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Pharmacological Separation of the Expression of Tissue Transglutaminase and Apoptosis after Chemotherapeutic Treatment of HepG2 Cells

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ABSTRACT

Chemotherapeutic drugs are known to eliminate cancer cells by inducing apoptosis. Tissue transglutaminase (tTG), a frequent player in apoptotic processes, is markedly induced in drug-resistant cancer cells. To better understand the action of apoptosis-inducing drugs, our study elucidates changes in the expression of tTG in the early phase of cell death, before the downstream events of apoptosis. We demonstrate that HepG2 cells uniformly induce both tTG mRNA and enzyme activity upon treatment with cisplatin, doxorubicin, and bleomycin, chemotherapeutic agents with different modes of action. The expression of fas ligand, caspase3 and baxα changes differentially or remain unaffected. tTG expression did not change significantly after administration of either the peroxisome proliferator activated receptor-α agonist WY14643 or the retinoid X receptor-specific analog LG 100268. However, both compounds blocked drug-induced tTG induction without affecting the extent of cell death. The pleiotropic cytokine interleukin-6 effectively rescued hepatoma cells from apoptosis while tTG induction still took place, along with the induction of antiapoptotic transcripts bcl-x_L, gp130, and her2/neu. These results suggest that the induction of tTG, although present in drug-induced apoptosis, is pharmacologically dissociable from the early, initiating events of apoptosis. Blocking the induction of tTG during drug-induced cell death may alleviate limiting side effects of anticancer agents, including fibrosis and neuropathies.

The family of transglutaminase enzymes catalyze the Ca^{2+}-dependent cross-linking of proteins using protein bound glutaminic γ-carboxamide groups and primary amino groups of lysines in polypeptide chains or polyamines (Lorand and Conrad, 1984). Transglutaminases have been implicated in a wide variety of biological phenomena encompassing the stabilization and protection of cell and tissue integrity, including a role in the scaffold formation of apoptotic bodies and cornified envelopes, hepatic fibrogenesis, and tissue remodeling (Fesus et al., 1987; Mirza et al., 1997; Aeschlimann and Thomazy, 2000). Furthermore, the identification of tissue transglutaminase (tTG) as a GTP-binding protein raises the idea of other possible roles in signal transduction events, including the initiation phase of programmed cell death (Nakaoka et al., 1994; Melino and Piacentini, 1998).

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One of the unexpected observations to emerge from early studies on the comparison of transglutaminase activity of normal and neoplastic cells was that exposure of several tumor cell lines to antineoplastic drugs was associated with a marked increase in transglutaminase (TG) activity (Piacentini et al., 1993; Furuya and Isaacs, 1994; Lokshin et al., 1995). Because overexpression of tTG in transformed cells causes cell death, it has been suggested that induction of the enzyme may contribute to the cytotoxic effect of chemotherapeutic drugs (Gentile et al., 1992; Furuya and Isaacs, 1994; Melino et al., 1994). In addition, it has been demonstrated that some cytotoxic drugs, such as bleomycin, are TG inhibitors and can interfere with TG-mediated conjugation of polyamines (Griffin et al., 1978). Russel and Womble (1982) have shown that this inhibition can also contribute to drug-induced cytotoxicity. In contrast, several studies have suggested that the induction of tTG in cells treated with antineoplastic drugs may play a role in drug resistance of these cells. Doxorubicin-resistant human breast and lung carcinoma cells showed a much higher level of tTG expression

ABBREVIATIONS: tTG, tissue transglutaminase; TG, transglutaminase; Cis, cisplatin; Dox, doxorubicin; Bleo, bleomycin; RT, reverse transcriptase; PCR, polymerase chain reaction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; IL-6, Interleukin-6; PPAR, peroxisome proliferator activated receptor; RXR, retinoid X receptor; NFκB, nuclear factor κB.

than drug-sensitive ones (Mehta, 1994; Han and Park, 1999b).

To clarify the role of tTG in the cytotoxic activity of three unrelated antineoplastic drugs frequently used in the chemotherapy of human malignancies, cisplatin (Cis), doxorubicin (Dox) and bleomycin (Bleo), we have studied the molecular and regulatory events that take place in the 'window' of time that precedes the execution phase of apoptosis. Therefore, we applied response modifiers as tools to pharmacologically perturb enzyme induction and cell death and characterized transcriptional changes in the early phase of drug-induced apoptosis.

We report herein that, upon application of various anticancer treatments, tTG undergoes uniform transcriptional up-regulation followed by the induction of enzyme cross-linking activity, occurring before apoptosis. Stimulation with PPARα or RXR agonists causes abrogation of tTG induction but still allows drug-induced cell death. Treatment of cells with interleukin-6 (IL-6) before the addition of anticancer agents effectively prevents cell death and up-regulates gp130, her2/neu (erbB2), and bcl-x_L, but does not interfere with induction of tTG. These findings suggest that tTG is involved in the apoptotic phenotype but is pharmacologically dissociable from the early phase of apoptosis.

