Biochemical Pharmacology, Vol. 46, No. 4, pp. 615–620, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

ARE LETHAL EFFECTS OF NITRACRINE ON L5178Y CELL SUBLINES ASSOCIATED WITH DNA–PROTEIN CROSSLINKS?

MAŁGORZATA WALICKA,* LESZEK SZMIGIERO,† EWA CIESIELSKA† and IWONA GRĄDZKA

Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, ul. Dorodna 16, 03-195 Warsaw, and †Department of General Chemistry, Institute of Physiology and Biochemistry, Medical University of Lodz, ul. Lindleya 6, 90-131 Lodz, Poland

(*Received* 4 *January* 1993; *accepted* 13 *May* 1993)

Abstract—Nitracrine (Ledakrin, 1-nitro-9-(3,3-*N*,*N*-dimethylaminopropylamino)-acridine) is of interest as a DNA intercalator and alkylator with very high cytotoxic potency, especially against hypoxic cells. DNA–DNA crosslinks [Konopa *et al.*, *Chem Biol Interact* **43**: 175–197, 1983; Pawlak *et al.*, *Cancer Res* **44**: 4289–4296, 1984] or DNA–protein crosslinks (DPCs) [Woynarowski *et al.*, *Biochem Pharmacol* **38**: 4095–4101, 1989; Szmigiero and Studzian, *Biochim Biophys Acta* **1008**: 339–345, 1989] are related to the toxicity of the drug. The cytotoxic effect of and DNA damage induced by nitracrine were measured in two sublines of mouse lymphoma L5178Y, LY-R (resistant to ionizing radiation) and LY-S (sensitive to ionizing radiation). LY-R cells were more sensitive to nitracrine (D_{10} = 0.11 μM) than LY-S (D_{10} = 0.35 μM) when treated for 1 hr at 37°. To a DNA–DNA crosslinking agent, mitomycin C, the comparative sensitivity was opposite. LY-R cells were more resistant to this drug than LY-S cells (D_{10} = 7.1 vs 2.3 μM). DNA damage induced by nitracrine was measured by the alkaline elution method and by nitrocellulose filter binding assay. Nitracrine treatment with biologically relevant concentrations (0.1–3.0 μM, 1 hr, 37°) induced only DPCs. Interstrand crosslinks and DNA breaks were not detected. Nitracrine produced about two times more DPCs in LY-R cells than in LY-S cells. Both sublines removed 50% of initial lesions during 2 hr post-treatment incubation. The greater sensitivity of LY-R cells to nitracrine is thus not related to the efficiency of DNA repair, but may be a consequence of enhanced initial damage in the form of DPCs. This finding is consistent with the latter lesion being responsible for the cytotoxicity of nitracrine.

Nitracrine (Ledakrin, 1-nitro-9-(3,3-*N*,*N*-dimethylaminopropylamino)-acridine) has been used clinically as an antitumour drug in Poland [1] and is of current interest as a hypoxia-selective cytotoxin [2] and radiosensitizer of hypoxic cells [3]. The drug undergoes metabolic reductive activation [4–6] and forms macromolecular adducts [4, 5], the amount of which correlates with the cytotoxic effect [5]. In cultured cells interstrand crosslinks (ISCs‡), DNA–protein crosslinks (DPCs) and single strand breaks (SSBs) were reported to occur [7–12], and ISCs were related to cytotoxicity [8]. Recently, DPCs were found to be an important factor in nitracrine cytotoxicity, whereas ISCs and SSBs were not detected, when small, pharmacologically relevant doses of the drug were used [9, 10]. Thus, the nature of the DNA lesions responsible for cytotoxicity of nitracrine is a matter of controversy. To test whether ISCs or DPCs are critical chromatin lesions responsible for the cytotoxicity of nitracrine, we examined survival, DNA damage and repair in two mouse lymphoma L5178Y cell sublines which differ in sensitivity to genotoxic agents [13]. The cytotoxic effect of nitracrine was compared with that of mitomycin C (MMC), an agent which produces mainly ISCs in DNA [14].

