, human colon carcinoma cell line. Also, combination of IFN-α with 2-deoxycoformycin and 2-chlorodeoxyadenosine have been tested by Talpaz et al (20) and Piro et al (21). Reiter et al (17) reported synergistic antiproliferative effect of IFN-α in combination with 5-FU on cervical carcinoma cell line, ME180, and AIDS-related Kaposi's sarcoma. Cytotoxic effect of combination of IFN-α and 5-FU has also been tested by Wadler et al (22) on patients with esophagus cancer and they found that this combination use of 5-FU and IFN-α caused DNA fragmentation. Sugimachi and their colleagues (23) reported effective therapy with 5-FU on rectal carcinomas.

Results presented in this paper add new findings about the antiproliferative effects of IFN-α and its combinations with 5-FU and HU on human Burkitt lymphoma cells and human erythroblastic leukemia cells. These results may aid scientists in the treatment of neoplasias with IFN-α and shed light on its use in combination with other drug regimens.

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