Materials and Methods

Cell Cultures. HepG2 cells were obtained from the European Collection of Animal Cell Cultures and grown in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (Life Technologies, Gaithersburg, MD), 2 mM glutamine, and 2 mM Na-pyruvate but no antibiotics and maintained in an incubator with 5% CO_2 at 37°C and 90% humidity. Cells were routinely passaged twice a week by trypsinization (0.1% trypsin plus 0.02% EDTA in phosphate-buffered saline) and used for experiments when reaching 30 to 40% confluence.

Induction and Modulation of Apoptosis. HepG2 cells were treated with cisplatin (EBEWE Pharmaceuticals, Unterach, Austria) at a dose range of 1 to 100 μg/ml, doxorubicin (Farmitalia Carloerba, Milan, Italy) at a dose range of 20 ng/ml to 20 μg/ml, or bleomycin (Nippon Kayaku, Tokyo, Japan) at a dose range of 60 μg/ml to 3 mg/ml to determine optimal concentrations to induce apoptotic but not necrotic cell death most abundantly at the time point of maximal effect. Puromycin (Sigma) was applied at 10 μg/ml, WY14643 (pirinixic acid, Sigma) was used at a dose of 10 μM. LG100268 was obtained from Ligand Pharmaceuticals and applied at a concentration of 1 μM. Interleukin-6 (a kind gift from Dr. András Falus) was applied at a concentration of 20 U/ml. In each case, at least two independent experiments were performed.

RNA Preparation and Quantitative RT-PCR. Human liver RNA was purchased from CLONTECH (Palo Alto, CA). Total RNA was isolated from 10^6 HepG2 cells after appropriate treatment using the RNeasy kit from Qiagen (Chatsworth, CA). After DNase treatment, reverse transcription was performed at 50°C for 30 min from 100 ng of total RNA using Superscript II reverse transcriptase and specific reverse primers. Quantification based on real-time monitoring of amplification was carried out using an ABI 7700. All determinations were done in triplicate with one control reaction containing no RT enzyme to test for potential DNA contamination. Values of transcripts in unknown samples were obtained by interpolating Ct (PCR cycles to threshold) values on a standard curve derived from known amounts of cognate, amplicon-specific synthetic RNAs. Absolute numbers of mRNA molecules have been normalized to cyclophilin to correct for differences in RNA concentration. Sequences of the primers and TaqMan probes used for subsequent amplification reactions are summarized in Table 1. Synthetic sRNA standards were synthesized as described previously (Ahuja et al., 2001).

Protein and Enzyme Activity Assays. Protein concentrations were determined by the Bradford method with necessary reagents obtained from Bio-Rad Laboratories (Hercules, CA). Tissue transglutaminase activity was measured by the rate of incorporation of [^{3}H]putrescine into *N,N*-dimethylated casein. Reaction mixtures consisted of the following in a total volume of 100 μl: 50 μl of crude cell homogenate, 10 μl of *N,N*-dimethylcasein (40 mg/ml), 20 μl of [1,4(n)-^{3}H] putrescine (30 Ci/mmol), 10 μl of 250 mM Tris·HCl, pH 7.5, containing 150 mM β-MEA and 10 μl $CaCl_2$ (50 mM). The reaction was initiated by the addition of $CaCl_2$ and incubated at 37°C. After 5 and 10 min elapsed, a 25-μl sample was taken, dropped on filter paper, and precipitated in cold trichloroacetic acid; washed

TABLE 1
Primers and TaqMan probes used for transcript quantification

Transcript Name	Sequence	Accession No.
Cyclophilin	(52+) ACGGCGAGCCCTTGG (117−) TTTCTGCTGTCTTTGGGACCT (69+) FAM-CGCGTCTCCTTTGAGCTGTTTGCA-TAMRA	Y00052
tTG	(529+) CTGGGCCACTTCATTTTGC (610−) ACTCCTGCCGCTCCTCTTC (564−) FAM-TCCAGGTACACAGCATCCGCTGGG-TAMRA	M55153
Casp3	(669+) AGAAGTCTAACTGGAAAACCCAAA (781−) TTATGACACGCCATGTCATCA (777−) FAM-GACACGCCATGTCATCATCA-TAMRA	4757911
CD95L	(606+) CAGGCAAGTCCAACTCAAGG (676−) TCCAGAAAGCAGGACAATTCC (627+) FAM-CCATGCCTCTGGAATGGGAAGACA-TAMRA	U08137
Baxα	(335+) CCCTTTTCTACTTTGCCAGC (396−) CAGTTCCGGCACCTTGG (356) FAM-AACTGGTGCTCAAGGCCCTGTGC-TAMRA	L22473
bcl-x_L	(457+) TGACATCCCAGCTCCACAT (532−) TCCCGGAAGAGTTCATTCAC (477−) FAM-ACCCCAGGGACIGCATATCAGAGCTT-TAMRA	L20121
gp130	(1650+) CTGTATCACAGACTGGCAACAAG (1728−) GCATTTGCTCTCTGCTAAGTTCC (1676+) FAM-ATGGTACCGTGCATCGCACCTATTTAAGA-TAMRA	M57230
Her2/neu	(681+) CCAGCTGGCTCTCACACTG (754−) AGCCCTTACACATCGGAGAAC (704−) FAM-AGGCCCGAGAGCGGTTGGTGT-TAMRA	NM_004448