* Corresponding author.

‡ Abbreviations: LY-R, L5178Y-R murine lymphoma cell subline; LY-S, L5178Y-S murine lymphoma cell subline; DPC, DNA–protein crosslink; ISC, interstrand crosslink; SSB, single strand break; D_{10}, drug concentration lowering cell survival to 10% of control; PBS, phosphate-buffered saline; SSC, 0.15 M NaCl, 15 mM sodium citrate; MMC, mitomycin C.

MATERIALS AND METHODS

Cell culture and cytotoxicity assay. LY-R and LY-S cells are the original L5178Y sublines, resistant and sensitive to ionizing radiation described by Alexander and Mikulski [15]. LY-S cells have not changed their phenotype in 30 years of *in vitro* cultivation. To retain the LY-R cell phenotype, alternating passages *in vitro* and *in vivo* are necessary [13]. The difference in response of the two sublines to a variety of cytotoxic agents is presented in Table 1. LY cells were cultured as a suspension in Fischer's medium with 8% bovine serum and antibiotics [16]. Cell densities were maintained in the range 2.5×10^4–8×10^4 cells/mL, as measured in a Bürker haemocytometer. LY-R cells had a doubling time of 11 hr; LY-S cells, 10 hr.

Nitracrine was kindly donated by Professor J. Konopa (Technical University of Gdansk, Poland). A stock solution of the drug, 1 μM, was prepared in saline, stored at −20° and diluted before cell treatment.

Table 1. Sensitivities of LY-R and LY-S cells to some genotoxic agents

Agent	D_{10} values* LY-R	LY-S	Reference
γ-Rays	3.26 Gy	1.26 Gy	21
Bleomycin	260 μg/mL	54 μM/mL	†
MMS	33 μM	20 μM	22
MNU	1.33 μM	0.57 μM	22
Nitrogen mustard‡	0.60 μM	0.36 μM	23
MMC	7.1 μM	2.3 μM	This work
cis-PAD	3 μg/mL	12 μg/mL	16
UVC	2.4 J/m	9.1 J/m	24
Nitracrine	0.11 μM	0.35 μM	This work

* D_{10} values were estimated by the authors of the present work from survival curves presented in the references quoted. Drug treatments were for 1 hr at 37° with the exception of nitrogen mustard. The most recent literature possible was chosen.

† Walicka, unpublished data.

‡ Survival after a single administration of the drug, without subsequent medium change, was estimated in Ref. 23 from extrapolation of the growth curves, as described in Ref. 15.

MMS, methyl methane sulphonate; MNU, *N*-methyl-*N*-nitrosourea; *cis*-PAD, *cis*-dichlorobis(cyclopentylamine)-platinum(II); UVC, UV radiation, $\lambda = 254$ nm.

Mitomycin C (Lampro B.V., The Netherlands) was kindly provided by Dr M. Zdzienicka (State University of Leiden, The Netherlands). A stock solution of MMC, 1 mg/mL in saline, was stored at 8° and diluted before cell treatment.

For survival determinations exponentially growing cultures, density *ca.* 4×10^5 cells/mL, were treated with nitracrine or MMC for 1 hr at 37°. Suspensions were centrifuged, medium was changed and cells were cloned in agar-solidified medium [16]. Cloning efficiency of drug-untreated cells was 35–76% for LY-R cells and 59–100% for LY-S cells. In order to determine IC_{50} values, cultures at the initial densities of 5.0×10^4 and 2.5×10^4 cells/mL for LY-R and LY-S lines, respectively, were exposed continuously to nitracrine for 48 hr. Thereafter, density and viability of cell populations were determined by nigrosine staining.

