Potentiation of Antitumor Effects of Cisplatin by Tumor Necrosis Factor-β

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Recombinant tumor necrosis factor-β potentiated the inhibitory effect of cisplatin on intramuscularly transplanted GA-1 tumor and liver metastasis in mice. The antitumor effect was related to cytotoxic and cytostatic activities of the test preparations rather than to initiation of apoptosis.

Key Words: *tumor necrosis factor; cisplatin; mice; primary tumors; metastases; apoptosis*

Tumor necrosis factor (TNF) produced by activated macrophages and lymphocytes is the major immunomodulator and antiinflammatory cytokine. It is presented by 2 polypeptides (TNF-α and TNF-β). TNF was initially characterized as a protein inducing necrosis of experimental tumors in mice [4]. In clinical practice, TNF was used in combination with cytostatics. However, TNF-α caused pronounced side effects, including hypotension, pulmonary edema, and intravascular thromboses [4,9]. TNF-β possesses more pronounced antitumor activity and lower toxicity [6]. Here we studied the potentiating effect of TNF-β from recombinant *E. coli* SG 200-50/LT21 strain [5] on antitumor activity of cisplatin (complex platinum preparation). Experiments were performed on the model of transplanted tumors and liver metastases in mice. Inflammatory mediators TNF-α and TNF-β damage the endothelium of tumor blood vessels and increase vascular permeability (directly or via activation of macrophages and neutrophils producing peroxides and reactive oxygen species). It was hypothesized that TNF can increase the concentration of chemotherapeutics in tumor tissues. We studied the effects of TNF-β on functional activity of peritoneal macrophages and blood neutrophils in mice. Recent studies demonstrated that antitumor activity of chemotherapeutics is realized via induction of apoptosis in tumor cells. TNF acts as an apoptotic factor in some tumors [10]. We evaluated the contribution of cytostatic and apoptotic components into the antitumor effects of TNF-β and cisplatin on GA-1 tumor.

MATERIALS AND METHODS

Experiments were performed on 3-4-month-old male A/Sn mice (Institute of Cytology and Genetics). The animals were kept in cages (8 mice per cage) under natural light-dark conditions and *ad libitum* food and water supply. GA-1 tumor was primarily induced by *o*-aminoazotoluene and maintained as ascitic tumor in A/Sn mice [2]. Tumor cells (10^6 cells/ml physiological saline) were transplanted intramuscularly. After 1 month, transplanted tumors had a diameter of 1.3-1.5 cm and metastasized to the liver.

The mice were divided into 4 groups (the size of tumor nodules was taken into account). Group 1 animals served as the control. Group 2 and 4 mice received 2 times intravenous injections of 10^5 U TNF-β at 1-day intervals. Three hours after the last injection, group 4 animals intravenously received cisplatin in a dose of 12 mg/kg (St. Petersburg Chemical and Pharmaceutical Academy). Group 3 mice not treated with

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TABLE 1. Effects of TNF-β and Cisplatin on Growth of Intramuscularly Transplanted GA-1 Tumor and Liver Metastases in Mice (*M*±*m*)

Parameter	Control (*n*=7)	TNF-β (*n*=7)	Cisplatin (*n*=8)	TNF-β+cisplatin (*n*=8)
Body weight, % of control	103	99	97	95.5
Weight of muscle tumor, g	3.40±0.25	3.10±0.28	2.50±0.17	1.90±0.23*
Inhibition of tumor growth, %	—	8.8	26.5	44.1
Weight of liver metastases, mg	999.0±120.2	860.0±86.8	750.0±66.3	262.0±41.5+
Inhibition of metastasis growth, %	—	14	25	74

Note. *p<0.001 compared to the control and TNF-β-treated mice; +p<0.001 compared to other groups.

TNF received cisplatin in the same dose. The animals were decapitated 7 days after the last treatment. The weight of muscle tumors was estimated by the difference between the weights of affected and contralateral (intact) limbs. The weight of liver tumor was calculated as described elsewhere [3]; the hepatic index of control mice was taken as 4.5%. Transplanted tumors were fixed in 10% formalin. The number of mitotic and apoptotic cells was estimated morphometrically on paraffin slices [1]. The effects of TNF-β on phagocytosis in peritoneal macrophages and functional activity of blood neutrophils were studied using monolayer cultures [7] and HCT test [8], respectively.