FAM, 6-carboxy-fluorescein; TAMRA, 6-carboxy-tetramethyl-rhodamine.

intensively with 10% and 5% TCA and ethanol; and radioactivity of the filter was measured in a liquid scintillation counter. Enzyme activity was calculated as picomoles of [^{3}H]putrescine incorporated into casein in 1 min by one milligram of cellular protein.

Cytotoxicity Assay with MTT Staining of Viable Cells. The MTT assay is a colorimetric procedure based on the ability of viable cells to reduce a soluble yellow tetrazolium salt (MTT) to blue formazan crystals. Ten thousand cells were plated into wells of 96-well plates; 24 h later, cells were treated by various chemotherapeutic drugs. After 48 to 72 h, 5 mg/ml MTT was added and the plates were further incubated at 37°C for 3 h. After eluting the dye with isopropanol/formic acid, absorbance was determined using an automated plate reader at 540 nm and 620 nm. Results are the means of at least five independent measurements and expressed as the percentage of the absorbance of untreated control cells ± S.D.

Detection of Apoptosis. Assessment of apoptotic cells by flow cytometric analysis was carried out in a Coulter EPICS-XL apparatus using the System II software (Beckman Coulter, Fullerton, CA). Floating cells were collected by centrifugation at 200 g. Adherent cells were harvested by trypsinization with 1% trypsin for 2 min. All cells were washed in PBS, fixed in 70% ethanol, and stained with 50 μg/ml propidium-iodide for 30 min (Nicoletti et al., 1991). Cells showing lower DNA contents than those in the G_0 phase were considered apoptotic.

Statistical Analyses. Differences in transcript levels, enzyme activities, and cell death rates between the control group and various

