Glucocorticoids enhance cytotoxicity of cisplatin via suppression of NF-κB activation in the glucocorticoid receptor-rich human cervical carcinoma cell line SiHa

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Abstract

Glucocorticoids (GCs) are commonly co-administered with cisplatin in the treatment of patients with carcinomas to prevent drug-induced allergic reaction, nausea and vomiting. Although GC receptor (GR) is ubiquitous in carcinoma cells and has been linked to signal transduction pathways pertinent to cell growth and apoptosis, little is known regarding the possible effect of GC on the chemosensitivity of carcinomas. Our previous study demonstrated that dexamethasone (DEX) enhances the cytotoxicity to cisplatin in a GR-rich human cervical carcinoma cell line, SiHa. In this study, we found that this cisplatin cytotoxicity-enhancing effect of DEX correlated well with its effect on abrogating the cisplatin-induced activation of nuclear factor kappa B (NF-κB). RU486, a structural homologue of DEX, partially reversed this cytotoxicity-enhancing effect of DEX, a finding consistent with the well-known partial reversing effect of RU486 on DEX-induced NF-κB suppression. Furthermore, expression of a dominant-negative truncated IκBα gene in SiHa cells completely abolished the cisplatin cytotoxicity-enhancing effect of DEX. Our data suggest that the specific action of DEX on GR may enhance the cytotoxicity of cisplatin in selected GR-rich cancer cells by suppressing NF-κB activation.

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Introduction

Co-administration of glucocorticoids (GCs) with anticancer drugs such as cisplatin is a common clinical practice used to prevent drug-induced allergic reaction or nausea/vomiting (The Italian Group of Anticancer Research 1995, 2000). Although GCs are effective in inducing apoptosis via yet uncharacterized pathways in many hematological malignancies (Haskell 1995), they are generally not effective in the treatment of non-hematological solid tumors. However, some studies have shown that GC treatment may decrease the chemosensitivity (Chang *et al.* 1997*a*, 1997*b*, Naumann *et al.* 1998, Gassler *et al.* 2005, Wu *et al.* 2005) in non-hematological solid tumors. The possible mechanisms underlying the chemosensitivity-reducing effect of GC include: modulation of bcl-x expression (Chang *et al.* 1997), up-regulation of $p21^{Cip1}$ (Naumann *et al.* 1998), and induction of mitogen-activated protein kinase phosphatase-1 (MKP-1) (Wu *et al.* 2005).

In our previous study we examined 14 carcinoma cell lines and we found the majority of human carcinomas with high GC receptor (GR) content were affected by GC, either in their growth or in their sensitivity to chemotherapeutic agents (Lu *et al.* 2005). We demonstrated that dexamethasone (DEX) increased cisplatin chemosensitivity in SiHa, a human cervical carcinoma cell line with high GR content. DEX alone has no effect on the growth of SiHa cells (Lu *et al.* 2005). In the present study, we explored the mechanisms by which DEX causes a chemosensitizing effect to cisplatin in SiHa cells. The results showed that the mechanism appears to be related to its inhibition of cisplatin-induced nuclear factor kappa B (NF-κB) activation.

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Materials and Methods

Cell culture and chemicals

SiHa cells (human cervical carcinoma) were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium supplemented with 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma), and 10% heat-inactivated fetal bovine serum (Life Technologies Inc.). Cisplatin was obtained from Pharmacia-Upjohn (Kalamazoo, MI, USA). DEX and RU486 were purchased from Sigma and [^{3}H] DEX (specific activity 35–50 Ci/mmol) was purchased from Blossom Biotechnologies Inc. (Blossom, TX, USA).

Cytotoxicity assay

The *in vitro* growth inhibitory effect of the drugs was determined by the MTT assay as previously described with slight modification (Carmichael *et al.* 1987). Briefly, cells were plated in 96-well plates at 5×10^3 cells/well. After overnight incubation, various concentrations of drugs were added in triplicate samples to each culture. Cells were exposed to drugs continuously. After 3–4 days of culture, when cells in drug-free wells reached 90% confluency, 50 μl of 2·5 mg/ml MTT (Sigma) in PBS was added to each well, followed by incubation for 4 h at 37 °C. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and the absorbance was determined with an ELISA reader (Molecular Devices, Orange County, CA, USA) at 540 nm. Absorbance values were normalized to the values obtained for the vehicle-treated cells to determine the percentage of surviving cells. Each assay was performed in triplicate. Trypan blue exclusion method was also applied to verify the results of cytotoxicity assay by MTT assay. SiHa cells were seeded at 3×10^5 cells/well in six-well culture plates. After overnight incubation, various concentrations of drugs were added in triplicate samples to each culture. Cells were exposed to drugs continuously. Then, the cells were counted by trypan blue exclusion method using a hemocytometer.

