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Research paper

Cisplatin at clinically relevant concentrations enhances interleukin-2 synthesis by human primary blood lymphocytes

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Cytotoxic drugs influence the expression of certain genes in cancer cells. Cisplatin has recently been shown to modulate interleukin (IL)-1 and tumor necrosis factor (TNF)-α production in macrophages. In this study, we wanted to investigate whether cisplatin interferes with the IL-2, IL-2 receptor (IL-2R), interferon (IFN)-γ, and TNF-α expression in phytohemagglutinin-stimulated human peripheral blood lymphocytes. IL-2 was analyzed in a bioassay, while IFN-γ and TNF-α were measured by ELISA. Northern blots were performed to quantify steady-state cytokine mRNA levels. Furthermore, T cell subsets and IL-2R surface expression were analyzed by means of flow cytometry. A maximum stimulatory effect on IL-2 production (1.8-fold increase) was observed with cisplatin at 5–10 μM while IFN-γ and TNF-α synthesis and IL-2R density were unaffected. However, cisplatin-treated cells displayed enhanced IL-2, IL-2R, IFN-γ and TNF-α mRNA levels compared to drug-free controls. Cisplatin did not prolong cytokine mRNA half-life as revealed with the transcriptional inhibitor actinomycin D. In contrast to an inhibited growth of $CD4^+$ T lymphocytes, $CD3^+CD8^+$ cell density was unaffected at intermediate cisplatin concentrations (10 μM). Bleomycin, carboplatin, doxorubicin, novobiocin or etoposide, which were included for comparison, did not interfere with IL-2 expression. Our data imply that cisplatin most likely stimulated cytokine transcription via a putative stress-induced signaling pathway. [© 1999 Lippincott Williams & Wilkins.]

Key words: Cisplatin, cytokines, DNA damage response, interleukin-2, peripheral blood lymphocytes.

Introduction

Cis-diamminedichloro-platinum(II) (cisplatin) is commonly used for the treatment of human cancers. The basis for the therapeutic effectiveness of cisplatin is not fully understood but its cytotoxic activity against cancer cells has been shown to be mediated through the formation of cisplatin-DNA adducts.[1,2] It covalently modifies DNA, and as a secondary action inhibits topoisomerases resulting in lesions that are thought to lead to induction of apoptosis by inhibiting DNA and RNA synthesis. Additionally, cisplatin negatively influences transcription by interfering with the opening of repressive chromatin structures and by blocking transcription factor binding directly.[3] It has recently been suggested that proteins which bind to cisplatin-damaged DNA play a role in the cytotoxic action of the drug.[4]

During treatment of mammalian cells with cisplatin or other cytotoxic drugs, a DNA damage response (DDR) is generally triggered resulting in activation of an array of different genes.[5,6] Immediate early gene induction is initially observed during a DDR, and consists of the oncogene family with amongst others c-*jun*, c-*fos*, and c-*myc*.[7] Interestingly, cisplatin has been shown to trigger human c-*myc* and a 'cisplatin responsive' region has been defined within the 5′ flanking sequence of the human c-*myc* promoter.[8] Both rat fibroblasts and mouse erythroleukemia cells up-regulate c-*myc* activity in the presence of cisplatin at 5×10^{-5} M.[9] A similar enhancement of the c-*myc* promoter activity is also observed with doxorubicin-treated cells.[10] However, a chloramphenicol acetyl transferase (CAT) construct carrying 5′ flanking sequences of the c-H*ras*1 gene with promoter/enhancer function is not increased by cisplatin when examined in the same cell line.[11]

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Late induced genes such as cytokines are also influenced by DNA damaging agents. For example, murine or human macrophages exposed to cisplatin *in vitro* synthesize more interleukin (IL)-1 and tumor necrosis factor (TNF)-α compared to untreated cells.[12,13] This phenomenon has been studied in more detail using an experimental model consisting of murine peritoneal macrophages. Interestingly, the secretion of various cytolytic factors, like hydrogen peroxide, superoxide anion, IL-1α, TNF-α, lysozyme and, finally, β-*N*-hexoseaminidase, is increased with both cisplatin or carboplatin.[14,15] In cultures with human monocytes incubated in the presence or absence of lipopolysaccharide, cisplatin inhibits IL-1 receptor (IL-1R) antagonist expression, both at the protein and mRNA levels, proving disparate effects on IL-1 regulation.[16]

