Stable Inhibition of Nuclear Factor κB in Cancer Cells Does Not Increase Sensitivity to Cytotoxic Drugs

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Abstract

Several reports indicated that nuclear factor κB (NF-κB) activation by cytokines, cytotoxic drugs, or ionizing radiation protects cells against apoptosis. Therefore, we investigated the consequence of NF-κB inhibition on the efficiency of antineoplastic agents. HPB, HCT116, MCF7, and OVCAR-3 cells stably expressing a dominant negative IκBα inhibitor showed a decreased NF-κB activation following treatment with tumor necrosis factor α and various chemotherapeutic agents. However, there was no difference in survival between parental cells and cells expressing mutated IκBα. These studies suggest that, at least in these cell lines, stable NF-κB inhibition did not modify the response to cytotoxic drugs.

Introduction

NF-κB is an ubiquitous transcription factor that is involved in the control of the expression of many genes (1). NF-κB DNA-binding activity is induced after in vitro cellular treatment with a number of anticancer agents, such as ionizing radiation, topoisomerase I and II inhibitors, antimetabolites, taxanes, and Vinca alkaloids (2–7). However, the role of NF-κB activation following exposure to cytotoxic drugs remains controversial. It has been reported that NF-κB activation after treatment with TNF-α, Dauno, or ionizing radiation protects cells against apoptosis (8–11). Moreover, an antiapoptotic function for NF-κB was also demonstrated in B lymphocytes, breast cancer cells, Hodgkin’s disease cells, embryonic or regenerating liver, Ras-transformed cells, and insulin-treated CHO cells (12–18). It has, thus, been suggested that the inhibition of NF-κB could trigger apoptosis in cancer cells or sensitize them to cytotoxic drug-induced cell death.

However, we reported that stable NF-κB inhibition in MCF7 breast cancer cells did not influence cellular mortality after treatment with TNF-α (19), and others have shown that NF-κB activation by hyperoxia did not protect cells from apoptosis (20). To determine whether NF-κB inhibitors could be used in cancer therapy, we investigated the cytotoxic effect of a number of NF-κB-inducing drugs in the presence of a stable NF-κB inhibitor. The data presented here indicate that NF-κB inhibition prevents NF-κB activation after TNF-α or cytotoxic drug treatment without affecting cell survival.

Materials and Methods

Cell Lines and Reagents. HCT116 human colon carcinoma cells (ATCC CCL 247), HCT116-MT cells, MCF7 breast cancer cells, and MCF7-MT cells were described previously (5, 19).

OVCAR-3 ovarian carcinoma cells and OVCAR-3-MT cells were maintained in RPMI 1640 supplemented with 10% FCS, 1% 200 μM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. OVCAR-3-MT cells were obtained by stable transfection of OVCAR-3 cells with a linearized pCRII-CMV plasmid encoding the IκBα protein mutated at Ser-32 and Ser-36 (a gift from Dr. A. Israel, Institut Pasteur, Paris, France; Ref. 21) and cultivated in the presence of geneticin (500 μg/ml G418, active concentration; Life Technologies, Inc.). HPB lymphoid T cells and HPB-MT cells were a gift from Dr. M. Körner (Laboratoire d’Immunologie Cellulaire, Hôpital de la Pitié-Salpêtrière, Paris, France; Ref. 22).

Individual clones of all of the transfected cell lines were isolated and used throughout this study.

VLB (Eli Lilly Benelux, Brussels, Belgium), TAX (Bristol-Myers Squibb, Brussels, Belgium), Dauno (Rhone-Poulenc Rorer, Brussels, Belgium), CPT (Sigma Chemical Co., Bornem, Belgium), and MMC (Christiaens Pharma, Brussels, Belgium) were stored in stock solutions and added to cell cultures as indicated. Dauno, TAX, VLB, MMC, and CPT solutions were protected from light.

The human recombinant TNF-α (specific activity, >1.0 × 10^8 units/mg) was purchased from Boehringer Mannheim (Germany).

Nuclear Protein Extraction and EMSA. Nuclear protein extraction for EMSA was described previously (23). The palindromic κB probe was: 5’-TTGGCAACGGCAGGGGAATTCCCCTCTCCTTA-3’.

