*Anti-Cancer Drugs*, **2**, pp. 597–601

# The effect of cisplatin and carboplatin on c-*myc* promoter in erythroleukemic cells

**Aristides Eliopoulos, David J Kerr and Demetrios A Spandidos[CA]**

A Eliopoulos is at the Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas Constantinou Avenue, Athens 11635, Greece. DJ Kerr is at the CRC Department of Medical Oncology, University of Glasgow, Alexander Stone Building, Garscube Estate, Glasgow G61 1BD, UK. DA Spandidos is at the Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas Constantinou Avenue, Athens 11635 (where correspondence should be addressed) and the Medical School, University of Crete, Heraklion, Greece.

**The recombinant plasmids p324, p330 and p323 carrying 5′ flanking c-*myc* sequences linked to the reporter gene chloramphenicol acetyl-transferase (*cat*) were introduced into the mouse erythroleukemia cell line F412B2TK$^-$ and stable transfectants resistant to geneticin were obtained. The effects of two platinum coordination compounds, cisplatin and carboplatin, were studied using a wide range of drug concentrations. It was found that cisplatin stimulates *cat* gene expression with maximum effect at $5 \times 10^{-5}$ M concentration, while carboplatin at concentrations from $1 \times 10^{-6}$ to $1 \times 10^{-4}$ M did not have any effect. These results demonstrate that cisplatin stimulates transcription from the c-*myc* promoter in erythroleukemia cells and may support the use of carboplatin as compared to cisplatin in the treatment of cancer patients.**

*Key words:* Carboplatin, cisplatin, c-*myc*.

## Introduction

Cisplatin [*cis*-diammine-dichloro-platinum (II)] and carboplatin [*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum (II)] are two platinum coordination compounds used in the chemotherapy of several types of human tumours including squamous carcinoma of head and neck, testicular, ovarian and lung cancer.[1–3]

Despite similar degrees of anti-tumor activity of these two compounds, clinical studies suggest that carboplatin is myelotoxic and lacks much of the renal toxicity, ototoxicity and nephrotoxicity of the parent compound, cisplatin.[3]

The molecular pharmacology of these agents is complex; however, their cytotoxicity has been correlated with the formation of mono- and bihydroxylated platinum intermediates which then bind to the $N^7$ residue of guanine, causing intra- and interstrand DNA cross-links.[4,5] Cisplatin and carboplatin follow a similar aquation reaction, but this reaction is much slower with carboplatin.[6]

The different patterns of toxicity of the two compounds could also be explained by their different pharmacokinetics within the body.[6,7] *In vivo* experiments have shown a prolonged availability of free (ultrafilterable) carboplatin in the blood plasma compared with that of free cisplatin, due to differences in their binding to plasma proteins.[8,9]

The c-*myc* proto-oncogene encodes two nuclear phosphoproteins p62 and p64 involved in the control of cellular proliferation and differentiation.[10] Altered gene expression of c-*myc* has often been implicated in initiation and maintenance of malignancy in chickens, rodents, rats and humans.[11–13]

Mechanisms responsible for activation of the oncogenic potential of c-*myc* include proviral insertion, chromosomal translocation and gene amplification, and lead to quantitative rather than qualitative changes in *myc* protein. The regulation of c-*myc* gene expression is complex and is controlled not only at the level of transcription, by both positive and negative *cis*-acting regulatory elements, but also at a post-transcriptional level including stabilization of c-*myc* RNA (for a review see Cole[10]).

[CA] Corresponding Author

In the present study we show that in mouse erythroleukemia cells cisplatin, but not carboplatin, stimulates expression from the human c-*myc* promoter.

## Materials and methods

### Recombinant plasmids and cell lines

The mouse erythroleukemia cell line F412B2TK$^{-}$[14] can be transfected with high efficiency using the calcium phosphate technique as previously described.[15,16]

Plasmids p324, p330 and p323 (Figure 1) carrying 5′ c-*myc* flanking sequences linked to the reporter *cat* gene were introduced into the F412B2TK$^{-}$ cell line to obtain stable, geneticin-resistant transfectants, F324-1, F330-3 and F323-1, respectively.

