Acta Biochimica Polonica

Vol. 41 No. 2/1994

QUARTERLY

Thiol dependent inhibition of mouse leukemia L1210 DNA topoisomerase I by nitracrine*

Ewa Ciesielska and Leszek Szmigiero

Department of General Chemistry, Institute of Physiology and Biochemistry, Medical Academy, Lindleya 6, 90–131 Łódź, Poland

Key words: Nitracrine, topoisomerase I, L1210 cells

Nitracrine [NA[1], Ledakrin, 1-nitro-9-(3,3-*N,N*-dimethylamino-propylamino)-acridine] is of current interest as a hypoxia-selective cytotoxic agent [1]. The drug acquires its DNA and protein binding propensity upon reductive activation leading to formation of macromolecular adducts, which seem to be responsible for its cytotoxicity [1, 2]. The enzymatic activation of NA can be replaced in a cell-free system by the reaction with thiols. A transient product of the thiol-NA reaction binds to DNA and forms covalent adducts decreasing its template activity [3]. It was demonstrated in cultured cells that NA induces DNA interstrand crosslinks [4], DNA single strand breaks [5], and DNA-protein crosslinks [6, 7]. Although high crosslinking potency of the drug seems to be linked with its cytotoxicity, the critical target in chromatin responsible for lethal effects of NA remains unidentified. The ability of NA to form DNA-protein crosslinks and single strand breaks may suggest that this drug blocks topoisomerase I or/and topoisomerase II at the "cleavable complex" step [8]. However according to Woynarowski's *et al.* [9] results with L1210 cells, topoisomerase II is unlikely to be inhibited by the drug. The inhibitory effect of NA on topoisomerase I (topo I) has not been tested so far.

The aim of the current work was to test whether NA may disturb catalytic action of topo I from L1210 cells. The effect of the drug on topo I was compared with that of C-137, its biologically inactive *des*-nitro analog.

The inhibitory effect of both acridines on topo I was studied in a cell-free system containing pBR322 DNA (at least 70% of supercoiled form) and nuclear extract from L1210 cells. Nuclei were extracted according to Filipski *et al.* [10]. The topo I assay measuring relaxation of the supercoiled substrate by nuclear extract was performed according to Hertzberg *et al.* [11], under conditions promoting the processive mode of topo I action. Reaction mixtures (usually 25 µl) contained 0.5 µg of pBR322 DNA and 0.5 unit of topo I. One unit fully relaxed 1 µg of substrate after 30 min at 37°C. Relaxation was carried out for 30 min at 37°C and the reaction was stopped by adding sodium dodecylsulfate and proteinase K. After another 30 min at 37°C, 5 µl of gel loading buffer (Tris/HCl 0.01 M, pH 8, glycerol 30%, bromophenol blue 0.05%) was added. Samples were loaded on 0.8% agarose gel and electrophoresis was carried out for 2 h at 4 V/cm. Gels were stained with 0.5 µM ethidium bromide and photographed under UV light. The negative of the gel photograph was scanned with a Hoefer Scientific Instrument GS 300 densitometer, and percentage of the relaxed DNA form relative to total DNA for each lane was quantitated. To form NA-DNA adducts the drug was preincu-

*This work was supported by Grant No. 502-11-82 of Medical Academy in Łódź.

[1]Abbreviations: DTT, dithiothreitol; NA, nitracrine; topo I, topoisomerase I.