Since cisplatin has been shown to modulate immediate early gene expression as well as monokine (IL-1 and TNF-α) synthesis,[8,12-15] we thought it important to investigate whether cisplatin interferes with human T cell cytokine production. Other chemotherapeutic drugs (i.e. bleomycin, carboplatin, doxorubicin, novobiocin and etoposide) were included for comparison. An increased IL-2 production was detected in cultures with phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBL) incubated with cisplatin at clinically achievable concentrations (up to 7×10^{-6} M)[17,18] as compared to untreated cells. In contrast, interferon (IFN)-γ and TNF-α production was unaffected or inhibited at high cisplatin concentrations. The cytokine steady-state gene transcripts IL-2, IL-2 receptor (IL-2R), IFN-γ and TNF-α were all enhanced in cisplatin-treated cells. However, the mRNA increase was not secondary to an augmented stability suggesting that cisplatin interacted with the transcriptional apparatus. Finally, analysis of T cell subsets revealed that $CD4^+$ T lymphocytes were more sensitive to cisplatin than $CD8^+$ T lymphocytes.

Materials and methods

Reagents

PHA (Wellcome, Dartford, UK) was dissolved in RPMI 1640 medium (Gibco, Paisley, UK). Actinomycin D, purchased from Boehringer-Mannheim (Mannheim, Germany), was used at a final concentration of 10 μg/ml. Doxorubicin (Adriamycin) and bleomycin were kindly provided by Pharmacia & Upjohn (Stockholm, Sweden) and Lundbeck (Helsingborg, Sweden), respectively. Etoposide (VP-16-213; Vepesid) and carboplatin (Paraplatin) were from Bristol-Myers Squibb (Bromma, Sweden). Cisplatin was obtained from Nycomed (Stockholm, Sweden) and novobiocin was bought from Sigma (St Louis, MO). All drugs were solubilized according to the manufacturers' instructions. Cytotoxic drug concentrations tested in this study are indicated in Table 1.

Cells and culture conditions

Human PBL were isolated from buffy coats with citrate or from heparinized blood from healthy donors by centrifugation on a step gradient of Ficoll-Isopaque (Lymphoprep; Pharmacia, Uppsala, Sweden).[19] PBLs (10^6/ml) were incubated in a humidified 5% CO_2 atmosphere in RPMI 1640 medium containing HEPES buffer supplemented with 10% heat-inactivated fetal calf serum, glutamine and gentamicin (12 μg/ml).

Cytokine assays, flow cytometry and antibodies

For IL-2 analysis, all supernatants (approximate volume 600 μl) were dialyzed against 50 ml culture medium over night. The biological activity of IL-2 in supernatants was analyzed by means of IL-2 dependent stimulation of proliferation of the murine cytolytic T

Table 1. Cytotoxic drug concentrations used in this study.

Compound	Concentration ranges tested (M)	Mechanism of action
Bleomycin	$3.5 \times 10^{-8} - 1.4 \times 10^{-5}$	DNA binding (partial interchalation)
Carboplatin	$1.0 \times 10^{-6} - 5.0 \times 10^{-4}$	DNA binding (inhibition of topoisomerase II)
Cisplatin	$1.0 \times 10^{-7} - 5.0 \times 10^{-4}$	DNA binding (inhibition of topoisomerase II)
Doxorubicin	$1.8 \times 10^{-10} - 1.8 \times 10^{-5}$	DNA binding (inhibition of topoisomerase II)
Etoposide	$1.0 \times 10^{-6} - 1.0 \times 10^{-4}$	Inhibition of topoisomerase II
Novobiocin	$1.0 \times 10^{-7} - 2.5 \times 10^{-4}$	Inhibition of topoisomerase I

lymphocyte line CTLL-2 as described previously.[19] IFN-γ and TNF-α was measured by enzyme-linked immunoassay (ELISA) (Genzyme, Boston, MA).