Measurement of GR content

The GR content was measured by a whole-cell binding assay as previously described with minor modification (Harmon & Thompson 1981). Briefly, cells with 90% confluency were subcultured and allowed to grow overnight, and then trypsinized and suspended in culture medium containing 10% fetal bovine serum (pH 7·2) to a density of $1 \sim 10 \times 10^6$ cells/ml. Cells were incubated for 1 hr 37 °C with various concentrations of [3H] DEX from 1 to 100 nM in the presence or absence of 10 μM unlabeled DEX. Cells were then harvested by centrifugation at 1200 ***g*** for 1 min. Cells were then washed three times in 3·0 ml of Hank's balanced salt solution (Sigma) and finally suspended in 1·6 ml of the same solution. A 0·2-ml aliquot of this suspension was used for the determination of cell number, and 1·0 ml was assayed for radioactivity by a liquid scintillation counter (Beckman LS 6500; Beckman Instruments Inc., Fullerton, CA, USA). The presence of at least 200-fold excess of unlabeled DEX effectively competed out all of the binding of [^{3}H] DEX to specific GR. The difference in disintegrations/minute per cell between those samples incubated with [^{3}H] DEX alone and those incubated with a 200-fold excess of unlabeled DEX represented the binding of [^{3}H] DEX to specific GR. Using the specific activity of [^{3}H] DEX, the number of receptors/cell was calculated, assuming that each receptor binds to one DEX molecule.

Western blot analysis

Cells were plated in 6 cm dishes at a density of 1×10^6 cells/dish. After incubation with DEX for the indicated time periods, the cells were harvested. Whole cell lysates and nuclear extracts were prepared according to previously described methods (Staal *et al.* 1990). Protein concentration was determined by Bradford assay (Bradford 1976). Antibodies used in immunoblotting were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), including anti-NF-κB p65 (sc-372), anti-IκBα (sc-371), anti-Bax (sc-493), anti-Bcl-2 (sc-492), anti-Bcl-X_L (sc-1041), anti-p21^{Cip1} (sc-817), anti-MKP-1 (sc-1102), anti-phospho-ERK (sc-7383), and anti-GR (sc-1003). Signals were visualized with an enhanced chemiluminescence kit (Amersham) followed by exposure to X-ray films.

Electrophoretic mobility shift assay (EMSA) for NF-κB

[α-^{32}P]dCTP end-labeled double-stranded oligo-deoxyribonucleotides (5′-GGATTGGGACTTTCCCCTCC-3′ and 3′-CCTAACCCTGAAAGGGGAGG-5′) were used as the binding substrates for NF-κB. The preparation of nuclear extracts for EMSA was performed according to a previously described method (Andrews & Faller 1991). Nuclear extracts of SiHa cells (10 μg/assay) were incubated with 10 000 c.p.m. of probe (0·1 to 0·5 ng) and 1 μg poly(dI-dC) for 30 min at room temperature with a final reaction mixture of 15 μl containing 20 mM HEPES (pH 7·5), 100 mM KCl, 0·2 mM EDTA, 20% glycerol, 1 mM dithiothreitol and 1 μg/μl BSA. Samples were analyzed in a 5% polyacrylamide gel with 0·25 x TBE as running buffer, and run at room temperature at 150 V for 2–2·5 h. The nuclear extract from tumor necrosis factor-alpha (TNF-α)-treated SiHa cells was used as positive control. Antibody to p65 (Rel A) was added to the reaction mixture before the addition of labeled probe for supershift analysis. After electrophoresis, gels were dried and autoradiographed for 12 h at − 70 °C.

Transfection of reporter plasmid and measurement of luciferase and reporter gene activity

The luciferase reporter plasmid, pM-Luc, contains the 1·4-kb MMTV LTR which encompasses the natural glucocorticoid reponse element (GRE) sequences (Scheidereit *et al.* 1983). The other luciferase reporter plasmid, pRκB-Luc, contains five NF-κB sites followed by a TATA box. These plasmids both contain the hygromycin resistance gene from SV2 hygro. SiHa cells were transfected by Lipofectamine 2000 (Life Technologies Inc) according to the manufacturer's protocol. The stable clone was selected by 400 μg/ml hygromycin for 20 days. Single cell clones were obtained by limiting dilution of the hygromycin-resistant cells. The SiHa/κB-reporter cell line was selected on the basis of TNFα-induced luciferase activity and constitutive β-galactosidase activity. For each time point, 1×10^5 SiHa/κB-reporter cells were stimulated with 10 ng/ml TNFα or cisplatin (20 and 200 μM) and incubated for an additional 6 h. Reporter gene activity was determined with the reporter luciferase assay system (Packard, the Netherlands).

Transfection of dominant-negative IκBα

The dominant-negative truncated IκBα (dnIκBα) cDNA was constructed by deletion of amino acid residues 1 to 70, which contain the phosphorylation sites (serine residues 32 and 36) of IκB kinases and ubiquitin binding sites (lysine residues 21 and 22). This cDNA was inserted into the vector pRCMV (Invitrogen) followed by the CMV promoter. The empty vector was used for the generation of control cells. SiHa cells were transfected by Lipofectamine 2000 (Life Technologies, Inc.) according to the manufacturer's protocol. The stably transfected SiHa cells were pooled by G418 selection for 20 days after transfection. The experiments examining the effect of DEX on the growth of these cells were performed within 30 days of each transfection.