### Cat assays

Cells were grown exponentially in Ham's medium containing 10% fetal calf serum (Fcs) and assayed for *cat* activity as previously described.[17]

### Assay for cell proliferation

Cell proliferation was estimated using the rapid colorimetric assay of Mossman.[18] This assay has been described in detail previously.[19] In brief, 10 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma) in phosphate buffered saline was added to 100 μl medium containing F324-1 cells exposed to cisplatin and carboplatin for various lengths of time (0, 24, 48, 72, 96 and 120 h). The plates were incubated at 37°C for 4 h and then 110 μl of 0.04 N HCl in isopropanol was added to each well. Optical density (OD) was recorded at 540 nm using a Titertek Flow MicroELIZA reader.

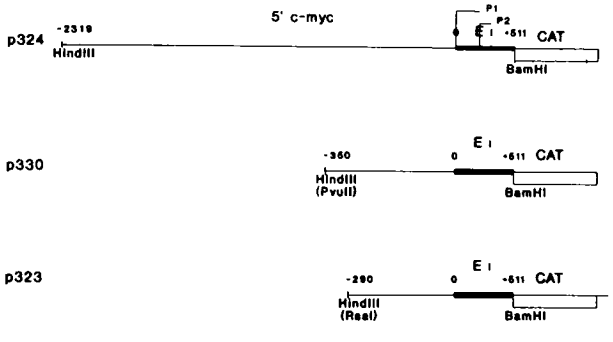


**Figure 1.** A 2830 bp *Hind*III–*Bam*HI fragment from human c-*myc* containing 511 bp of 5′ flanking sequence was linked to the coding sequence of the *cat* gene. 5′ terminal deletions of the *myc* promoter were constructed by *Pvu*II or *Rsa*I cleavage followed by *Hind*III linker addition and similarly linked to the *cat* gene. The hybrid *myc/cat* constructs were then cloned into the multi-cloning site of vector p304 containing the *aph* gene.

## Results

The effect of cisplatin and carboplatin on F324-1, F330-3 and F323-1 cells, harboring the recombinant plasmids p324, p330 and p323, respectively, was examined. As shown in the autoradiograph in Figure 2(a) and the histogram in Figure 2(b), the amount of *cat* produced in cell line F324-1 varies with the amount of cisplatin administered to the cells whereas it remains constant over a wide range ($1 \times 10^{-6}$ to $1 \times 10^{-4}$ M) of carboplatin concentrations. At the optimum concentration of $5 \times 10^{-5}$ M, cisplatin causes a 7.4-fold increase in *cat* activity. At higher doses ($1 \times 10^{-4}$ M cisplatin) *cat* activity is shown to decrease, perhaps due to the toxic effects of the drug. However, a greater concentration ($1 \times 10^{-3}$ M) of carboplatin is required to increase the amount of *cat* by 3.7-fold (lane 11, Figure 2a).

F330-3 and F323-1 cell lines also responded to cisplatin at the optimum concentration of $5 \times 10^{-5}$ M showing a 3.9- and 2.1-fold increase in *cat* gene expression, respectively (Figure 3a and b).

As shown in the autoradiograph in Figure 4(a) and the histogram in Figure 4(b), the optimal time period of exposure to cisplatin is 24 h, the *cat* activity from F324-1 increased 7.4-fold. An exposure longer than 24 h resulted in a dramatic decrease in *cat* activity due to cell death (data not shown).

The cytotoxic effect of cisplatin and carboplatin was measured by a rapid cell proliferation assay, for various time intervals (0, 24, 48, 72, 96 and 120 h), and a range of drug concentrations (from $1 \times 10^{-6}$ to $1 \times 10^{-4}$ M). As depicted in Figure 5(a and b), carboplatin inhibits cell proliferation but is less effective than cisplatin.