FITC-conjugated mouse anti-human CD3, CD4, CD8, CD45RA and CD25 mAbs were from Becton Dickinson (San Jose, CA). Mouse IgG1 and mouse IgG2a were included as negative controls (Dakopatts, Glostrup, Denmark). Cells were stained according to standard protocols and analyzed in a FACSort flow cytometry device (Becton Dickinson).

Thymidine incorporation and cell number determination

To quantify DNA synthesis, PBL were pulsed with [methyl-^{3}H]thymidine (1 μCi; TRK 120; specific activity 5 Ci/mmol; Amersham, Little Chalfont, UK) during the last 18 h of incubation. Radioactivity was measured in a scintillation counter. For cell number determination, viable lymphocytes were counted in a Bürker chamber using Trypan blue (Sigma). The sensitivity of PBL was defined as the drug concentration yielding a 50% decrease in cell growth (IC_{50}).

RNA isolation, Northern blots and cDNA

Total RNA was prepared as reported previously.[19] Briefly, RNA (10–20 μg) was loaded onto formaldehyde-agarose gels and blotted to nylon filters (Hybond-N$^+$; Amersham) as described by the manufacturer. Filters were hybridized according to standard protocols and exposed for 24–72 h to preflashed X-ray film (XAR-5; Kodak, Rochester, NY) at -70°C using intensifying screens. Autoradiographs were quantified by scanning laser densitometry. The gene-specific probes used to probe RNA blots were isolated from agarose gels after digestion of the plasmids in which they were propagated with appropriate restriction endonucleases. The IL-2-specific probe containing the entire coding region, IL-2R, TNF-α and β-actin cDNAs have been described earlier.[19] The IFN-γ probe was a 1 kb *Pst*I fragment isolated from p52 and was kindly provided by Genentech (South San Fransisco, CA).[19] To obtain specific cDNA fragments, plasmids were digested with appropriate restriction enzymes and purified from agarose gels (Geneclean; Bio101, La Jolla, CA). Fragments were labeled with [^{32}P]dCTP (specific activity 3000 Ci/mmol) by random priming (Amersham) and free nucleotides were separated on spin-columns (Costar, Cambridge, MA) containing Sephadex G-50 fine (Pharmacia).

Statistics

Student's *t*-test for paired data was used for statistical calculations. Statistical significance was set at $p \leqslant 0.05$.

Results

Effects of cisplatin on lymphocyte cell growth and DNA synthesis

To analyze the cell-inhibitory effects of cisplatin on PBL

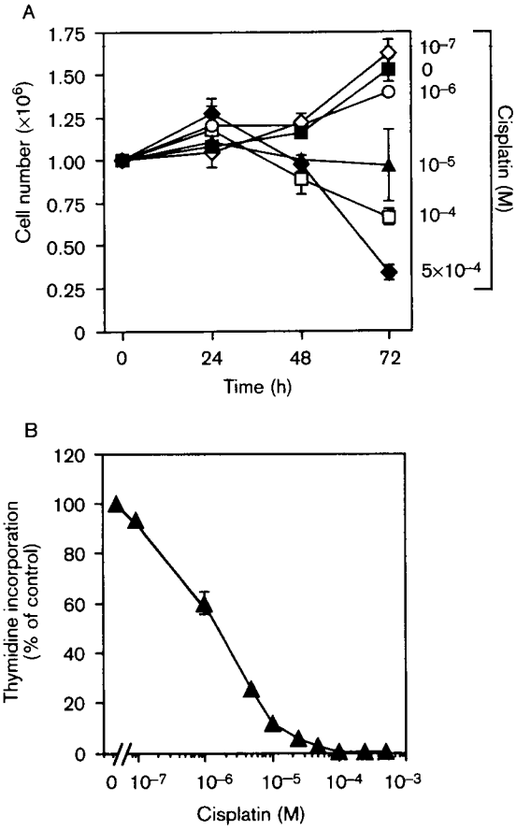

Figure 1. Cisplatin inhibited lymphocyte cell growth and thymidine incorporation. Relative cell growth with or without cisplatin was determined after 24, 48 and 72 h (A). [^{3}H]Thymidine incorporation was analyzed at 72 h (B). Human PBL (10^6/ml) were incubated with PHA (1 μg/ml) and different concentrations of cisplatin (10^{-7} to 5×10^{-4} M). Mitogen and cisplatin were added at culture initiation. At the indicated times cells were harvested, stained with Trypan blue and counted in a Bürker chamber. Blue-stained cells (i.e. dead PBL) were excluded. Cells were pulsed with [^{3}H]thymidine for 18 h. Error bars indicate SEM. The thymidine incorporation (mean $\pm$ SD) was 181 627 $\pm$ 65, 022 c.p.m. in untreated cells. PBL isolated from four different blood donors were analyzed at two separate occasions.