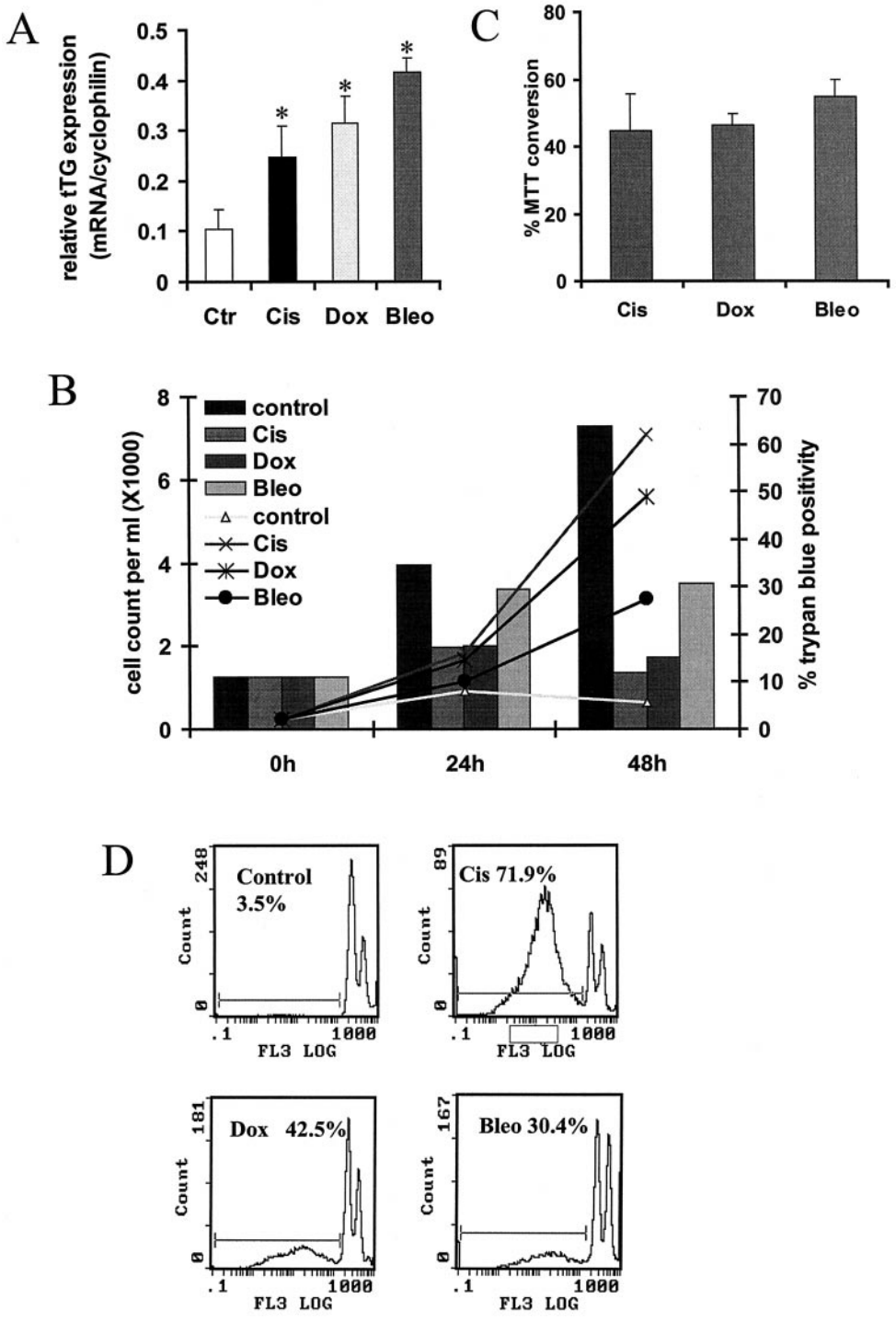

Fig. 1. Effect of the administration of 10 μg/ml cisplatin (Cis), 2 μg/ml doxorubicin (Dox) or 0.6 mg/ml bleomycin (Bleo) on tTG mRNA levels and apoptosis of HepG2 cells compared with untreated control cells (Ctr, $^*p < 0.05$). A, comparison of tTG transcript levels in HepG2 cells treated with antineoplastic drugs for 6 h (normalized to cyclophilin and given as mean ± SD). B, illustration of the progression of viable cell counts (bars) after 24 and 48 h of treatment with chemotherapeutic drugs and the accumulation of trypan blue positive cells (lines). C, comparison of MTT positivity as a measure of viability in cell cultures treated with Cis, Dox, or Bleo for 48 h, relative to untreated ones (100%). D, flow cytometric analysis of propidium iodide-stained HepG2 cells after exposure to Cis, Dox, or Bleo; ratios of the gated apoptotic populations are shown.

treatment groups were tested by one-way analysis of variance test with Tukey's post hoc analysis. When normal distribution could not be assumed despite logarithmic transformation of the data, the corresponding nonparametric test (i.e., Kluskal-Wallis analysis of variance on ranks) was performed. Student's t test was applied for pairwise comparison of treatments. All statistical analyses were carried out using SigmaStat (Jandel, San Rafael, CA) software systems. p-Values < 0.05 were considered statistically significant.

Results

Induction of tTG in Relation to Apoptosis. The tTG enzyme is activated and induced in liver cells in several models of apoptosis (Fesus et al., 1987; Cummings, 1996). To investigate the molecular mechanisms of different antineoplastic drugs in relation to apoptosis, we treated nonconfluent cultures of HepG2 hepatoma cells with 10 μg/ml cisplatin, 2 μg/ml doxorubicin, or 0.6 mg/ml bleomycin. Six hours later, gene expression was quantified in total RNA extracts from these cells using real-time, quantitative RT-PCR. According to multiple determinations, tTG transcripts were present in a low abundance in HepG2 cells, approximately 0.01% of cyclophilin, a highly abundant 'housekeeping' gene.

Each drug markedly induced transcript levels of tTG with the applied treatment ($p < 0.01$; Fig. 1A). However, inductions were abrogated by simultaneous administration of the protein synthesis inhibitor puromycin (10 μg/ml, data not shown), suggesting the involvement of a mediating factor. The up-regulation of tTG occurring after 6 h preceded any morphological or biochemical signs of apoptosis. Cytotoxic effects first occurred after 24 h of treatment by cisplatin with significant inhibition of cell proliferation and an increase in cell population unable to exclude trypan blue stain ($p < 0.05$; Fig. 1B). Similar but more delayed effects were observed after treatments with doxorubicin and bleomycin. These findings were consistent with determinations of viability 48 h later by MTT assays (Fig. 1C) or uptake of neutral red dye (not shown). The type of underlying cell death in each case was apoptotic, as indicated by the corresponding fraction of hypochromic nuclei in flow cytometric analysis (Fig. 1D) and the morphology involving cell shrinkage, cytoplasmic blebbing, and nuclear condensation (not shown).

Modulation of tTG Expression and Cell Death by Response Modifiers. Having determined the induction of tTG at the transcriptional level, we then measured cross-linking activity in whole-cell lysates to confirm that the induction of RNA resulted in the production of functional enzyme upon drug treatment. As expected, the induction of enzyme activity followed a trend similar to that of the change at the transcript level (Fig. 2A). The most pronounced induction was measured after bleomycin treatment. The observed response, to a more modest extent, could be elicited in HeLa cervical carcinoma and K562 erythroleukemia cells, human cell lines derived from different sources of tissue (Fig. 2B).