Alkaline elution. Cultures at 1.5×10^5 and 2.0×10^5 cells/mL for LY-R and LY-S cells, respectively, were labelled with 0.02 μCi/mL of [^{14}C]thymidine (55 mCi/mmol, Amersham, U.K.) for 21 hr, and then the suspensions at 4×10^5 cells/mL were treated with nitracrine for 1 hr, 37°, without washing out the radioactive label. Suspensions were centrifuged, pellets were washed with phosphate-buffered saline (PBS) to remove traces of the drug and the alkaline elution procedure was performed essentially as described in Ref. 17 except that the pumping rate was 0.1 mL/min and tetraethylammonium hydroxide (the Sigma Chemical Co., St Louis, MO, U.S.A.) was used in place of tetrapropylammonium in the elution buffer. Fractions of 6 mL were collected at 1 hr intervals for 5 hr. SSBs and ISCs were assayed using polycarbonate filters, pore size 1 μm (Nuclepore, Pleasanton, U.S.A.). For DPC measurement, type DM-800 filters, pore size 0.8 μm (Gelman, U.S.A.), were used. In the ISC and DPC assay cells received 300 rad (3 Gy) and 3000 rad (30 Gy), respectively [17].

The DPC frequency (P_{CD}) in rad equivalents was calculated by the formula:

$$P_{CD} = \left(\sqrt{\frac{1}{1-R_1}} - \sqrt{\frac{1}{1-R_0}}\right) \times 3000 \text{ rad}$$

where R_1 and R_0 are the extrapolated fractions of slowly eluting DNA in drug-treated and control cells, respectively [17].

Nitrocellulose filter binding assay. The procedure of Strniste and Rall [18] with slight modification was applied. Cell cultures were labelled for 21 hr with 0.02 μCi/mL of [^{14}C]thymidine or 0.05 μCi/mL of [^{3}H]thymidine (25 mCi/mmol, Amersham, U.K.), then drug treated, washed with medium and resuspended in cold PBS for measurement of the initial damage, or incubated in fresh, prewarmed medium to allow repair of damage. For a single measurement a pellet of 2×10^6 cells in PBS was resuspended in 2 mL of SSC (0.15 M NaCl, 15 mM sodium citrate) + 10 mM EDTA buffer, pH 7.5. One tenth of a millilitre of this suspension was taken for measurement of the total radioactivity of DNA per sample, applied to a nitrocellulose filter (0.45 μm, Sartorius, Germany) and rinsed with 5 mL of 1 mM Tris + 1 mM EDTA, pH 7.8. Cells in the remaining suspension were lysed with 0.1 mL of 20% *N*-laurylsarcosine (No. α-5000, Sigma) in SSC + EDTA buffer followed by 1.9 mL of the saturated (~6 M) NaCl in SSC + EDTA buffer. Samples were incubated for 20 min at 60° to precipitate DNA–protein complexes and cooled; 3.6 mL of cell lysates were loaded on filters and rinsed with 5 mL of 3 M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.8, and subsequently with 10 mL of 1 mM Tris, 1 mM EDTA, pH 7.8. Filters with the samples of non-lysed cells as well as with DNA–protein complex precipitates were air-dried and radioactivity was counted in a toluene-based scintillator. Percentage of the DNA retained on filters was calculated from the formula:

$$\% \text{ of DNA retained} = \frac{0.057 \times S_L}{S_0} \times 100\%$$

where S_L = cpm of a sample after lysis and S_0 = cpm of a sample before lysis.

RESULTS

Survival curves of LY cells exposed to nitracrine are shown in Fig. 1A. In terms of D_{10} (drug concentration lowering cell survival to 10% of control) values LY-R cells were 3.4 times more sensitive to the drug than LY-S cells. LY-S cells sensitivity to nitracrine was similar to that of other rodent, Chinese hamster AA8 cells, as a very close D_{10} value can be read from the data of Wilson *et al.* [5].