Results

DEX enhanced the cytotoxicity of cisplatin in GR-rich carcinoma cell line SiHa

Cytotoxicity effect of DEX and cisplatin was first examined by MTT assay. Pretreatment of SiHa cells with 1 μM DEX for 24 h decreased the IC_{50} of cisplatin from $18{\cdot}6 \pm 1{\cdot}9$ μM to $9{\cdot}7 \pm 2{\cdot}0$ μM. DEX alone, up to 20 μM, was not toxic to SiHa cells. This cytotoxicity-enhancing effect of DEX in SiHa cells could be observed even at a pretreatment dose of 1 nM (Fig. 1A) or with concurrent treatment of DEX and cisplatin (Fig. 1B). Similar results of cytotoxicity-enhancing effect of DEX in SiHa cells was also noted by trypan blue exclusion method (Fig. 1C).

SiHa cells were found to contain approximately $8{\cdot}1 \times 10^4$ receptors/cell (Fig. 1D). The GR content of human lymphocytes was within the reported normal range (~2500–5400 sites/cell) (Lippman *et al.* 1978), and served as an internal control.

RU486 partially reversed the cytotoxicity-enhancing effect of DEX

RU486, a structural homologue of DEX, was reported to have a differential effect on inhibition of the two major DEX-mediated cellular pathways (Beck *et al.* 1993, McEwan *et al.* 1997, Van der Burg *et al.* 1997, Wissink *et al.* 1998). RU486 usually blocks the DEX-mediated regulation of GRE-containing downstream genes completely, but only partially reverses the DEX-mediated suppression of NF-κB activity. DEX-induced cisplatin chemosensitization, in the presence of RU486, was examined in SiHa cells. The cytotoxicity-enhancing effect of DEX in SiHa cells was partially reversed ($IC_{50} = 13{\cdot}9 \pm 0{\cdot}8$ μM) by pretreatment with an equal concentration of RU486 (Fig. 2A). On the other hand, when the effect of transcription activity of DEX on GRE was examined in SiHa cells transfected with pM-Luc, which contains the MMTV-Luc reporter gene, pretreatment with RU486 completely blocked the induction of luciferase activity (Fig. 2B). These results suggested that the cytotoxicity-enhancing effect of DEX was not mediated via regulation of the expression of downstream genes governed by GRE. Other signal transduction pathways such as NF-κB, which DEX regulates via a direct protein–protein interaction with activated GR, should be investigated.

DEX suppressed cisplatin-induced NF-κB activation in SiHa cells

To explore the mechanism responsible for the chemosensitizing effect of DEX on SiHa cells, EMSA assay of NF-κB DNA binding activity and reporter luciferase assay of NF-κB transcription activity were performed. As shown in Fig. 3A, NF-κB DNA binding activity transiently increased after exposure to 20 μM (IC_{50}) cisplatin. This NF-κB DNA binding activity was blocked by pre-treatment with 1 μM DEX (Fig. 3B). While RU486 could only partially reverse the effect of DEX, on the suppression of NF-κB DNA binding activity. RU486 had an intrinsic effect on the suppression of NF-κB DNA binding activity (Fig. 3B). The transactivating activity of NF-κB on its *cis* elements was further verified in SiHa cells, stably transfected by a reporter construct containing five NF-κB binding sites. Treatment with cisplatin (20–200 μM) resulted in the induction of luciferase activity, which could be repressed by pretreatment with DEX. Again, RU486 could partially reverse the effect of DEX