Because PPARα is the predominant form of its family of nuclear receptors in liver cells (Sterchele et al., 1996), we investigated the effect of WY14643, a potent PPARα compound, on the expression of tTG. We found that when preadministered 12 h before antineoplastic drug treatment, WY14643 was very effective in blocking the up-regulation of tTG resulting from antineoplastic drug treatment (Fig. 3A). This effect was even greater with LG100268 (268), an RXR-specific retinoid analog. However, combined treatment of HepG2 cells with either compound and bleomycin or doxorubicin did not result in increased survival (not shown).

The next set of experiments were designed and carried out to investigate the effect of chemotherapeutic treatments on tTG induction in the absence of concomitant cell death. IL-6 is a known survival factor for a number of cell types (Kerst et al., 1993; Fujio et al., 1997). Without affecting proliferation of HepG2 cells, when applied for 24 h before administration of anticancer drugs, IL-6 efficiently diminished cell death in cells subsequently treated for 48 h with bleomycin and doxorubicin (Fig. 3B), but not cisplatin. At the same time, induction of tTG was not abrogated (Fig. 3C). In fact, bleomycin showed a synergistic effect with IL-6 to induce tTG.

Expression Pattern of Other Apoptosis-Related Genes. To evaluate whether the induction of tTG was coincident with changes of other genes in HepG2 cells, we quantified the transcripts of genes commonly regarded as key effectors in apoptotic cells. These included fasL, caspase3, and bax α along with genes with antiapoptotic potential, bcl-x_L, the signal transducer gp130, and her2/neu (Table 2). Our results show that both cisplatin and bleomycin selectively induce tTG and fasL. Expression levels of caspase 3

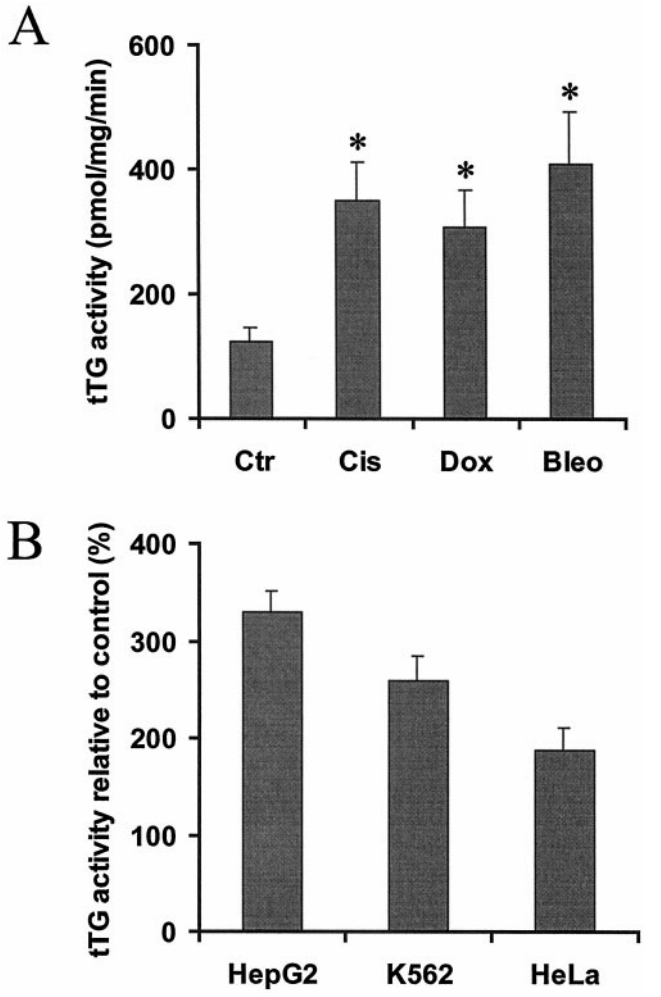

Fig. 2. Tissue transglutaminase activity in human cell lines following chemotherapeutic treatments. A, comparison of tTG cross-linking activity in lysates of HepG2 cells treated with antineoplastic drugs for 48 h (values shown as mean ± SD, *$p < 0.05$). B, comparison of tTG activity in human cell lines HepG2, K562, and HeLa 48 h after the addition of 0.6 mg/ml Bleo.

were unchanged and bax α was induced only by cisplatin. As opposed to tTG, the expression of the receptor tyrosine kinases gp130 and her2/neu were down-regulated by all three drugs. Also, bcl-x_L transcript levels decreased upon treatment with cisplatin and doxorubicin but not bleomycin. Induction of both tTG and fasL mRNAs were transitory, because transcript levels already decreased 12 h later, whereas changes in bcl-x_L and her2 expression showed a continuous trend over 18 h. Interestingly, IL-6 pretreatment resulted in marked and sustained induction of gp130 when combined with either anticancer drug (Fig. 4A), paralleled by a smaller induction of her2/neu mRNA (Fig. 4B). IL-6 pretreatment also up-regulated bcl-x_L expression, maintaining its induced state even after drug treatments (Fig. 4C). Administration of WY14643 or LG100268 did not have a similar effect (data not shown).