Determination of Cellular Viability. Stably transfected cells or untransfected cells were seeded at the concentration of 10^4 cells (OVCAR-3 and OVCAR-3-MT), 7 × 10^3 cells (HCT116 and HCT116-MT), or 8 × 10^3 cells (HPB and HPB-MT) per well in flat-bottomed 96-well plates in 0.2 ml of medium (for OVCAR-3, OVCAR-3-MT, HCT116, and HCT116-MT) or 0.15 ml of medium (for HPB and HPB-MT). OVCAR-3, OVCAR-3-MT, HCT116, and HCT116-MT cells were cultivated in medium containing the appropriate drug at the indicated concentration, and the medium was replaced after 48 h. For HPB and HPB-MT cells, 24 and 72 h after cell seeding, respectively, 50 μl of medium containing the appropriate drug were added to obtain the final concentration.

After 48 or 96 h of incubation with the drug, cell viability was measured by a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells (Boehringer Mannheim).

Results

Four cancer cell lines stably expressing a mutated, noninducible IκBα inhibitor were used in this study. HCT116 colorectal adenoca...
carcinoma cells, MCF7 breast cancer cells, and HPB T lymphoid cells that were stably transfectected with an expression vector coding for an IκBα protein carrying mutations of Ser-32 and Ser-36 (mIκBα, MT cells) have been described previously (5, 19, 22). OVCAR-3 ovarian carcinoma cells were stably transfected with the same mIκBα construct to generate the OVCAR-3-MT cells. It has been reported previously that such an IκBα mutant can completely abolish NF-κB activation by a number of stimulating agents (19, 21, 22, 24). Indeed, we confirmed by EMSA that, after TNF-α stimulation, NF-κB DNA-binding activity was strongly induced in parental cells but was markedly inhibited in mIκBα stably expressing cells (Fig. 1; Ref. 19). The specificity of the observed bandshift was checked by competition experiments with cold wild-type and mutated probes (data not shown).

HCT116, OVCAR-3, and HPB parental and transfected cells were treated with MMC, CPT, Dauno, TAX, or VLB, and NF-κB DNA-binding activity was analyzed (Fig. 2). These drugs were chosen because they represent major classes of clinically used chemotherapeutic agents: inhibitors of topoisomerase I and II, antitumor antibiotics, taxanes, and Vinca alkaloids. MMC activated NF-κB only in OVCAR-3 cells, whereas CPT did not generate any NF-κB DNA-binding activity in OVCAR-3 cells. TAX, Dauno, and VLB induced NF-κB in the three parental cell lines. NF-κB activation by Dauno in HCT116 cells had been demonstrated previously (5). However, the induction of NF-κB by cytotoxic drugs was much weaker than that observed after TNF-α stimulation (the autoradiographies shown in Fig. 2 were exposed overnight, with the exception of Dauno-treated OVCAR-3 cells, whereas the one shown on Fig. 1 was exposed for 7 h). High concentrations of TAX (25 μM) were needed to observe a significant NF-κB induction. Similarly, 10 nm VLB failed to induce NF-κB in OVCAR-3 or HCT116 cells (data not shown). Again, NF-κB induction by these cytotoxic drugs was almost completely abolished in cells expressing mIκBα (Fig. 2).

To determine whether stable inhibition of the NF-κB transcription factor could increase the cytotoxic effect of TNF-α, parental and mIκBα-transfected HPB, HCT116, and OVCAR-3 cells were incubated in the presence of increasing TNF-α concentrations, and cell viability was measured after 48 (data not shown) and 96 h (Fig. 3). Under these conditions, we could demonstrate that NF-κB inhibition did not significantly increase cell death after treatment of the transfected cell lines with 1–100 ng/ml TNF-α for 96 h (Fig. 3). These data confirmed our previous report showing that stable expression of mIκBα in MCF7 cells inhibited NF-κB activation but not TNF-α-induced apoptosis (19).

The same experiment was then repeated in the presence of the anticancer drugs MMC, TAX, CPT, Dauno, and VLB. The cytotoxic drugs concentrations were chosen to cover their usual therapeutic levels. Moreover, with the exception of TAX, these con-
centrations were identical (MMC, CPT, and Dauno) or very close (VLB) to those which induced nuclear NF-κB activity (Fig. 2). Treatment with increasing concentrations of MMC (0.5–3 μM) influenced similarly the viabilities of parental and mIkBα-expressing cells (Fig. 4A). Only transfected HCT116 cells showed a small but not significant decrease in survival as compared to parental cells.

Treatment with TAX (0.0001–0.1 μM; Fig. 4B), VLB (0.01–10 nM; Fig. 4C), CPT (0.001–1 μM; Fig. 4D) or Dauno (0.1–2 μM; Fig. 4E) had exactly the same cytotoxic effect on parental and mIkBα-transfected OVCAR-3 or HPB cells. Again, with all these drugs, we observed a cytotoxic effect that was slightly higher in transfected HCT116 than in parental cells, but such an effect was not significant and was dose dependent. Survival curves established for shorter times of treatment (48 h) and for higher concentrations showed again the same sensitivity of the parental and the transfected cells to the cytotoxic agents (data not shown). Moreover, in our experimental conditions, inhibition of NF-κB failed to facilitate cell death after treatment with other chemotherapeutic agents (5-fluorouracil, etoposide, epirubicine, and amsidine; data not shown).