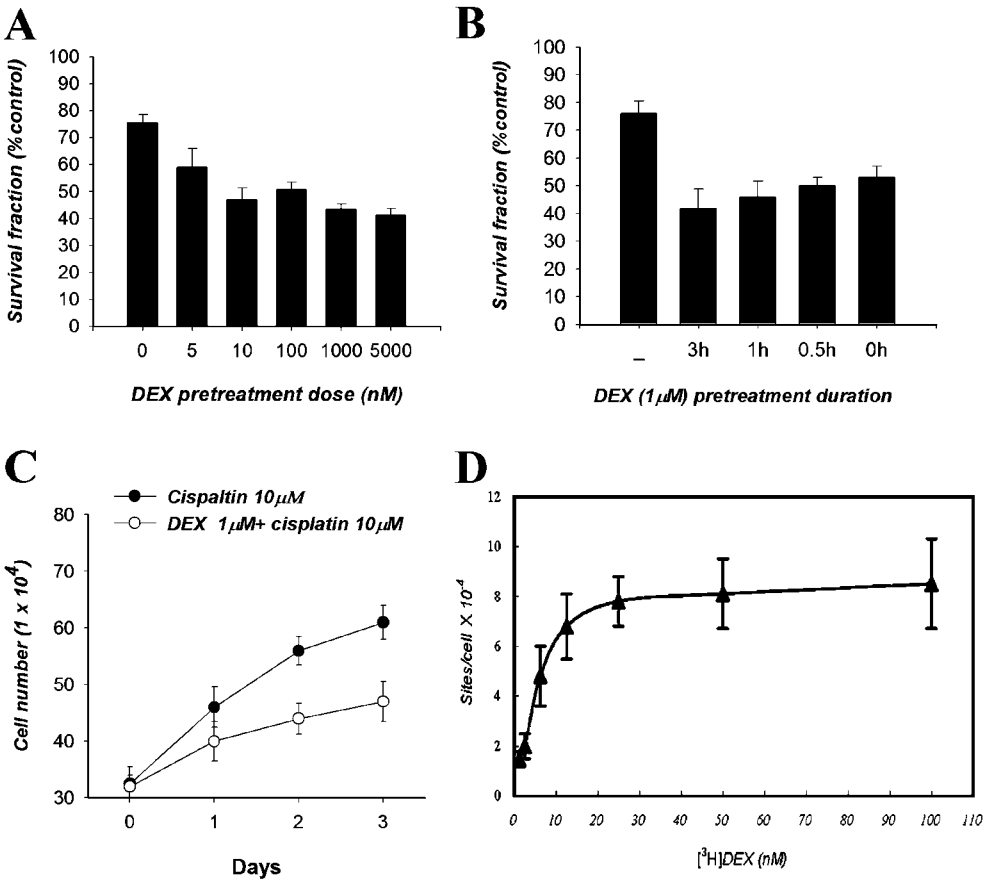


Figure 1 Effect of DEX on the cisplatin chemosensitivity of SiHa cells. (A) DEX pretreatment had a dose-dependent effect on chemosensitization up to 1000 nM. SiHa cells were pre-treated with various concentrations of DEX for 24 h, and then exposed to 10 μM of cisplatin for 3 days. Cell numbers were measured by MTT assay and were plotted as a percentage of the control (cells not exposed to drugs). The chemosensitizing effect could still be observed as low as 5 nM concentration. (B) Effect of DEX pretreatment duration on cisplatin chemosensitization in SiHa cells. Cells were pretreated with 1 μM of DEX for various durations, and then exposed to 10 μM of cisplatin for 3 days. Cell numbers were measured by MTT assay. A chemosensitizing effect of DEX could still be observed even when cells were co-incubated with cisplatin simultaneously. All values represent mean ± S.D. of experiments in 6 separate wells. (C) Using trypan blue exclusion method, SiHa cells pretreated with DEX for 24 h were still more sensitive to cisplatin. (D) Saturation binding to steroid receptors in SiHa cells. Specific binding was determined as described in Materials and Methods.

on the repression of NF-κB activity, while it had an intrinsic effect on the suppression of NF-κB activity (Fig. 3C). The effect of DEX on IκB expression in SiHa cells was also examined. Western blot analysis of whole-cell protein showed that DEX did not up-regulate the expression of IκB in SiHa cells (Fig. 3D).

Inhibition of NF-κB activation blocks the cytotoxicity-enhancing effect of DEX in SiHa cells

To further examine the role of NF-κB in the cytotoxicity-enhancing effect of DEX, we generated a recombinant plasmid containing dominant negative IκBα (dnIκBα) gene. This dnIκBα protein does not contain the residues necessary for signal-induced phosphorylation and proteasome-mediated degradation of IκBα, thereby preventing dissociation and translocation of NF-κB to the nucleus. The expression of the dnIκBα in pooled stably transfected SiHa cells was verified by Western blot analysis. As shown in Fig. 4A, the control pRCMV-transfected SiHa cells contained only the endogenous wild-type IκBα protein, while the dnIκBα pRCMV-transfected SiHa cells contained an additional band representing the truncated exogenous IκBα protein. Results of EMSA showed that NF-κB binding activity was markedly suppressed in the dnIκBα pRCMV-transfected cells after either TNF-α or cisplatin treatment (Fig. 4B). In addition, as shown in Fig. 4C, the cytotoxicity-enhancing effect of DEX was