Taken together, these results demonstrate that chemotherapeutic agents uniformly induce tTG in HepG2 cells. The proinflammatory cytokine IL-6 suppressed cell death without decreasing tTG induction. Using ligands of a nuclear receptor heterodimer, drug-induced apoptosis took place without any induction of tTG. The expression of gp130, her2/neu, and bcl-x_L is coordinately suppressed by the used drugs but is induced in parallel to increased survival elicited by IL-6.

Discussion

In this study, we report that in HepG2 cells, DNA-damaging chemotherapeutics elicit uniform induction of a marker of apoptosis, tTG, well before manifest cell death takes place. The finding seems to represent a general effect of antineo-

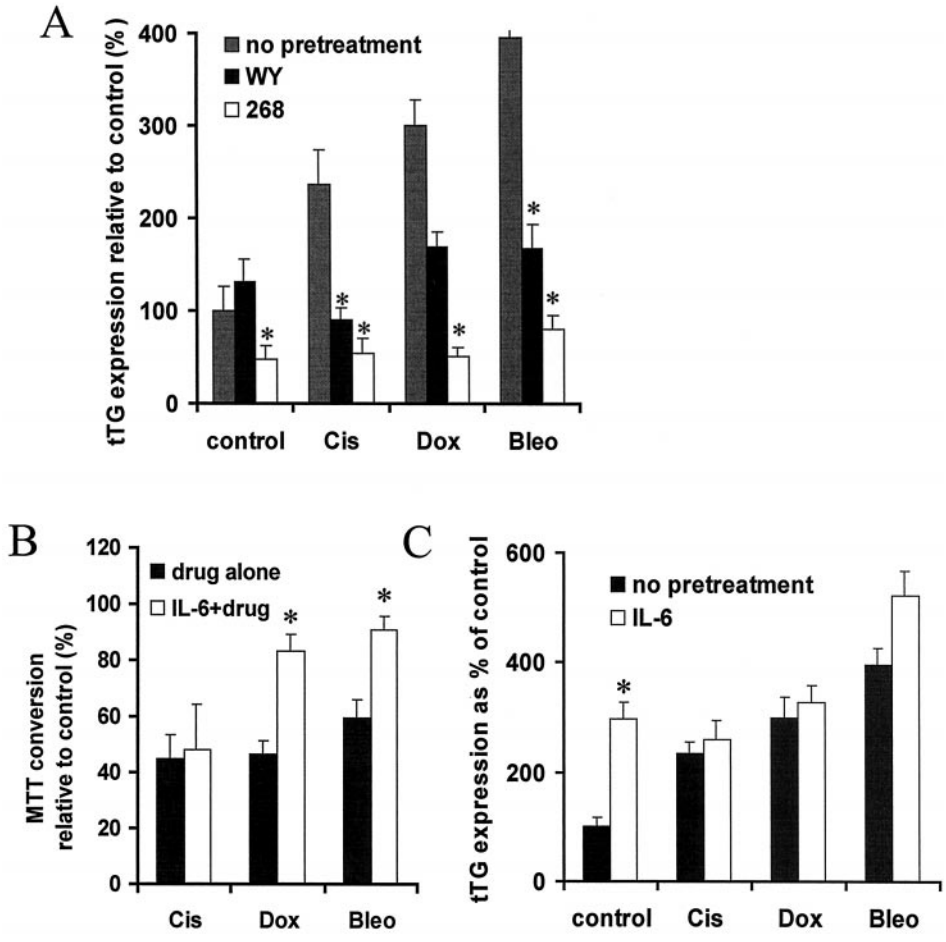

Fig. 3. Effect of the pretreatment of HepG2 cells with PPARα and RXR ligands and IL-6 on tTG expression and cell viability following antineoplastic treatment. A, comparison of the effects of a 12-h pretreatment with vehicle only (▨), 10 μM WY14643 (■), or 1 μM LG100268 (□) on tTG mRNA levels subsequently induced by antineoplastic drugs at 6 h. Normalized values are expressed as fraction of untreated controls (*$p < 0.05$). B, comparison of MTT reduction capacity in cells without (■) or with (□) preadministration of 20U/ml IL-6 before chemotherapeutic treatment. Measurements were made 48 h after the addition of drugs. Values are shown as mean ± SD (*$p < 0.05$). C, comparison of the effects of 24-h pretreatment with vehicle only (■) or 20U/ml IL-6 (□) on tTG mRNA levels induced by antineoplastic drugs. Normalized values are expressed as fraction of untreated controls.