Continuous, 48-hr exposure gave an $IC_{50} = 1$ nM for LY-R cells and 4 nM for LY-S cells (data not

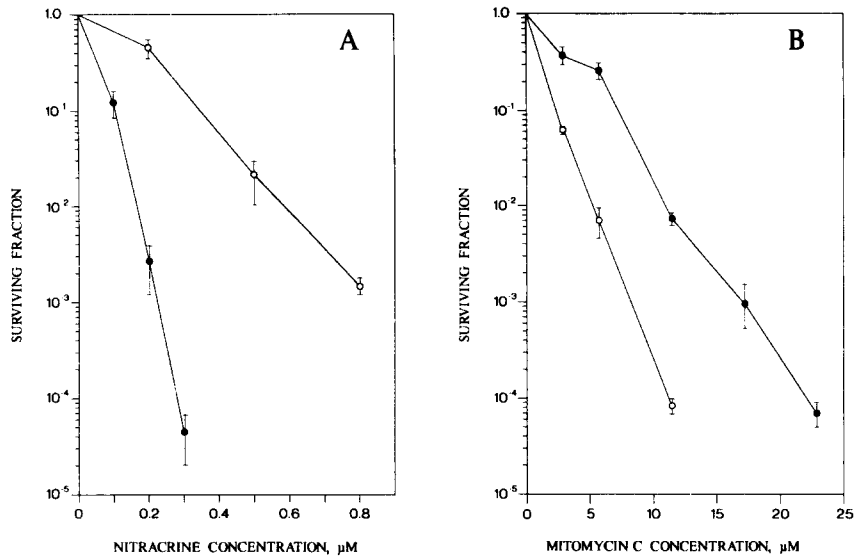

Fig. 1. Survival of LY cells treated with nitracrine or MMC in the medium for 1 hr at 37°. Mean values of three to four experiments with standard errors are shown. (●) LY-R cells, (○) LY-S cells. Panel A nitracrine, panel B MMC.

shown). Thus, LY cells seem to be more sensitive to the drug than other mouse lymphoma L1210 cells for which the IC_{50} was reported to be 7.6 nM [9].

Earlier works on the nature of cytotoxic damage inflicted by nitracrine [7, 8] suggested a strong relationship between cytotoxicity and DNA–DNA interstrand crosslinking. Hence, we present in Fig. 1B the data on sensitivity of LY cell lines to the reference crosslinking agent MMC. In terms of D_{10} dose LY-R cells were three times more resistant to MMC than LY-S cells.

To identify the DNA lesions involved in the

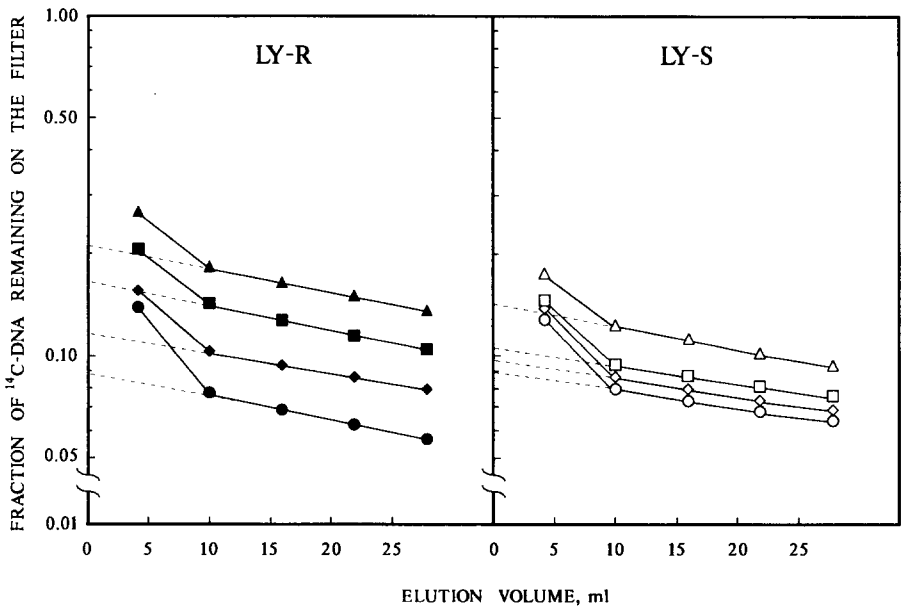

Fig. 2. Alkaline elution profiles of DNA from control and nitracrine-treated LY cells. Cells were incubated with different drug concentrations for 1 hr at 37° and subjected to the alkaline elution procedure. (●, ○) Control DNA. Nitracrine concentrations: (◆, ◇) 0.5 μM, (■, □) 1 μM, (▲, △) 2 μM.