## Discussion

The c-*myc* oncogene is implicated in the genesis of a number of human neoplasia (for a review see Field and Spandidos[20]). Recently, the importance of c-*myc* expression in the modulation of cellular response to

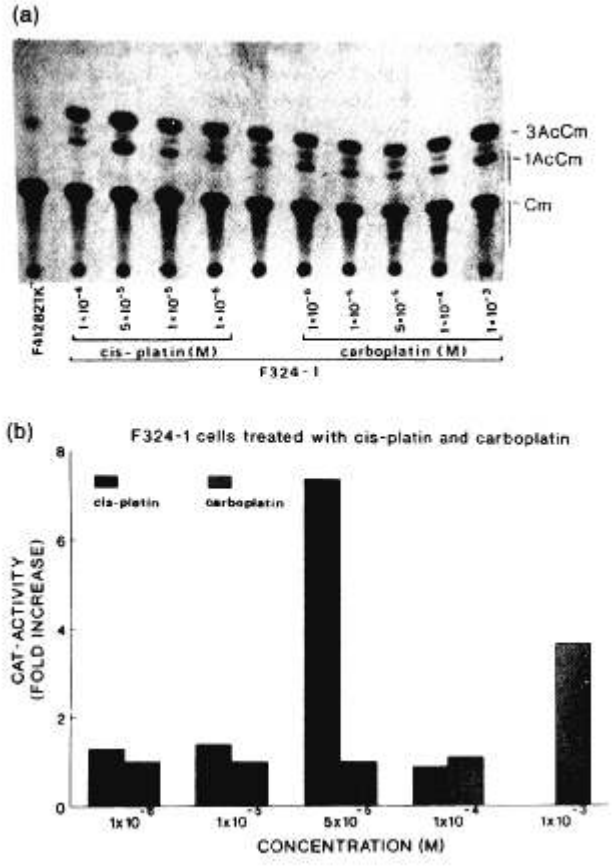


**Figure 2.** Induction of *cat* activity by cisplatin and carboplatin. (a) Chromatogram of a representative *cat* assay with extracts from parental F412B2TK$^-$ and transfectant F324-1 cells with and without treatment by cisplatin (lanes 2–5) and carboplatin (lanes 7–11). (b) Histogram of recorded *cat* activities. F324-1 cells were plated at $1.5 \times 10^6$ cells/75 cm$^2$ flask in Ham's SF12 containing 10% FCS at 37°C. At 24 h later the medium was replaced with Ham's SF12 containing 0.5% FCS and left for another 24 h at 37°C. The medium was then changed to Ham's SF12 containing 5% FCS and varying concentrations of drug (cisplatin or carboplatin). Cells were harvested 24 h later and tested for *cat* activity as described in Materials and methods. *Cat* activity was 1.0 nmol acetylated chloramphenicol/$\mu$g protein per hour of incubation. An average from three experiments is given. Standard deviation was less than 3% of the average.