TABLE 2
Transcript levels of apoptotic and antiapoptotic genes after various chemotherapeutic treatments

	tTG		FasL		Caspase 3	±	Bax α	±	bcl-x_L	±	gp130	±	Her2/neu	±
	×1000	±	×1000	±										
Human liver RNA	7.8	*2.2*	9.9	*1.2*	1.1	*0.1*	1.6	*0.1*	n.a.		57.0		12.2	
HepG2—untreated	10.5	*4.0*	4.5	*4.0*	1.4	*0.2*	3.6	*0.3*	1.8	*0.2*	3.8	*0.2*	8.6	*0.3*
Cisplatin 6 h	24.7	*6.2*	28.5	*13.4*	1.4	*0.1*	6.4	*0.6*	1.2	*0.1*	2.1	*0.1*	6.4	*0.0*
Cisplatin 18 h	9.6	*3.1*	0.0	*0.0*	0.9	*0.7*	4.6	*0.9*	0.9	*n.a.*	n.a.		4.7	*n.a.*
Doxorubicin 6 h	31.4	*5.5*	2.6	*3.2*	0.8	*0.1*	2.8	*0.2*	0.6	*0.0*	1.9	*0.1*	4.4	*0.1*
Doxorubicin 18 h	7.8	*0.6*	4.2	*1.4*	1.1	*0.0*	1.4	*0.1*	0.7	*0.2*	n.a.		2.4	*0.3*
Bleomycin 6 h	41.5	*2.9*	11.2	*9.5*	1.6	*0.1*	4.4	*0.5*	2.7	*0.1*	3.1	*0.3*	4.9	*1.2*
Bleomycin 18 h	19.2	*1.3*	1.9	*1.0*	0.9	*0.1*	4.5	*0.9*	3.1	*1.1*	n.a.		2.3	*0.0*

Values shown are transcript levels expressed as percentage of cyclophilin; S.D. values are italic.

plastic agents, because drugs with different modes of action elicited induction of tTG in different cell types.

The expression and activity of proapoptotic genes (tTG, fasL, caspase 3, bax) are regulated at several different levels (Balajthy et al., 1997; Nicholson and Thornberry, 1997; Villunger et al., 1997; Wood and Newcomb, 2000). Therefore, it comes as no surprise that chemotherapeutic treatment fails to alter the mRNA pool of many apoptotic genes (Table 2). Antiapoptotic transcripts, however, undergo correlated changes; surprisingly, these appear in a coordinated fashion with tTG. The induction of gp130 and her2/neu along with bcl-x_L by IL-6 may be manifestations of an orchestrated process to protect the cell from apoptotic triggers. Her2/neu belongs to the same family of EGF receptors and is often up-regulated or amplified in breast cancers. It has also been implicated in chemoresistance (Tsai et al., 1993) and shown to associate with the Fas receptor (Shen and Novak, 1997), suggesting a possible connection to Fas-dependent cell death. Recent findings imply that Her2/neu may be involved in the IL-6 signaling pathway via gp130, its newly identified heterodimerization partner, to generate a receptor that has potent antiapoptotic activity in a variety of cell types (Qiu et al., 1998; Chien, 1999). Our data imply that expression of her2/neu and gp130 in HepG2 cells treated with chemotherapeutic drugs is directed by the same factor and provide further evidence of an autocrine regulatory loop existing in HepG2 cells. The identification of these mechanisms and their functional relationship to the participation of tTG in drug-induced apoptosis remain the subject of further studies.

Based on the information accumulated over the years about the possible connections of transglutaminase enzymes and drug-induced cytotoxic effects (Griffin et al., 1978; Russell and Womble, 1982; Piacentini et al., 1993; Han and Park, 1999a), important knowledge can be provided by studies clarifying the role of tTG on the molecular mechanisms of chemotherapeutic agents. Our results suggest, that although the induction of tTG is a frequent component of the apoptotic phenotype, it is pharmacologically dissociable from the early phase of apoptosis. Using agonists of a nuclear receptor heterodimer as response modifiers, the effects of cytotoxic drugs on tTG could be manipulated independently from concomitant cell death. The multiplicity of simultaneous processes occurring in the course of apoptosis can allow for cell death without early tTG up-regulation, whereas enhanced tTG induction can still permit rescue from cell death by alternative mechanisms. This result does not support the notion that tTG may be an essential player in the early, decision-making phase of apoptosis. On the other hand, the observation may be important in cancer therapy, because the ability to block the induction of tTG during drug-induced cell death may alleviate some of the limiting side effects of anticancer agents linked to transglutaminase activity, including fibrosis and neuropathies (Toida et al., 1991; Mirza et al., 1997; Igarashi et al., 1998).