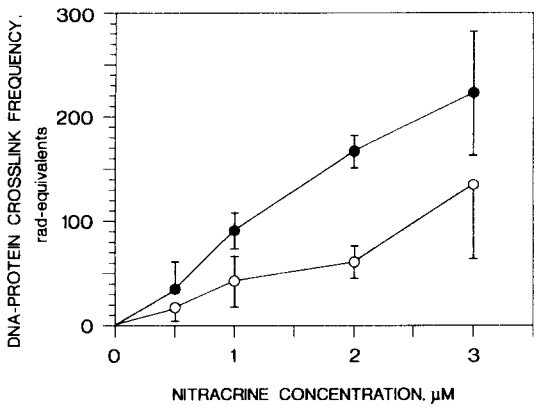

Fig. 3. DPCs in LY cells were measured by the alkaline elution method. Mean values of 11 experiments and standard errors are shown. (●) LY-R cells, (○) LY-S cells.

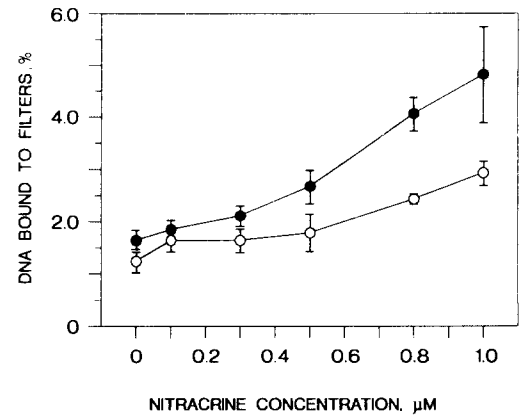

Fig. 4. DPCs in LY cells were measured by the nitrocellulose filter binding method. Mean values of 3–14 experiments with standard errors are shown. (●) LY-R cells, (○) LY-S cells.

toxicity of nitracrine we used the alkaline elution method which enables detection of SSBs, ISCs and DPCs [17]. We could not detect SSBs or ISCs in the DNA of either subline after incubation of cells with nitracrine for 1 hr at 37° in the concentration range 0.5–3.0 μM (data not shown). The only lesions found in that dose range were DPCs. In Fig. 2, DNA–protein crosslinking in both cell sublines is shown in the elution assay performed immediately after drug treatment. Cells were irradiated with a high γ-ray dose (30 Gy) to obtain short fragments of DNA and applied on filters having a high capacity to bind proteins. Then, the cells were lysed and DNA was eluted with the elution solvent at pH 12.1. Under these conditions polynucleotide single strands, which are not bound to the filter, elute rapidly; hence, DNA retention from control cells is very low [17]. The observed increase in retention of DNA from cells treated with nitracrine was dose-dependent and indicated the presence of DPCs. Although alkaline elution is recognised as one of the best methods for DPC measurement, the results were highly variable, especially at 3 μM. This is probably due to the high toxicity of nitracrine at this concentration and the presence of dead cells, which are known to produce some artefacts in alkaline elution assays [17].

To assess crosslinking caused by lower doses of nitracrine we used the more sensitive procedure based on the high adsorption of precipitated DNA–protein complexes on nitrocellulose [18]. In this method the fraction of DNA retained on the filter corresponds to DPC frequency. As can be seen in Figs 3 and 4, both methods gave similar results: nitracrine induced about two times more DPCs in LY-R cells as compared to LY-S cells.