We confirmed these data by treating parental or mIkBα-expressing MCF7 cells with two of the most active cytotoxic drugs in breast cancers, Adriamycin and TAX, as well as with CPT and etoposide (data not shown). Again, the cytotoxic effect of the drugs was very similar in parental and genetically modified cells.

Because NF-κB activation by cytotoxic drugs is transient and as in vivo cytotoxic treatments are usually performed with repeated short perfusions, we considered that the effect of NF-κB inhibition on cancer cell survival might be more significant and more clinically relevant after short drug exposures. Parental and mIkBα-transfected HCT116 or OVCAR-3 cells were incubated in the presence of increasing CPT, VLB, Dauno, or TNF-α concentrations for 4 h, either on a single day or on 3 consecutive days. Cell viability was measured 48 h after the last treatment. In these experimental conditions, there was no significant difference in the survival of parental or mIkBα-transfected cells (data not shown).

To confirm that NF-κB inhibition did not modify cytotoxic drugs-induced apoptosis, we evaluated apoptosis in parental or mIkBα-transfected HCT116 cells after treatment with CPT or TAX for 24 h. In these conditions, an obvious DNA ladder was observed in parental as well as in transfected (MT) treated cells (data not shown). Similarly, untransfected HCT116 and HCT116-MT cells were incubated for 24 h with or without Dauno and TAX, fixed, and analyzed with a terminal deoxynucleotidyl transferase-mediated nick end labeling test. In these conditions, we did not observe any difference in the number of apoptotic cells between untransfected and mIkBα-expressing cells (data not shown).

Discussion

Recently, several reports indicated that NF-κB constitutive activity in cancer cells as well as NF-κB activation by TNF-α, anticancer drugs or ionizing radiation could protect cells against apoptosis and would, therefore, favor cancer cell survival and resistance to chemotherapy, probably by controlling the expression of apoptosis inhibitors (8–11, 14, 18). It was, thus, crucial to confirm the antiapoptotic NF-κB role in different experimental settings to determine whether the development of NF-κB inhibitors for cancer treatment had to be pursued. In this paper, a gene construct coding for a stable and specific NF-κB inhibitor (mIkBα) was stably transfected in various cell lines. Although TNF-α and the studied drugs could efficiently induce NF-κB DNA-binding activity in parental untransfected cells, NF-κB inhibition did not increase the cytotoxic effect of these drugs in any of the treated cell lines.

Our work, thus, contradicts previous reports demonstrating that NF-κB activation protected against apoptosis following treatment of mouse embryo fibroblast, NIH3T3, Jurkat, and HT1080 cells with TNF-α or after treatment of HT1080 cells with Dauno or ionizing radiation (8, 10, 11). Such differences might be explained by cell type specificities. The mechanisms leading to cellular resistance to cytotoxic drugs are numerous and include increased P-glycoprotein, multidrug resistance-associated protein, or lung-resistance protein expression; p53 mutation; increased Bcl-2 or Bcl-XL expression; increased glutathione levels; altered topoisomerase II activity; alteration of the ceramide pathway; increased A20 expression; and altered DNA repair (25, 26). Here, we used several cell lines, including adenosarcoma and leukemia cells and numerous drugs to circumvent specific mechanisms for resistance. Therefore, if our work does not formally exclude any implication for NF-κB in resistance to chemotherapy, it certainly indicates that this transcription factor does not play a central role in such a mechanism. Thus, we believe that NF-κB inhibitors are unlikely to become a major tool for the treatment of a large number of cancers. However, it will probably be necessary to determine, for each individual cancer, the molecular characteristics of the transformed cells, including p53 status, P-glycoprotein expression, oncogene expression, and possibly NF-κB nuclear activity to determine the treatment sensitivity of the cells and, thus, to define the most appropriate therapeutic combination.
Fig. 4. NF-κB inhibition does not increase cell killing by cytotoxic drugs. OVCAR-3, HPB, and HCT116 cells, either untransfected or stably transfected with an expression vector coding for the mIkBa protein (MT cells), were left untreated or were incubated for 96 h with increasing concentrations of MMC (A), TAX (B), VLB (C), CPT (D), or Dauno (E). Cell viability was estimated with the WST-1 test. Data points, means of three different measures; bars, SD.
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References

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