The common factor to regulate tTG induction upon antineoplastic treatment of cancer cells remains to be elucidated. In macrophages, NFκB signaling was shown to be involved in tTG induction by tumor necrosis factor-α (Kuncio et al.,

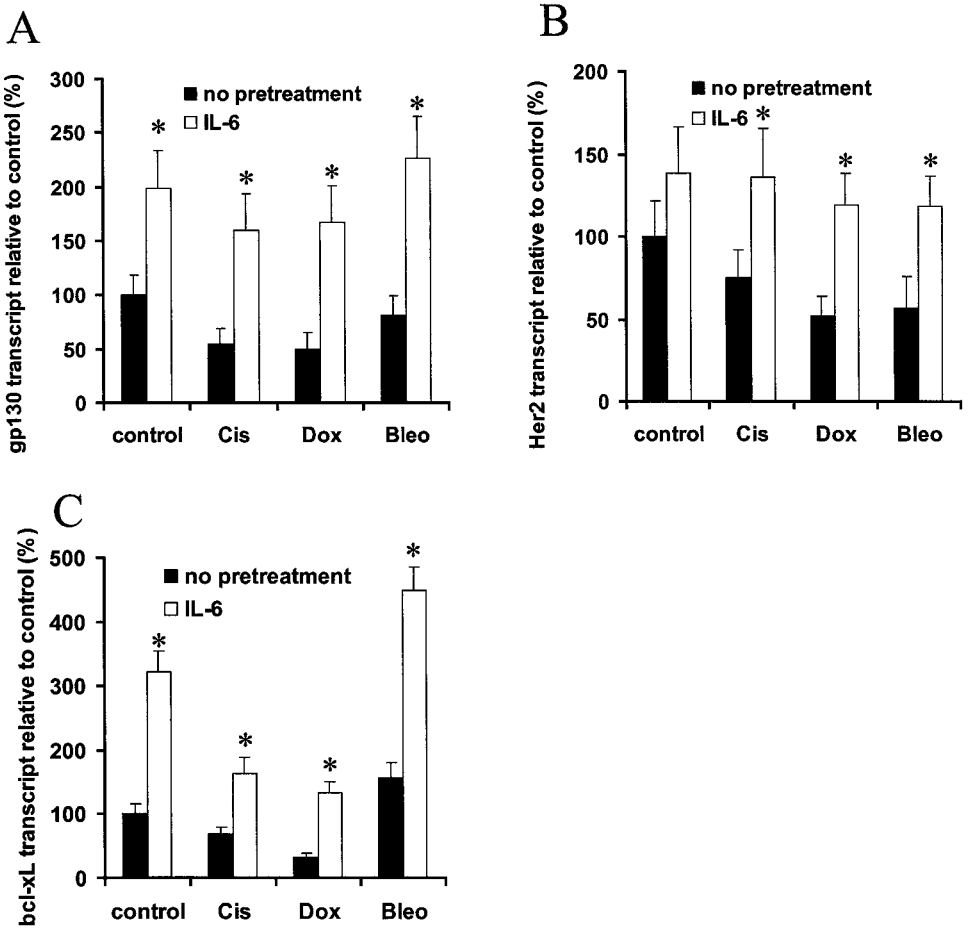

Fig. 4. Effect of the pretreatment of HepG2 cells with IL-6 on drug-induced changes in transcript levels of antiapoptotic genes. Normalized values are expressed as fraction of untreated controls (* $p < 0.05$). A, comparison of the effects of 24-h pretreatment with vehicle only (■) or 20 U/ml IL-6 (□) on gp130 mRNA levels after antineoplastic drug treatment. B, comparison of the effects of 24-h pretreatment with vehicle only (■) or 20 U/ml IL-6 (□) on Her2/neu mRNA levels after antineoplastic drug treatment. C, comparison of the effects of 24-h pretreatment with vehicle only (■) or 20U/ml IL-6 (□) on bcl-x_L mRNA levels after antineoplastic drug treatment.

1998). NFκB signaling can be blocked by the activation of PPARs (Ricote et al., 1998). Because WY14643 and LG100268, PPARα and RXR agonists, respectively, interfere with the induction of tTG in HepG2 cells, we can hypothesize that NFκB participates in drug-dependent tTG up-regulation. Transcriptional regulation of fasL involves activator protein-1 and SV-40 promoter binding protein-1 sites and a functional role of NFκB (Holtz-Heppelmann et al., 1998; Kasibhatla et al., 1998), all of which are present in the tTG promoter. Whether NFκB acts as *cis*-regulatory element to retinoid or IL-6 response elements remains to be investigated. However, the differential effect of doxorubicin suggests that despite shared regulatory elements chemotherapeutic drugs may lack one common mechanism to trigger the molecular response in HepG2 cells.

Acknowledgments

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