in our experimental systems, cells were incubated with PHA and cisplatin at 10^{-7} to 5×10^{-4} M. No statistically significant inhibition or cell death was observed when Trypan blue-negative cells were counted at 24 and 48 h of incubation (Figure 1A). However, cells started dying at day 3 and IC_{50} was calculated to approximately 5×10^{-5} M. In addition, PBL cultures were pulsed with [^{3}H]thymidine for 18 h and specific thymidine incorporation was analyzed at 72 h (Figure 1B). A significant cisplatin-dependent inhibition up to 40% was observed at 10^{-6} M cisplatin, while the thymidine uptake was abolished at 5×10^{-5} M.

Increased IL-2 production in cells treated with cisplatin

Cisplatin has been shown to potentiate both IL-1 and TNF-α production in cultures with human monocytes.[15] To reveal whether cisplatin influenced IL-2 synthesis, we treated mitogen-activated PBL with cisplatin (10^{-7} to 5×10^{-4} M). Cell supernatants harvested from 24, 48 and 72 h cultures were analyzed for IL-2 content. Peak IL-2 values in untreated controls occurred at 24 h. At 10^{-7} M cisplatin, a statistically significant IL-2 increase was seen and up to 1.8-fold more IL-2 was detected at 10^{-5} M cisplatin as compared to drug-free controls (Figure 2A). In contrast to IL-2, IFN-γ secretion was not significantly increased but was inhibited by cisplatin at 10^{-5} M or higher concentrations (Figure 2B). TNF-α production was slightly increased, albeit not significantly, at cisplatin concentrations above 10^{-5} M (Figure 2C). Thus, amongst the cytokines analyzed, cisplatin enhanced only the IL-2 production in activated PBL.