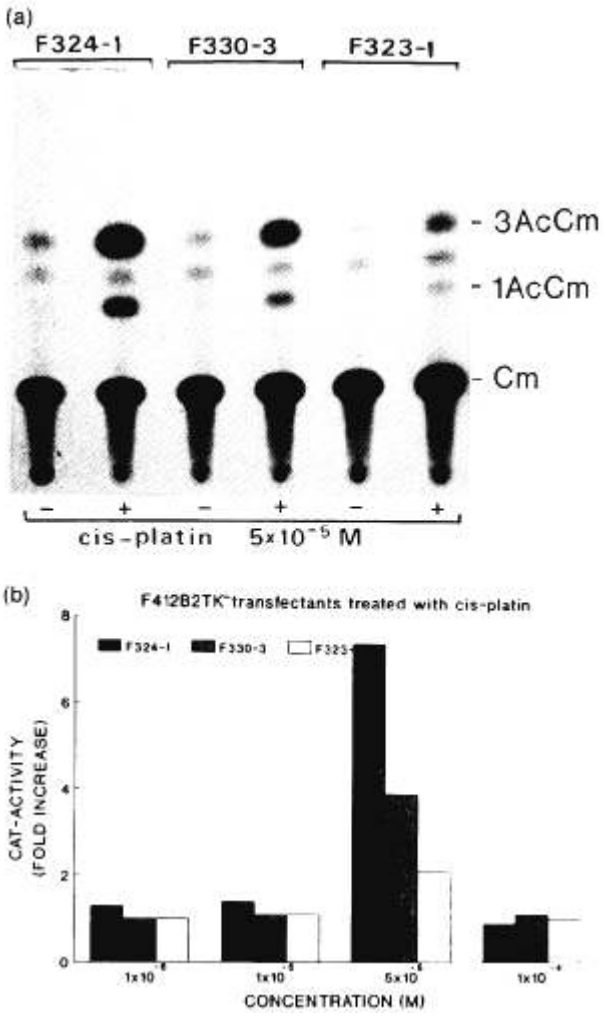


**Figure 3.** Induction of *cat* activity by cisplatin. (a) Chromatogram of representative *cat* assays with extracts from F324-1, F330-3 and F323-1 cells with and without treatment by $5 \times 10^{-5}$ M cisplatin. (b) *Cat* values were computed and are presented in histogram form as described in Figure 2.

therapeutic agents such as cisplatin has been reported.[21]

In the present study we have investigated the effect of cisplatin and carboplatin on the transcriptional activation of the c-*myc* promoter employing three stable transfectant mouse erythroleukemia cell lines carrying different deletions of the c-*myc* promoter. We have found that cisplatin at the optimum concentration of $5 \times 10^{-5}$ M induces a significant increase in *cat* activity (7.4-, 3.9- and 2.1-fold in F324-1, F330-3 and F323-1, respectively) while the carboplatin concentration needed to exert a smaller effect (3.7-fold increase) on F324-1 is $1 \times 10^{-3}$ M. At concentrations from $1 \times 10^{-6}$ to $1 \times 10^{-4}$ M the c-*myc* promoter does not respond to carboplatin.

The differences in cisplatin and carboplatin ability to interact with c-*myc* promoter could be partly explained by their differences in the kinetics of aquation which has been shown to affect the rate of binding to DNA.[6] Aquation of cisplatin is much faster than that of carboplatin and about a 100-fold and 20- to 40-fold dose of carboplatin is required

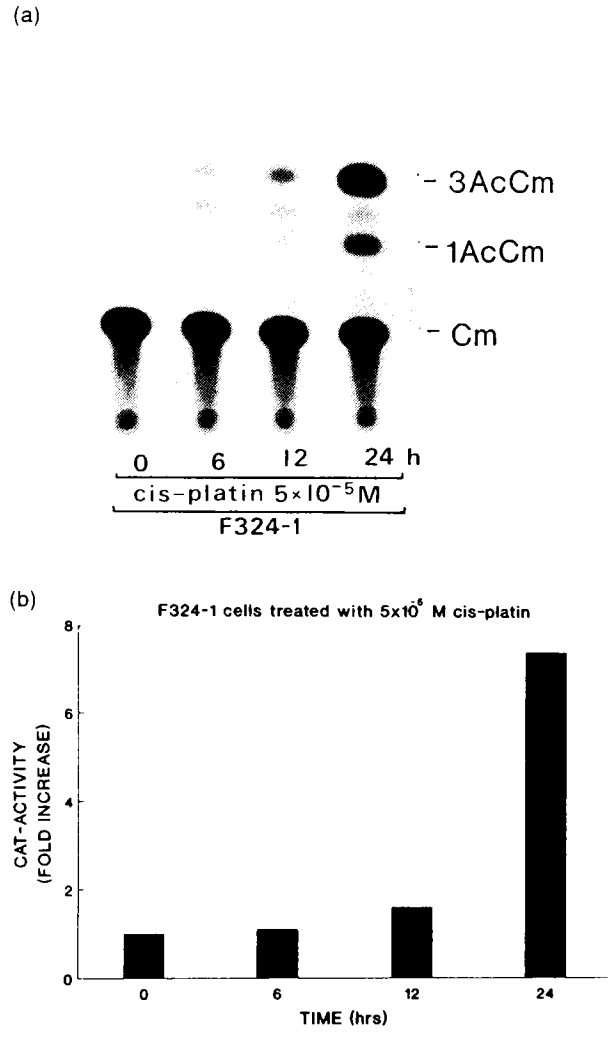


**Figure 4.** (a) Induction of *cat* activity by cisplatin at various times post-treatment. (b) *Cat* values were computed and are presented in histogram form as described above.