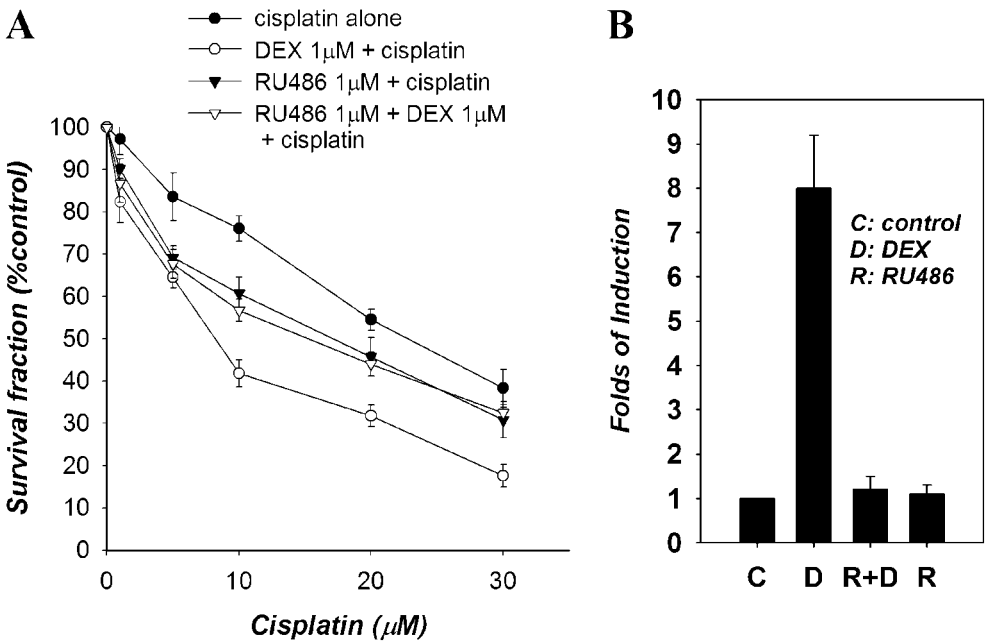


Figure 2 (A) Effect of RU486 on the DEX chemosensitizing effect in SiHa cells. Cell numbers were measured by MTT assay and plotted as a percentage of the control (cells not exposed to drugs). SiHa cells pretreated with DEX for 24 h were more sensitive to cisplatin. Co-pretreatment of cells with DEX and RU486 for 24 h partially abrogated the cisplatin chemosensitizing effect of DEX on SiHa cells. RU486 alone had partial cisplatin chemosensitizing effect. (B) Effect of RU486 on the DEX transactivation effect through GRE. SiHa cells were stably transfected with luciferase reporter plasmid containing MMTV LTR as described in Materials and Methods. The MMTV-Luc-transfected SiHa cells were pretreated with or without 1 μM DEX and with or without 1μM RU486 for 6 h. Luciferase activity was then assayed and represented as folds of the induction activity of the control. The DEX transactivation effect through GRE was completely inhibited by RU486 in SiHa cells. All values represent means ± S.D. of 3 experiments.

abolished in dnIκBα-pRCMV-transfected SiHa cells. The dnIκBα-pRCMV-transfected SiHa cells were also more sensitive to cisplatin as compared with the control pRCMV-transfected SiHa cells (Fig. 4C). These data confirmed that NF-κB plays a central role in the chemosensitizing effect of DEX on SiHa cells.

DEX has no effect on the expression of Bcl-2 family, $p21^{Cip1}$, MKP-1, and the activity of ERK

We examined other common possible mechanisms by which GC may decrease the cisplatin chemosensitivity (Chang *et al.* 1997, Naumann *et al.* 1998, Wu *et al.* 2005) of SiHa cells. Protein levels of Bax, Bcl-2, Bcl-$X_{L/S}$, $p21^{Cip1}$, MKP-1 and phospho-ERK1/2 were not changed by DEX treatment of SiHa cells (Fig. 5A). On the other hand, the expression of GR was not affected by the treatment of cisplatin in SiHa cells (Fig. 5B).

Discussion

This study has demonstrated that GC may affect the cytotoxicity of cisplatin in the GR-rich human carcinoma cell line, SiHa. Since GC is commonly co-administered with cisplatin, this influence of GC on cytotoxicity may affect the response to cisplatin treatment in carcinoma patients.

GC mediates its effects by binding to and activation of GR. Activated GR exerts its cellular effect by either transactivating its downstream GRE-containing genes or interacting with other transcriptional factors. In the former transactivation mechanism, GC binds to its cytoplasmic receptor, dimerizes, enters the nucleus, and finally binds to GREs to transactivate the target genes, such as tyrosine aminotransferase and alanine aminotransferase (Beato *et al.* 1995). However, some major effects of GC, including its anti-inflammatory and immunosuppressive effects, are achieved by regulating genes that do not contain GREs in their promoters (McEwan *et al.* 1997, Van der Burg *et al.* 1997, Cato & Wade 1996). This finding led to discovery of the second mechanism of action of GC, i.e. suppression of NF-κB activity by direct protein–protein interaction between activated GR and Rel A, the subunit of NF-κB (Ray & Prefontaine 1994, Scheinman *et al.* 1995*a*). Another minor mechanism of action of GC involves up-regulation of the expression of IκB gene, which then inactivates NF-κB (Auphan *et al.*