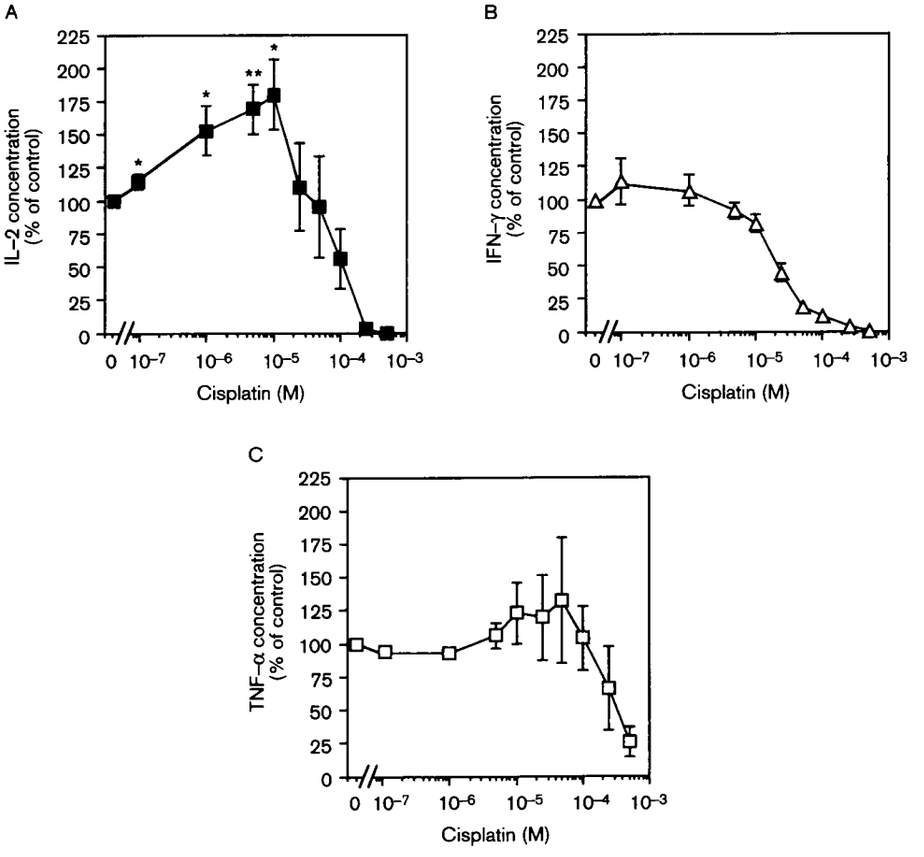

Figure 2. Increased IL-2 production by stimulated human PBL incubated with cisplatin. IL-2 (A), IFN-γ (B) and TNF-α (C) were analyzed in PBL cultures treated with cisplatin (10^{-7} to 5×10^{-4} M) for 24 h. Culture conditions were as described in Figure 1. Dialyzed supernatants were analyzed for IL-2 bioactivity using an IL-2-dependent stimulation of proliferation of the murine cytolytic T cell line CTLL-2. IFN-γ and TNF-α were analyzed by ELISA. Four different human blood donors were included in two separate experiments. *$p<0.05$ and **$p<0.01$.

Enhanced cytokine gene transcript levels by cisplatin

The up-regulated IL-2 production raised the question whether an mRNA increase paralleled the protein synthesis. To examine steady-state mRNA levels in PBL treated with cisplatin for 24 h, total RNA was isolated and subjected to Northern blots that were sequentially hybridized with specific ^{32}P-labeled cDNA probes (Figure 3). When cisplatin-treated cells were compared to drug-free controls, a 2- to 2.5-fold increase of IL-2, IL-2R and IFN-γ steady-state mRNA levels was detected at 10^{-6} M cisplatin (Figure 3B). Furthermore, up to 3.5-fold more IL-2R and IFN-γ mRNAs were observed at 5×10^{-5} M cisplatin compared to controls without any drug. TNF-α mRNA levels were slightly augmented; a 1.5-fold increase was detected at the highest cisplatin concentration tested.

As bleomycin and doxorubicin have been reported to potentiate cytokine synthesis by stimulated lymphocytes *in vitro*,[20] these DNA binding drugs were included. In addition, etoposide, carboplatin and, finally, novobiocin were tested (Table 1). In contrast to cisplatin, doxorubicin or the other cytotoxic drugs did not modulate cytokine mRNA levels. Thus, in our expermental system with human PBL, cisplatin appeared to be unique in that this drug enhanced IL-2 mRNA that was further translated into functionally bioactive protein.

Mitomycin C and other DNA damaging drugs have been shown to increase the stability of IL-1 and IL-6 transcripts in human monocytes.[21] To exclude that an mRNA stabilization occurred with cisplatin, PBL were incubated with 4 μg/ml PHA for 6 h at what time the transcriptional inhibitor actinomycin D (10 μg/ml) was added with or without cisplatin at 10^{-5} M. IL-2 mRNA levels were examined 2 and 4 h later. This experimental protocol has been shown to be applicable for studying IL-2 mRNA turnover in human PBL.[19] Interestingly, the IL-2 mRNA half-life in PBL exposed to cisplatin was not altered compared to untreated control cells (data not shown). Taken together, cisplatin most likely triggered IL-2 transcription without influencing mRNA stability.

T helper cells were more sensitive to cisplatin than suppressor/cytotoxic T cells

It is well documented that certain T cell subsets inhibit T helper function.[22] An attractive explanation for the cisplatin-dependent increased IL-2 synthesis would be that a distinct T cell suppressor population was more sensitive for cisplatin than the main IL-2 producing lymphocytes ($CD3^+CD4^+$). To reveal whether $CD4^+$ and $CD8^+$ T cells were differently influenced by cisplatin, lymphocytes were incubated with mitogen