In experiments on repair of DNA damage the concentrations of drug were chosen to be as low as possible to minimize the early appearance of nigrosine-stained dead cells. The drug concentrations investigated were 0.3 and 0.5 μM for LY-R and LY-S cells, respectively, which gave similar initial DPC frequencies (Fig. 4). As can be seen in Fig. 5

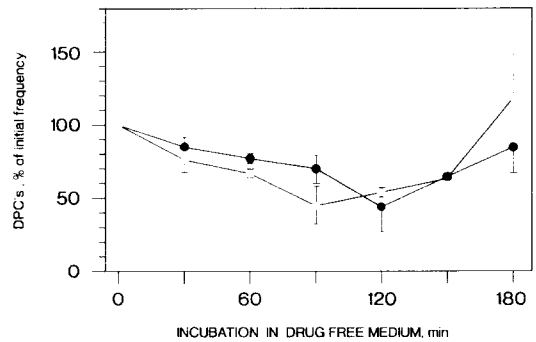

Fig. 5. Removal of DPCs from LY cells measured by the nitrocellulose filter binding method. Cells were treated for 1 hr at 37° with 0.3 and 0.5 μM nitracrine for LY-R and LY-S cells, respectively, and then incubated in drug-free medium. Mean values of three to four experiments with standard errors are shown, except for 150 min where results of a single experiment are shown. (●) LY-R cells, (○) LY-S cells.

during the first 120 min following drug treatment the DPC frequency decreased in both cell sublines. A significant increase in DPC level began at 180 min and persisted until 48 hr. This increase was probably related to the presence of dead cells in the population, as measured by nigrosine staining (data not shown). Results shown in Fig. 5 indicate that the cells of both sublines removed 50% of the initial DPCs in 2 hr, with LY-S cells exhibiting a higher rate of removal in the first 90 min.

DISCUSSION

This study aimed to determine the type of DNA damage produced by nitracrine which correlates with the cytotoxicity of the drug. The cytotoxicity depends on the metabolic activation of the drug [4, 5] which

is, almost certainly, enzymatic nitroreduction [6] resulting in covalent binding of nitracrine to DNA and other macromolecules. Recently, adduct formation by nitracrine was confirmed by means of the ^{32}P-post-labelling technique [19].

In earlier studies [7–9], SSBs, DPCs and ISCs were reported to be the DNA lesions induced by nitracrine in mammalian cells *in vivo* and in cell cultures. However, the doses of drug used in these studies were beyond the range of biological relevance. Recent data on HeLa [10] and L1210 [9] cells indicate that smaller doses of nitracrine induce only DPCs.

We decided to examine the types of initial DNA lesions produced by nitracrine in the mouse lymphoma sublines, LY-R and LY-S, with different sensitivities to genotoxic agents (Table 1). Designations -R (resistant) and -S (sensitive) concern their sensitivity to ionizing radiation [15]. The cells of these sublines were shown to differ 3.4 times, in terms of D_{10}, in their sensitivity to nitracrine (Fig. 1A). Their response to MMC, a DNA–DNA crosslinking agent, was the opposite: LY-R cells were three times more resistant (Fig. 1B). This result clearly indicates that LY-R cells deal with ISCs better than LY-S cells. The fact that both LY sublines differed in their sensitivity to nitracrine gave the possibility of examining the role of DNA damage and repair in the cytotoxic action of the drug.

DNA lesions produced by nitracrine were measured by the alkaline elution and filter binding methods. We did not find lesions other than DPCs in cells treated with nitracrine at concentrations of 0.1–3 μM (1 hr treatment at 37°). This does not necessarily mean that DPC is the sole DNA injury produced by low concentrations of the drug. Alkaline elution does not measure drug–DNA monoadducts, which are also good candidates for the cytotoxic lesion. The lack of ISCs among initial lesions inflicted by nitracrine together with the resistance of LY-R cells to MMC suggests that ISCs are not involved in nitracrine toxicity in LY cells. However, the absence of ISCs in DNA from LY cells treated with low drug doses may be due to alkali lability of the drug adducts responsible for ISC formation, which Konopa and co-workers [7, 8] demonstrated using DNA renaturation. Although these methods are less sensitive than DNA alkaline elution and require a high nitracrine concentration and long exposure times to detect crosslinks, they avoid exposure of DNA to alkali. Further research using sensitive methods based on agents other than alkali agents causing DNA strand separation should clarify this controversy.