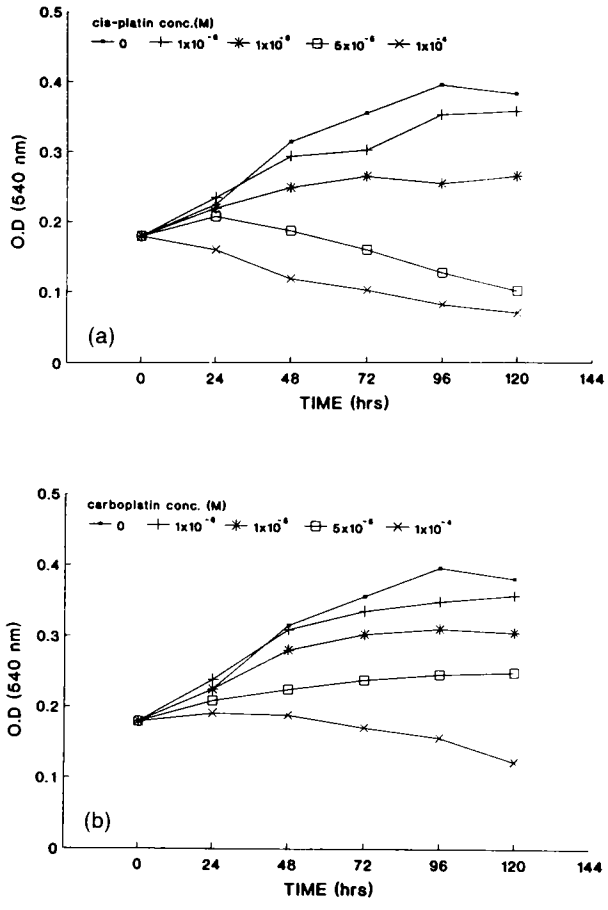


**Figure 5.** Cell proliferation in response to cisplatin (a) and carboplatin (b) at various times of exposure. Exponentially growing 4 x $10^3$ F324-1 cells were plated in 96-well tissue culture clusters (Costar) in Ham's SF12–10% FCS medium in the presence of the indicated drug concentration. At the times shown, cell proliferation was measured by Mossman's rapid colorimetric assay.

*in vitro* and *in vivo*, respectively, to produce equal binding.[6,9] This could also account for the fact that a greater ($1 \times 10^{-3}$ M) carboplatin concentration is required to activate the c-*myc* promoter.

It is possible that activation of c-*myc* is beneficial as it could increase cell cycling time and therefore reduce the amount of time which the cell has to repair the Pt-DNA lesion before mitosis.

Moreover, whereas in fibroblasts a cisplatin responsive element has been localized in the region between −350 and −290 of the c-*myc* promoter,[23] it is of interest that in mouse erythroleukemic cells the +1 to −290 promoter region (cell line F323-1) still responds to cisplatin. This suggests a different mechanism, possibly involving different proteins in the mouse erythroleukemia cells as compared with fibroblasts. We are now studying the proteins which mediate the cisplatin interaction with the c-*myc* promoter in the two cell systems.

## Acknowledgments

We would like to thank Vassilis Zoumpourlis for his help with the experiment described in Figure 4 and his interest in our work.