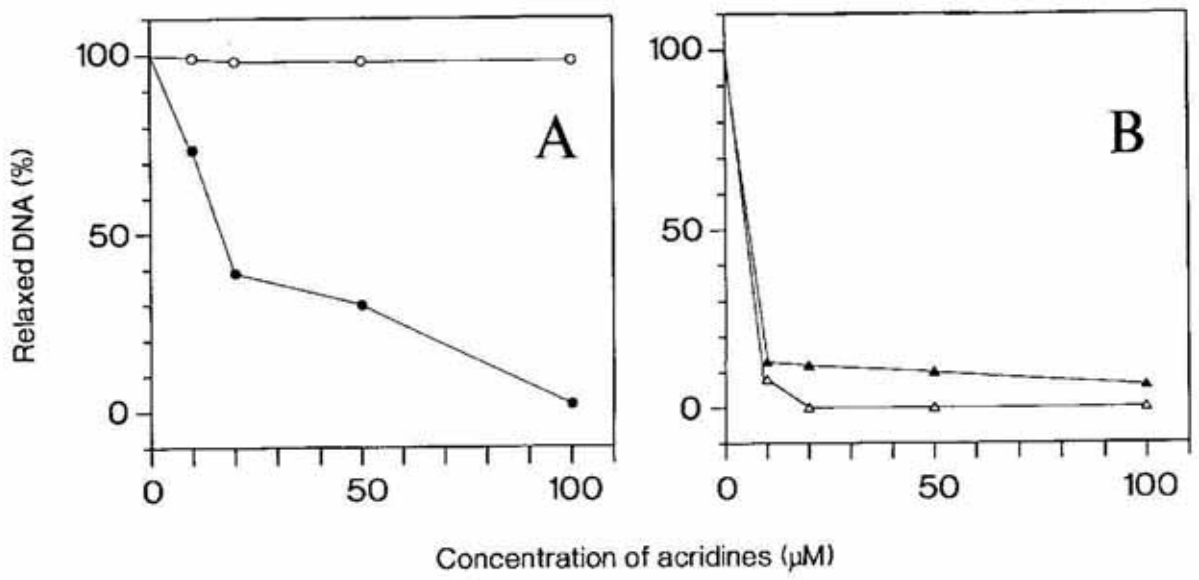

Fig. 1. Effect of dithiothreitol on inhibition of topoisomerase I from L1210 cells by nitracrine and C-137. Assay without dithiothreitol (○, △); pBR322 DNA preincubated with increasing concentrations of the drugs for 30 min at 37°C in the presence of 2 mM dithiothreitol (●, ▲). Panel A — nitracrine, panel B — C-137.

bated with DNA for 30 min at room temperature in the presence of 2 mM dithiothreitol (DTT) prior to topo I addition.

As shown in Fig. 1A, NA exhibited a very low inhibitory effect on relaxation when the reaction was carried out in the absence of DTT. Preincubation of NA for 30 min in the presence of 2 mM DTT resulted in inhibition of the topo I catalyzed relaxation of supercoiled DNA (Fig. 1A). C-137, the *des*-nitro analog of NA, inhibited relaxation of DNA to a greater extent than did the parent compound, and its action was not dependent on DTT (Fig. 1B).

The effect of either acridine observed in the absence of thiol seems to be related to the drugs intercalation properties. As it was demonstrated by Pommier *et al.* [12] several intercalating acridines inhibited topo I in a cell-free system. C-137 with its flat acridine ring is a typical intercalating dye and its relatively strong effect on topo I activity was rather expected. NA, of a "butterfly" like molecule, is not flat and cannot form a strong intercalating complex with DNA (see Gniazdowski *et al.* [13] for review). As the NA-DNA intercalation complex is characterized by a low binding constant and low number of binding sites in DNA [13] it seems clear that NA, a poor intercalator, does not interfere efficiently with topo I action (Fig. 1, without DTT).

Preincubation of DNA with NA in the presence of DTT resulted in a significant enhancement of the inhibition (Fig. 1A). No similar enhancement was observed in the case of C-137 (Fig. 1B). This result demonstrates a necessity for reduction of the nitro group in NA molecule for its inhibitory action, and is consistent with earlier studies on inhibitory effect of NA and its analogs on RNA synthesis *in vitro* [13]. The exact mechanism of topo I inhibition by NA in the presence of DTT remains unknown. However, our results suggest that thiol dependent activation of the drug leads to the formation of the drug-DNA adducts which are an impediment to topo I action. The most interesting question: whether topo I could be a cellular target for nitracrine, cannot be answered at this stage of our work. Especially as it is not known whether NA traps specifically the enzyme in the "cleavable" complex, or whether it affects the affinity of topo I to the substrate.

REFERENCES

1. Wilson, W.R., Denny, W.A., Stewart, G.M., Fenn, A. & Probert, J.C. (1986) *Int. J. Radiat. Oncol. Biol. Phys.* **12**, 1235–1238.
2. Pawlak, J.W. & Konopa, J. (1979) *Biochem. Pharmacol.* **28**, 3391–3402.

3. Gniazdowski, M., Ciesielska, E. & Szmigiero, L. (1981) *Chem. -Biol. Interact.* **34**, 355–366.
4. Konopa, J., Pawlak, J.W. & Pawlak, K. (1983) *Chem. -Biol. Interact.* **43**, 175–197.
5. Woynarowski, J.M., Bartoszek, A. & Konopa, J. (1984) *Chem.- Biol. Interact.* **49**, 311–328.
6. Szmigiero, L. & Studzian, K. (1989) *Biochim. Biophys. Acta* **1008**, 339–345.
7. Walicka, M., Szmigiero, L., Ciesielska, E. & Grądzka, I. (1993) *Biochem. Pharmacol.* **46**, 615–620.
8. Zhang, H., D'Arpa, P. & Liu, L.F. (1990) *Cancer Cells* **2**, 23–24.
9. Woynarowski, J.M., McNamee, H., Szmigiero, L., Beerman, A. & Konopa, J. (1989) *Biochem. Pharmacol.* **38**, 4095–4101.
10. Filipski, J., Yin, I. & Kohn, K.W. (1983) *Biochim. Biophys. Acta* **741**, 116–122.
11. Hertzberg, R.P., Caranfa, M.J. & Hecht, S.M. (1989) *Biochemistry* **28**, 4629–4638.
12. Pommier, Y., Covey, J.M., Kerrigan, D., Markovits, J. & Pham, R. (1987) *Nucleic Acids Res.* **15**, 6713–6731.
13. Gniazdowski, M., Filipski, J. & Chorąży, M. (1979) in *Antibiotics, Mechanism of Action of Antieukariotic and Antiviral Compounds* (Hahn, F.E., ed.) vol. 5, pp. 275–297, Springer Verlag, Berlin.