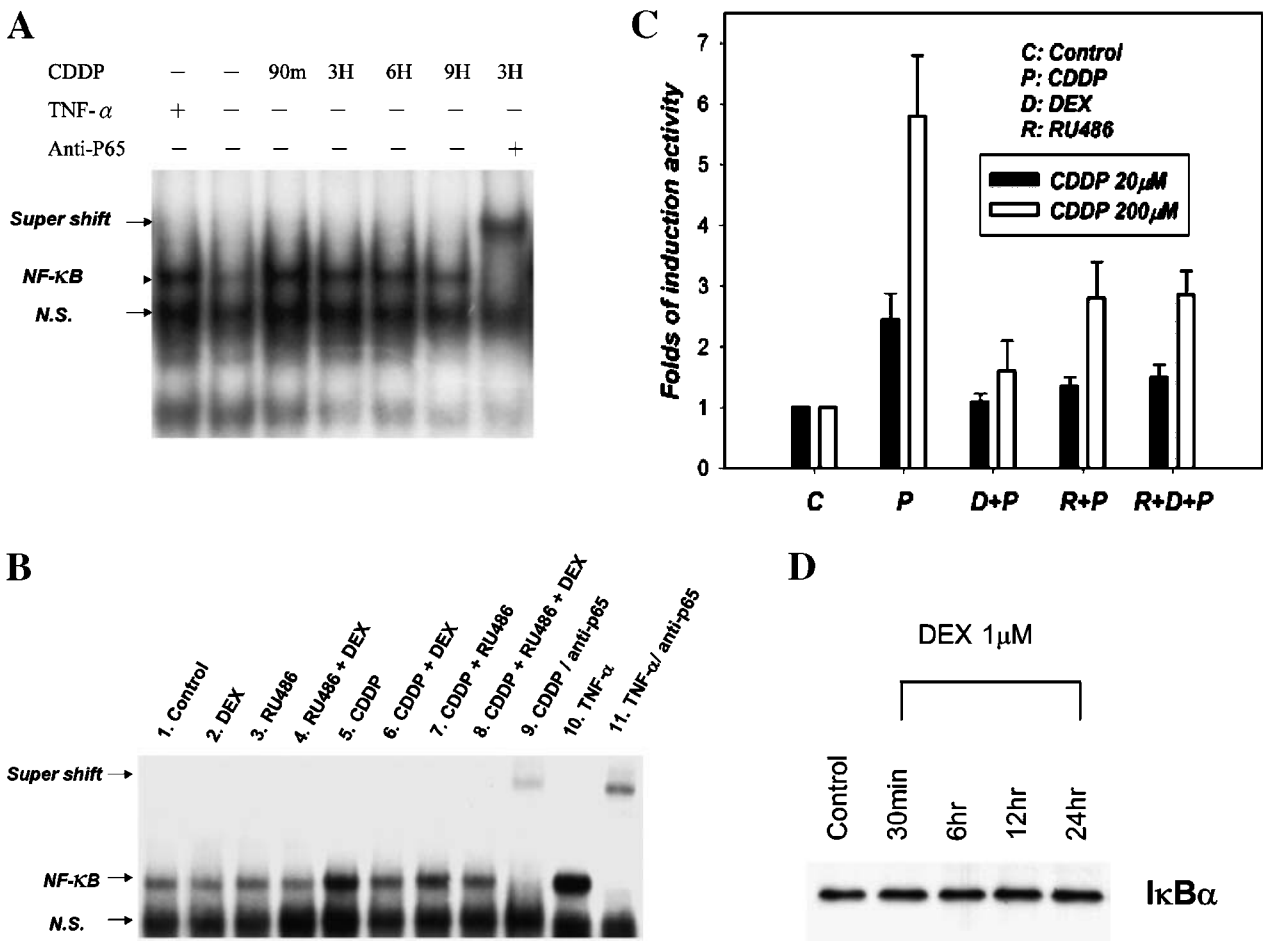
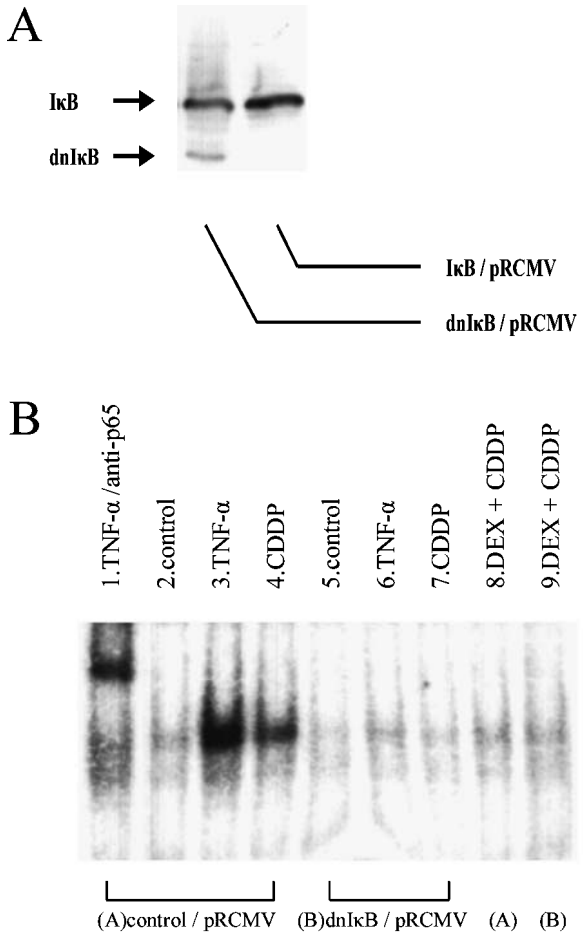