Since nitracrine induces about two times more DPCs in LY-R cells than in LY-S cells (Figs 3 and 4) we suggest that protein-associated DNA lesions are related to the cytotoxicity of nitracrine. The role of DPC repair in the sensitivity of LY sublines may be evaluated from the data presented in Fig. 5. DPC removal in LY cells started immediately after drug treatment as in previously studied HeLa [10] and L1210 [9] cells. Complete repair was not observed, as it was masked by a second peak of DPCs that began 180 min after drug treatment. It is known that the highest frequency of DPCs and ISCs, induced by "classical" bifunctional alkylators, occurs 3–6 hr after drug treatment, and this is interpreted as a second arm reaction [20]. In the case of nitracrine the increase in DPC frequency seems to reflect the presence of dying cells, rather than a specific interaction of drug–DNA adduct with protein. This is suggested by a correlation between DPC level and the percentage of dead cells in the population (data not shown). Both sublines remove DPCs with similar efficiency. Thus, sensitivities of LY-S and LY-R cells are not related to crosslink removal but rather to the level of initial damage.

Several factors may contribute to the higher frequency of DPCs produced by nitracrine in LY-R cells. These are: drug transport through the cell membrane, metabolism of nitracrine and accessibility of DNA in chromatin to the molecules of active nitracrine metabolites. The data presented do not answer the question as to why nitracrine induces different levels of DPCs in the two LY sublines; they suggest, however, that cytotoxicity of nitracrine may be associated with DNA–protein crosslinking.

Acknowledgements—We thank Ms E. Godlewska for her excellent technical help. We appreciate critical reading of this manuscript by Profs I. Szumiel and M. Gniazdowski. This work was supported by Grant No. 4-1303-91-01 of the State Committee for Scientific Research.

REFERENCES

1. Gniazdowski M, Filipski J and Chorąży M, Nitracrine. In: *Antibiotics V/2. Mechanism of Action of Anti-eukaryotic and Antiviral Compounds* (Ed. Hahn F), pp. 275–297. Springer, Berlin, 1979.
2. Wilson WR, Denny WA, Twigden SJ, Baguley BC and Probert JC, Selective toxicity of nitracrine to hypoxic mammalian cells. *Br J Cancer* **49**: 215–223, 1984.
3. Roberts PB, Anderson RF and Wilson WR, Hypoxia-selective radiosensitization of mammalian cells by nitracrine, an electron-affinic DNA intercalator. *Int J Radiat Biol* **51**: 641–654, 1987.
4. Pawlak JW and Konopa J, *In vitro* binding of metabolically activated [^{14}C]-ledakrin, or 1-nitro-9-^{14}C-(3′-dimethylamino-*n*-propylamino)acridine, a new antitumor and DNA crosslinking agent, to macromolecules of subcellular fractions isolated from rat liver and HeLa cells. *Biochem Pharmacol* **28**: 3391–3402, 1979.
5. Wilson WR, Denny WA, Stewart GM, Fenn A and Probert JC, Reductive metabolism and hypoxia—selective metabolism of nitracrine. *Int J Radiat Oncol Biol Phys* **12**: 1235–1238, 1986.
6. Wilson WR and Anderson RF, The reaction between nitracrine and glutathione: implications for hypoxic cell radiosensitization and cytotoxicity. *Int J Radiat Oncol Biol Phys* **16**: 1001–1005, 1989.
7. Konopa J, Pawlak JW and Pawlak K, The mode of action of cytotoxic and antitumor 1-nitroacridines. III. *In vivo* intrastrand cross-linking of DNA of mammalian or bacterial cells by 1-nitro-acridines. *Chem Biol Interact* **43**: 175–197, 1983.
8. Pawlak K, Pawlak JW and Konopa JW, Cytotoxic and antitumour activity of 1-nitroacridines as an after effect of their interstrand DNA cross-linking. *Cancer Res* **44**: 4289–4296, 1984.
9. Woynarowski JM, McNamee H, Szmigiero L, Beerman A and Konopa J, Induction of DNA–protein crosslinks by antitumour 1-nitro-9-aminoacridines in L1210 leukemia cells. *Biochem Pharmacol* **38**: 4095–4101, 1989.