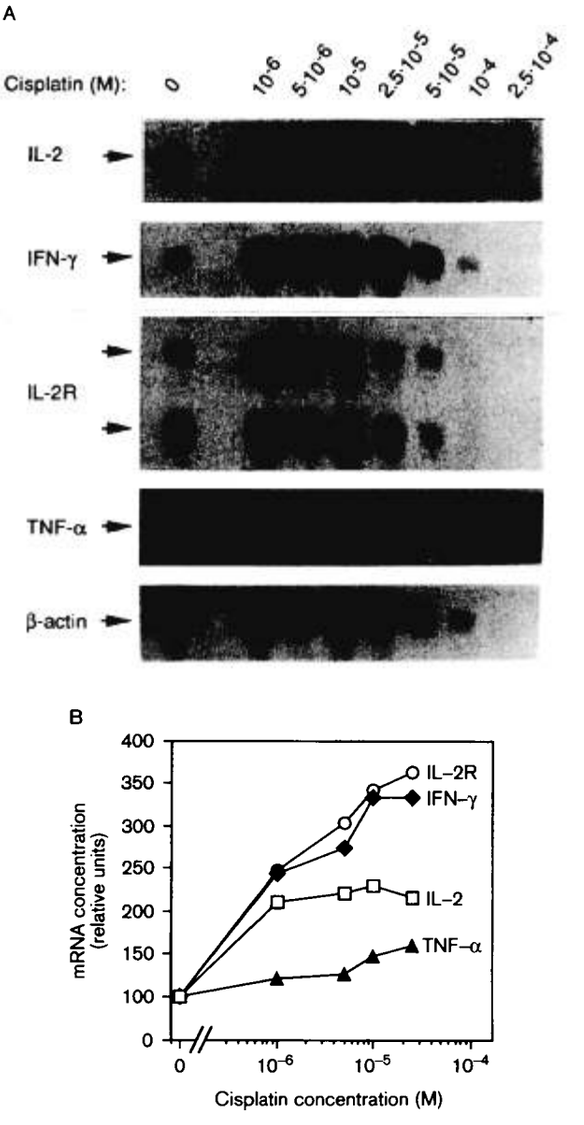

Figure 3. Enhanced cytokine mRNA levels in cisplatin-treated lymphocytes. Northern blots (A) and scanning data (B) are shown. IL-2, IFN-γ, IL-2R and TNF-α relative mRNA levels are indicated. Cisplatin (10^{-6} to 2.5×10^{-4} M) was added to PBL together with PHA (1 μg/ml) at culture initiation and harvested 24 h later. Total RNA was prepared, run on denaturing formaldehyde gels, blotted to nylon filters, hybridized with radioactive cDNA probes and finally exposed to X-ray films. Densitometry data for specific cytokine mRNAs were divided with corresponding values for β-actin. Cytokine mRNA concentrations were adjusted to values obtained from PHA-activated control cells without any cisplatin. The pattern of one typical blood donor out of four is shown.

and increasing concentrations of cisplatin for 3 days, labeled with specific mAbs and analyzed by flow cytometry (Figure 4A). Interestingly, $CD3^+CD4^+$ cells were selectively inhibited at 10^{-5} M cisplatin compared to $CD3^+CD8^+$ lymphocytes that were unaffected at this relatively low concentration. With cisplatin at 2×10^{-5} M and higher concentrations, both T cell subsets were inhibited to the same degree resulting in a normalized CD4/CD8 ratio (Figure 4B). Other lymphocyte surface receptors, i.e. $CD3^+CD25$ (IL-2Rα) and $CD3^+CD45RA$, were also analyzed. The density of these receptors was inhibited in a dose-dependent fashion by cisplatin (not shown). Thus, despite more IL-2R mRNA was detected in cisplatin-treated PBL (Figure 3B) it was not translated to mature protein and exposed at the cell surface.