Figure 3 Effect of DEX and RU486 on cisplatin-induced NF-κB activity. (A) Nuclear extract was prepared and EMSA was performed as described in the Materials and Methods. Exposure of SiHa cells to 20 μM cisplatin for 3 h resulted in activation of NF-κB activity. The TNF-α lane represents positive control. Supershift by anti-p65 antibody verifies the correct band of NF-κB. (B) SiHa cells were pretreated with or without 1 μM DEX and with or without 1 μM RU486 for 24 h. Cells were then exposed to 20 μM cisplatin for 3 h. DEX and RU486 had no effect on NF-κB (lane 2, 3, and 4). Cisplatin activated NF-κB (lane 5), but DEX pretreatment completely abolished cisplatin-induced NF-κB activation (lane 6). RU486 partially abrogated the effect of DEX, but RU486 also had an intrinsic effect in reversing cisplatin-induced NF-κB activation. (C) SiHa cells were stably transfected with luciferase reporter plasmid containing five NF-κB sites as described in Materials and Methods. The SiHa/κB-reporter cells were pretreated with or without 1 μM DEX and with or without 1μM RU486 for 24 h, then exposed to 20 or 200 μM cisplatin for 3 h. The luciferase activity was assayed and represented as folds of the induction activity of the control. All values represent means ± S.D. of 3 experiments. (D) Effect of DEX on IκBα expression. SiHa cells were exposed to 1 μM DEX for different durations before harvesting. Western blot analysis of the whole cell lysates was performed. The protein amount of IκBα was not changed after DEX treatment. CDDP, cisplatin; TNF-α, tissue necrosis factor-α, N.S., non-specific binding.

1995, Scheinman *et al.* 1995*b*). RU486, a structural homologue of DEX, which binds GR with a 20-fold greater affinity than DEX, may help differentiate the two major mechanisms of actions of GC. RU486-bound GR dimerizes and translocates to the nucleus as DEX-bound GR does, but fails to transactivate GRE-containing promoters and thus completely abolishes the regulatory effect of DEX on the expression of GRE-containing downstream genes (Lindemeyer *et al.* 1990, Segnitz & Gehring 1990, Beck *et al.* 1993). In contrast, while DEX-bound GR effectively inactivates NF-κB, RU486-bound GR also partially inhibits NF-κB due to an intrinsic NF-κB suppressing activity (McEwan *et al.* 1997, Van der Burg *et al.* 1997, Wissink *et al.* 1998). Therefore, if a particular effect of activated GR is mediated via GRE, the addition of excess RU486 would completely abolish it. In contrast, if the effect of the GR is mediated via inactivation of NF-κB, addition of excess RU486 would only partially reverse it. Accordingly, our data suggest that DEX affects cisplatin chemosensitivity of SiHa cells primarily via the suppression of NF-κB activity, because RU486 totally reversed the effect of DEX on GRE reporter (Fig. 2B) while it only partially reversed the cytotoxicity-enhancing effect of DEX (Fig. 2A). This hypothesis is further



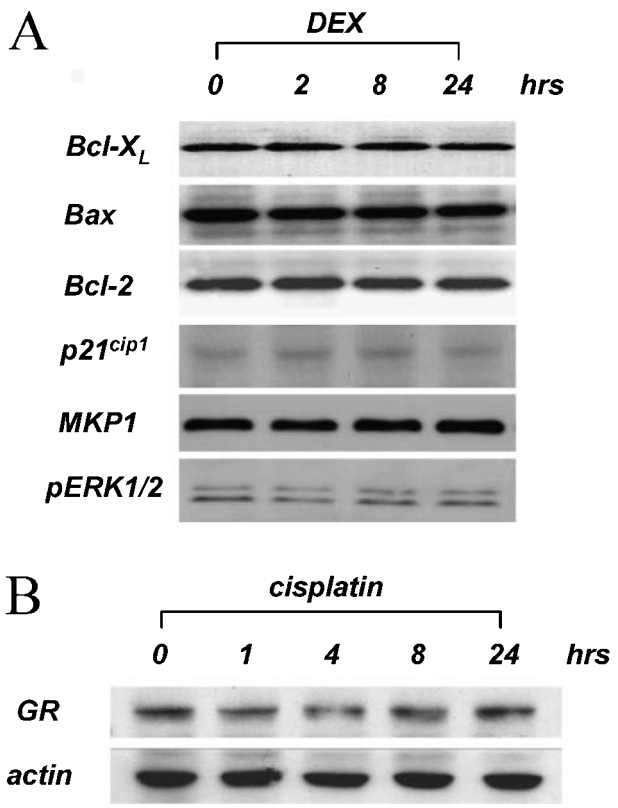


Figure 5 (A) Western blot analysis for Bcl-X_L, Bax, Bcl-2, $p21^{Cip1}$, MKP-1, and phospho-ERK1/2 in whole cell lysate of SiHa cells after DEX 1μM treatment for various durations. These proteins were not regulated by DEX in SiHa cells. (B) Western blot analysis for GR of SiHa cells after cisplatin 10μM treatment for various durations. The blots shown are representative of three experiments.