10. Szmigiero L and Studzian K, DNA damage and cytotoxicity of nitracrine in cultured HeLa cells. *Biochim Biophys Acta* **1008**: 339–345, 1989.
11. Woynarowski JM, Bartoszek A and Konopa J, DNA damage in HeLa S3 cells by an antitumor drug Ledakrin and other antitumor 1-nitro-9-aminoacridines. *Chem Biol Interact* **49**: 311–328, 1984.
12. Woynarowski JM and Bartoszek A, The mechanisms of inhibition of DNA replication in HeLa S cells by the antitumor drug Ledakrin and other antitumor 1-nitro-9-aminoacridines. *Biochim Biophys Acta* **825**: 244–253, 1985.
13. Beer JZ, Budzicka E, Niepokojczycka E, Rosiek O, Szumiel I and Walicka M, Loss of tumorigenicity with simultaneous changes in radiosensitivity and photosensitivity during *in vitro* growth of L5178Y murine lymphoma cells. *Cancer Res* **43**: 4736–4742, 1983.
14. Lawely PD, Approaches to chemical dosimetry in mutagenesis and carcinogenesis; the relevance of reactions of chemical mutagens and carcinogens with DNA. In: *Chemical Carcinogenesis and DNA* (Ed Grover PL), Vol. 1, pp. 1–36. CRC Press, Boca Raton, FL, 1979.
15. Alexander P and Mikulski ZB, Mouse lymphoma cells with different radiosensitivities. *Nature* **192**: 572–573, 1961.
16. Szumiel I, Response of two strains of L5178Y cells to cis-dichlorobis(cyclopentylamine)platinum (II). I. Cross-sensitivity to cis-PAD and UV-light. *Chem Biol Interact* **24**: 51–78, 1979.
17. Kohn KW, Principles and practice of DNA filter elution. *Pharmacol Ther* **49**: 55–77, 1991.
18. Strniste GF and Rall SC, Induction of stable protein deoxyribonucleic acid adducts in Chinese hamster cell chromatin by ultraviolet light. *Biochemistry* **15**: 1712–1719, 1976.
19. Bartoszek A and Konopa J, ^{32}P-Post-labeling analysis of DNA adduct formation by antitumor drug nitracrine (Ledakrin) and other nitroacridines in different biological systems. *Biochem Pharmacol* **38**: 1301–1312, 1989.
20. Kohn KW, Biological aspects of DNA damage by crosslinking agents. In: *Molecular Aspects of Anti-Cancer Drug Action* (Eds. Neidle S and Waring MJ), pp. 315–361. Macmillan, London, 1983.
21. Walicka M and Godlewska E, Radiation sensitivities are not related to the size of DNA supercoiled domain in L5178Y-R and L5178Y-S cells. *Int J Radiat Biol* **55**: 953–961, 1989.
22. Evans HH, Horng MF and Beer JZ, Lethal and mutagenic effects of radiation and alkylating agents on two strains of mouse L5178Y cells. *Mutat Res* **161**: 553–562, 1985.
23. Goldenberg GJ and Alexander P, The effects of nitrogen mustard and dimethyl myleran on murine leukemia cell lines of different radiosensitivity *in vitro*. *Cancer Res* **25**: 1401–1409, 1965.
24. Walicka M, Godlewska E and Mullenders LHF, DNA damage processing in L5178Y-R and L5178Y-S cells with different sensitivities to UV-C radiation. *Nukleonika* **37**: 59–70, 1992.