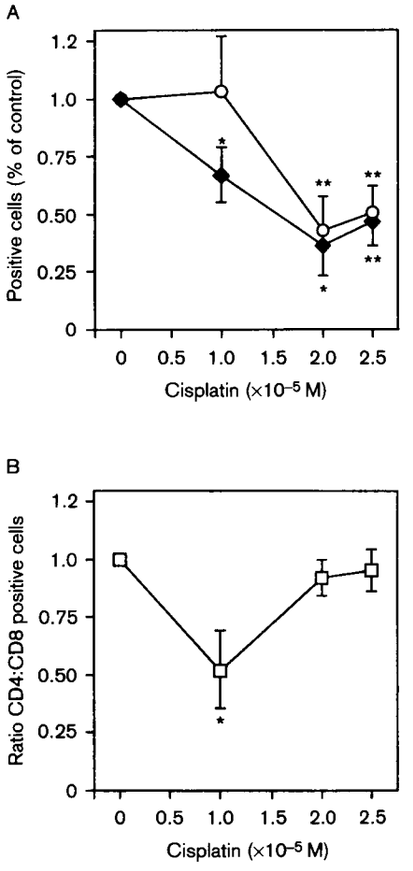

Figure 4. $CD4^+$ T cells were more sensitive to low cisplatin concentrations. $CD3^+CD4^+$ (◆) and $CD3^+CD8^+$ (○) lymphocyte subsets were analyzed by flow cytometry (A). In (B), the CD4/CD8 ratio (□) is indicated. PBL were incubated with mitogen (PHA 1 μg/ml) in addition to cisplatin at the indicated concentrations. After 72 h, cells were stained with specific mAbs and analyzed by flow cytometry. Results obtained from four various blood donors are indicated in the panels. $^*p<0.05$ and $^{**}p<0.01$.

Discussion

In the present investigation we show that cisplatin, amongst other chemotherapeutic drugs tested, increased IL-2 production both at the protein and mRNA levels (Figures 2 and 3). Peak IL-2 levels were observed at cisplatin concentrations between 10^{-6} and 10^{-5} M. Interestingly, this is within a concentration range that is readibly achievable in patients on cisplatin therapy.[17,18] In addition to an IL-2 mRNA increase, cisplatin enhanced IFN-γ, TNF-α and IL-2R steady-state mRNA levels without increasing protein concentrations detected in culture supernatants or at the cell surface (IL-2R). Most likely the effect of cisplatin was on the transcriptional apparatus as experiments with actinomycin D did not reveal any mRNA stabilization. A large body of published data supports this hypothesis. Early on it was reported that cisplatin (5×10^{-6} M) induces a 2.2-fold enhanced CAT expression when this reporter gene is linked to the long terminal repeat (LTR) of the human immunodeficiency virus (HIV)-1 promoter and examined in fibroblasts.[23] In parallel, cisplatin increases CAT activity in HeLa cells transfected with constructs containing the HIV and the adenovirus promoters.[6] Two other promoter/enhancer regions were investigated in the same study; the adenovirus 2 major late promoter (MLP) and the simian virus 40 (SV40) early region. Interestingly, these two promoters are strongly inhibited by cisplatin.

Cisplatin and doxorubicin bind to DNA, and as a secondary action inhibit topoisomerases followed by an increase of certain gene transcripts in eukaryotic cells.[8,9] The selective topoisomerase II inhibitor etoposide has been reported to induce one transcript, i.e. vimentin mRNA and protein, in the monocytic U937 cell line.[24] In contrast to the IL-2 promoting properties of cisplatin, we did not observe any IL-2 increase in PBL treated with either doxorubicin or etoposide. Doxorubicin has, however, been demonstrated to potentiate IL-2 production by activated rat spleen lymphocytes *in vitro*.[20,25] Stimulation of splenocytes obtained from rats administered doxorubicin *in vivo* also generates higher IL-2 concentrations compared to control cultures from untreated animals.[26]

The fluoroquinolones ciprofloxacin and CP-115,953 also inhibit mammalian topoisomerase II activity, albeit at different IC_{50} values; CP-115,953 stimulates DNA cleavage mediated by topoisomerase II with a potency approximately 600 times greater than that of ciprofloxacin. We have recently shown that these two fluoroquinolones enhance an array of cytokine gene transcripts in activated PBL.[19,27] In contrast to cipro-

floxacin, CP-115,953 does not induce any measurable IL-2 transcription in transiently transfected Jurkat T lymphoma cells. However, CP-115,953 is less potent than ciprofloxacin in inducing maximal cytokine production in PBL but the inhibitory effect on cell growth dominates. In parallel, when transfected Jurkat cells are incubated with cisplatin at 5×10^{-6} to 2.5×10^{-5} M any enhanced IL-2 transcription is not detected compared to untreated controls (Riesbeck, unpublished data). The effects of CP-115,953 on PBL are comparable with cisplatin in two ways: (i) both drugs strongly inhibit cell growth, but (ii) still induce cytokine mRNA in activated PBL. Taken together, these observations suggest that the cytokine-inducing capacity in human PBL by cisplatin may be associated with a reduced topoisomerase II activity that further results in a genotoxic stress response.