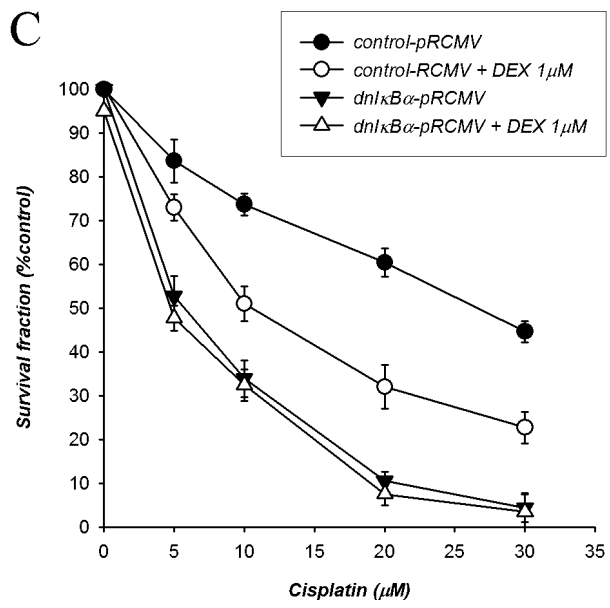


Figure 4 Effect of DEX on cisplatin-induced NF-κB activity in dominant negative IκB transfected SiHa cells. (A) Western blot analysis for IκBα in whole cell lysate of control-pRCMV-transfected SiHa cells and dnIκBα-pRCMV-transfected SiHa cells. An additional band in the lane of dnIκBα-pRCMV-transfected SiHa cells represents the exogenous truncated IκBα protein. (B) Nuclear extract was prepared and EMSA was performed as described in Materials and Methods. Exposure of control-pRCMV-transfected cells to cisplatin 20μM for 3 h or to TNF-α for 30 min resulted in activation of NF-κB (lane 3, 4), which was suppressible by DEX 1 μM pretreatment (lane 8). Super-shift by anti-p65 antibody verified the correct band of NF-κB (lane 1). The NF-κB activity was not increased in dnIκBα-pRCMV-transfected SiHa cells exposed to TNF-α and cisplatin (lane 6, 7). (C) Effect of DEX on the chemosensitivity in dominant negative IκB transfected SiHa cells. Cell numbers were measured by MTT assay and plotted as a percentage of the control (cells not exposed to the drugs). The control-pRCMV-transfected SiHa cells pretreated with DEX for 24 h were still more sensitive to cisplatin. However, the cytotoxicity-enhancing effect of DEX in dnIκBα-pRCMV-transfected SiHa cells was abolished.

supported by results of NF-κB activity assay (Fig. 3B and 3C). Western blot analysis of whole-cell protein showed that DEX did not up-regulate the expression of IκB in SiHa cells (Fig. 3D). Therefore, DEX suppressed cisplatin-induced NF-κB activation mainly through the protein–protein interaction between activated GCR and NF-κB in SiHa cells.

Activation of NF-κB has been implicated in mediating drug resistance of cancer cells. NF-κB could be activated by a variety of stresses, including oxidative stress and DNA damage (Quinto *et al.* 1993, Legrand-Poels *et al.* 1995, Piret & Piette 1996). Activated NF-κB may prevent the triggering of apoptosis, and thus result in drug resistance against DNA-damaging agents (Beg & Baltimore 1996, Van Antwerp *et al.* 1996, Wang *et al.* 1996). The molecular mechanism of NF-κB-mediated protection of cells remains unclear, but may involve the up-regulation of caspase inhibitors (Chu *et al.* 1997), and anti-apoptotic genes (*e.g.* cIAP-2, γGCS, Bcl-XL, A20 and Cyclin D2) (Shishodia & Aggarwal 2004). In this study, we have provided evidence that NF-κB plays an important role in mediating the drug resistance of SiHa cells. Suppression of NF-κB activity by dnIκBα not only abolished the cisplatin chemosensitizing effect of DEX, but the suppression of NF-κB activity by dnIκBα increased the chemosensitivity of SiHa cells to cisplatin (Fig. 4B and 4C).

Other studies have shown that GC decreases the chemosensitivity in non-hematological solid tumors cells through a variety of mechanisms, including modulation of bcl-x expression, up-regulation of $p21^{Cip1}$ and MKP-1 (Chang *et al.* 1997, Naumann *et al.* 1998, Wu *et al.* 2005). However, in the present study, we found that there was no change of the expression of the Bcl-2 family, $p21^{Cip1}$ and MKP-1 in SiHa cells after treatment with DEX. These diverse results might only be related to differences in the cell context. However, some cellular factors, such as steroid receptor co-regulators, may have played an important role in mediating the biochemical modulating effect of GC in carcinoma cells. It is apparent that a more comprehensive approach is needed to clarify the role of GC in the chemosensitivity of carcinoma cells.

In summary, we have demonstrated that DEX chemosensitizes SiHa cells to cisplatin. The mechanism of action of this effect appears to be related to its inhibition of cisplatin-induced NF-κB activation. Since the administration of high dose DEX is widely used for the prevention of cisplatin-induced nausea and vomiting, the possible effect of GC on the cytotoxicity of cisplatin may have clinical importance in selected carcinoma patients and deserves further investigation.

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