### References

1. Loehrer PJ, Einhorm LE. Cis-platin. *Ann Intern Med* 1984; **100**: 704–13.

2. Calvert AH, Harland SJ, Newell DR, *et al.* Early clinical studies with cis-diammine-1,1 cyclobutane dicarboxylate platinum II. *Cancer Chemother. Pharmacol* 1982; **9**: 140–47.
3. Bunn RA. Review of therapeutic trials of carboplatin in lung cancer. *Semin Oncol.* 1989; **16**: 27–33.
4. Pascoe JM, Roberts JJ. Interactions between mammalian cell DNA and inorganic platinum compounds, DNA interstrand cross-linking and cytotoxic properties of platinum II compounds. *Biochem Pharmacol* 1974; **23**: 1345–57.
5. Macquet JP, Butour JL. Modifications of the DNA secondary structure upon platinum binding: a proposed model. *Biochemie* 1978; **60**: 901–14.
6. Knox RJ, Friedlos F, Lydall DA, *et al.* Mechanism of cytotoxicity of anticancer platinum drugs: evidence that *cis*-diammine dichloroplatinum(II) and *cis*-diammine-(1,1-cyclobutanedicarboxylato) platinum (II) differ only in the kinetics of their interaction with DNA. *Cancer Res* 1986; **46**: 1972–9.
7. Curt GA, Erygiel JJ, Corden BJ, *et al.* A phase I and pharmacokinetic study of diammine cyclobutane dicarboxylato platinum. *Cancer Res* 1983; **43**: 4470–3.
8. Siddik ZH, Newell DR, Boxall FE, *et al.* The comparative pharmacokinetics of carboplatin and *cis*-platin in mice and rats. *Biochem Pharmacol* 1987; **36**: 1925–32.
9. Terheggen PM, Begg AC, Emondt JY, *et al.* Formation of interaction products of carboplatin with DNA *in vitro* and in cancer patients. *Br J Cancer* 1991; **63**: 195–200.
10. Cole MD. The *myc* oncogene: its role in transformation and differentiation. *Annu Rev Genet* 1986; **20**: 361–84.
11. Adams J, Gerondakis S, Webb E, *et al.* Cellular *myc* oncogene is altered by chromosome translocation to the immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. *Proc Natl Acad Sci USA* 1983; **80**: 1982–6.
12. Yokota J, Tsunetsugu-Yokota Y, Battifora H, *et al.* Alterations of *myc, myb* and *ras* proto-oncogenes in cancers are frequent and show clinical correlation. *Science* 1986; **231**: 261–4.
13. Field JK, Spandidos DA, Stell PM, *et al.* Elevated expression of the c-*myc* oncoprotein correlates with poor prognosis in head and neck squamous cell carcinoma. *Oncogene* 1989; **4**: 1463–8.
14. Spandidos DA, Paul J. Transfer of human globin genes to erythroleukemic mouse cells. *EMBO J* 1982; **1**: 15–20.
15. Graham FL, Van der Eb AJ. A technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 1973; **52**: 456–67.
16. Spandidos DA, Wilkie NM. Expression of exogenous DNA in mammalian cells. In: Hames BD, Higgins SJ, eds. *In vitro Transcription and Translation: A Practical Approach.* Oxford, IRL Press 1984: 1–48.
17. Gorman CM, Moffat LF, Howard BH. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* 1982; **2**: 1044–51.
18. Mossman T. Rapid colorimetric assay for cellular growth and survival: application of proliferation and cytotoxicity assay. *J Immunol Methods* 1983; **65**: 55–63.
19. Spandidos DA, Zoumpourlis V, Kotsinas A, *et al.* Transcriptional activation of the human immunodeficiency virus long terminal repeat sequences by *cis*-platin. *Genet Anal Techn Appl* 1990; **7**: 138–41.
20. Field JK, Spandidos DA. The role of *ras* and *myc* oncogenes in human solid tumours and their relevance in diagnosis and prognosis. *Anticancer Res* 1990; **10**: 1–22.
21. Sklar MD, Prochownik EV. Modulation of cisplatinum resistance in Friend erythroleukemia cells by c-*myc*. *Cancer Res* 1991; **51**: 2118–23.
22. Spandidos DA, Zoumpourlis V, Lang JC. *Cis*-platin responsive sequence in the human c-*myc* promoter. *Anticancer Res* 1991; **11**: 1353–6.

*(Received 9 September 1991; accepted 1 October 1991)*