The results were analyzed by Student's *t* test.

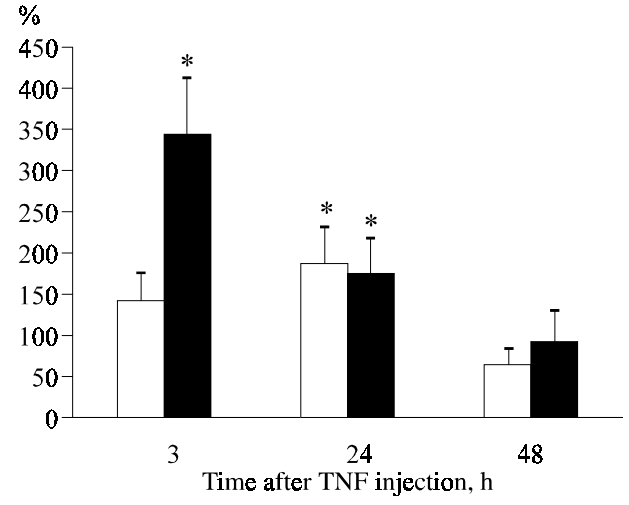

Fig. 1. Effect of TNF-β on functional activity of mouse peritoneal macrophages (% of macrophages phagocytizing sheep erythrocytes, light bars) and blood neutrophils (HCT-positive neutrophils, dark bars). p<0.05 compared to the control (100%).

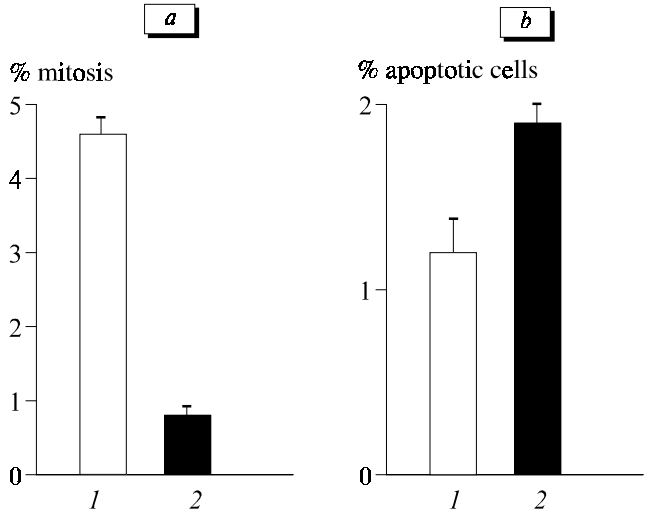

Fig. 2. Number of mitotic (*a*) and apoptotic cells (*b*) in mice with GA-1 tumors untreated (*1*) and treated with TNF-β and cisplatin (*2*).

RESULTS

TNF-β markedly increased phagocytic activity of macrophages and generation of peroxides and reactive oxygen species by neutrophils 3 and 24 h after treatment (Fig. 1). These data were taken into account in developing the treatment schemes for tumor mice (intervals between TNF and cisplatin injections).

TNF-β alone did not modulate the growth of transplanted tumors and liver metastases, but potentiated the antitumor effect of cisplatin from 26.5 to 44.1% (insignificant). The severity of metastatic damages to the liver decreased compared not only to the control, but also to mice treated with cisplatin (p<0.001). This potentiation of antitumor activity was not accompanied by potentiation of toxic effect, which was confirmed by an insignificant decrease in the body weight (4.5% of the control).

Mitotic activity of tumor cells in treated mice was 5 times lower than in the control, while the number of apoptotic cells in these animals 1.5-fold surpassed the control (Fig. 2). Probably, the antitumor effect was related to cytotoxic and cytostatic activities of test preparations, rather than to induction of apoptosis.

These results indicate that combined use of TNF-β and cytostatics potentiates antitumor activity of these drugs, but not their toxic and side effects.

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