The precise mechanism behind the cisplatin-induced stress response in mammalian cells is at present unclear. Recently Shishodia *et al.* demonstrated that cisplatin induces a pathway governed by protein kinase C as revealed in experiments using specific inhibitors.[28] Similarly, bone marrow-derived macrophages incubated with cisplatin show increased protein tyrosine phosphorylation and signal transduction through Ras and MAP kinase pathways.[21,30] The importance of NF-κB activation by neoplastic agents and the role of protein kinase C has recently been thoroughly examined in lung adenocarcinoma cells (A549). Doxorubicin, amongst other DDR inducing agents, causes NF-κB activation whereas other transcription factors such as AP-1, AP-2, CREB, SP-1 or TFIID are not activated.[31] Interestingly, doxorubicin-dependent protein kinase C activation results in removal of IκB from the NF-κB complex. Most stress-inducing compounds cause, however, an increase of reactive oxygen intermediates (ROI) resulting in *both* AP-1 (consisting of proteins from the c-Jun and c-Fos families) and NF-κB activation.[32]

Why did cisplatin up-regulate the IL-2 production in stimulated PBL while other chemotherapeutic drugs including carboplatin did not? Most likely a balance exists between cytotoxicity/inhibitory concentrations and the 'cytokine inducing window', which upon a DDR allows certain gene transcripts to be both transcribed and translated into functional proteins. It is intersting to note that the parent agents cisplatin and carboplatin differ regarding gene transcript enhancing activity. Cisplatin interferes with induction of the c-*myc* promoter and the LTR sequences of HIV-1, whereas carboplatin does not excert any effects.[11,33] Intriguingly, analysis of tissue culture supernatants collected from cisplatin- and carboplatin-treated macrophages shows enhanced IL-1α and TNF-α activity.[15]

Both tumour-specific and non-specific infiltrating lymphocytes (TIL) are detected in most solid tumors.[34] Different patterns of lymphocyte subsets are displayed depending on the particular tumor type.[35] The regimen of chemotherapy possibly influences the clinical outcome depending whether or not the cytotoxic drug interferes with the components of the immune system. Our findings on cisplatin-dependent increased IL-2 production in cultures with PBL suggest that cisplatin may be beneficial in generating LAK activity and potentiate anti-cancer therapy by interfering with the IL-2 producing cells in solid tumours. It has been demonstrated that cisplatin interacts directly with cytotoxic LAK cells although available data are contradictory. When human PBL are induced with IL-2 in the presence of cisplatin, an increased LAK activity is observed compared to cells induced in the absence of cisplatin.[36] In contrast, another laboratory reported that cisplatin decreases the generation of LAK cells when PBL are pre-incubated with the drug.[37] However, it was demonstrated in another study that the ability of PBL to generate functional LAK cells *ex vivo* is significantly augmented up to 7 days after a single dose of cisplatin injection when compared to that before drug administration.[38]

Conclusion

The collective data about effects of cytotoxic drugs on eukaryotic cells are divergent, further reflecting the complex DDR phenomenon. In this study, cisplatin was shown to increase IL-2 production in PHA-activated PBL. It is tempting to speculate that the interactions between certain chemotherapeutic agents and the immune system may have importance for the clinical outcome. For example, when rats bearing a highly antigenic fibrosarcoma are treated with the cytokine-promoting agents bleomycin or peplomycin, significantly longer survival is observed compared to liblomycin that reduces cytokine production.[39] However, it still remains to be proven that cisplatin is also immunostimulatory *in vivo*, i.e. that TIL can be triggered secondary to an increased IL-2 synthesis by T helper cells.

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