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DNA-protein crosslinks induced in HeLa cells by bis-1-nitroacridines*

Elżbieta Pastwa, Ewa Ciesielska, Kazimierz Studzian and Leszek Szmigiero

Department of General Chemistry, Institute of Physiology and Biochemistry, Medical University in Łódź, Lindleya 6, 90 - 131 Łódź, Poland

The anticancer drug nitracrine (Ledakrin, C-283) is a parent compound to 1-nitroacridine derivatives. The potent cytostatic activity of these compounds is probably due to their covalent binding to chromatin components [1 - 5]. Although the molecular mechanism in which nitracrine reacts with macromolecules remains unclear it has been suggested that interstrand crosslinks and/or DNA-protein crosslinks are lethal lesions produced by the drug [3 - 6]. In an effort to find antitumour agents of improved selectivity, 1-nitroacridine dimers with different types of linkers were synthesized [7]. The purpose of this work was to compare cytotoxicity of nitracrine and two new bis-1-nitroacridines 1 and 3 (see Fig. 1 for structures) as well as to analyse the DNA lesions produced by low, biologically relevant doses of the drugs in human cultured cells. To study the role of the nitro group in the cytotoxic activity of acridine dimers, their *des*-nitro analogues 2 and 4 were also tested. Nitracrine was a gift from Prof. Jerzy Konopa from the Technical University of Gdańsk (Poland). Bis-acridines 1 - 4 were synthesized in the Institute Gustave Roussy (France) and kindly donated by Dr Judith Markovits.

The cytotoxic effect of acridines was assayed by measuring their inhibitory effect on HeLa cells proliferation as described elsewhere [4]. DNA single strand breaks, DNA interstrand crosslinks and DNA-protein crosslinks (DPC[1]) were measured according to the alkaline elution procedure of Kohn *et al.* [8].

Cytotoxic activities of the acridines were dependent on the presence of the nitro group in position 1 of chromophore rings (Fig. 2). In terms of ED_{50} values, compounds 1 and 3 were about 9 times more active than their *des*-nitro analogues 2 and 4 (Table 1). Both of the 1-nitro substituted dimers exhibited lower cytotoxic activity than monomeric nitracrine (Fig. 2). The data obtained point clearly to the crucial role of the nitro groups in position 1 of the acridine rings of the dimers in their biological effect. It is commonly recognized that 1-nitro group and the presence of a substituent at position 9 are responsible for the biological activity of the drugs. It is somewhat surprising that the drugs bearing two potentially reactive sites in their molecules exhibited lower inhibitory effects on the cell growth than monomeric nitracrine did. Furthermore, it seems that flexibility of the linker between chromophores does not play a significant role in the cytotoxic activity of the dimers tested, as differences in the cytotoxic potencies of 1 (flexible linker) and 3 (rigid linker) were not substantial. On measuring the DNA damage we were unable to find any single strand breaks or interstrand crosslinks in the HeLa cells treated with any of compounds tested (not shown). Three 1-nitro substituted drugs: nitracrine, 1 and 3 induced, in the chromatin of HeLa cells, concentration dependent DPC (Table 1). *Des*-nitro analogues 2 and 4 were inactive in DPC formation (Table 1). Nitracrine was the most potent DNA-protein crosslinking agent and exibited the highest cy-

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[1]Abbreviations: DPC, DNA-protein crosslinks; ED_{50}, the dose which reduces the relative cell number to 50%

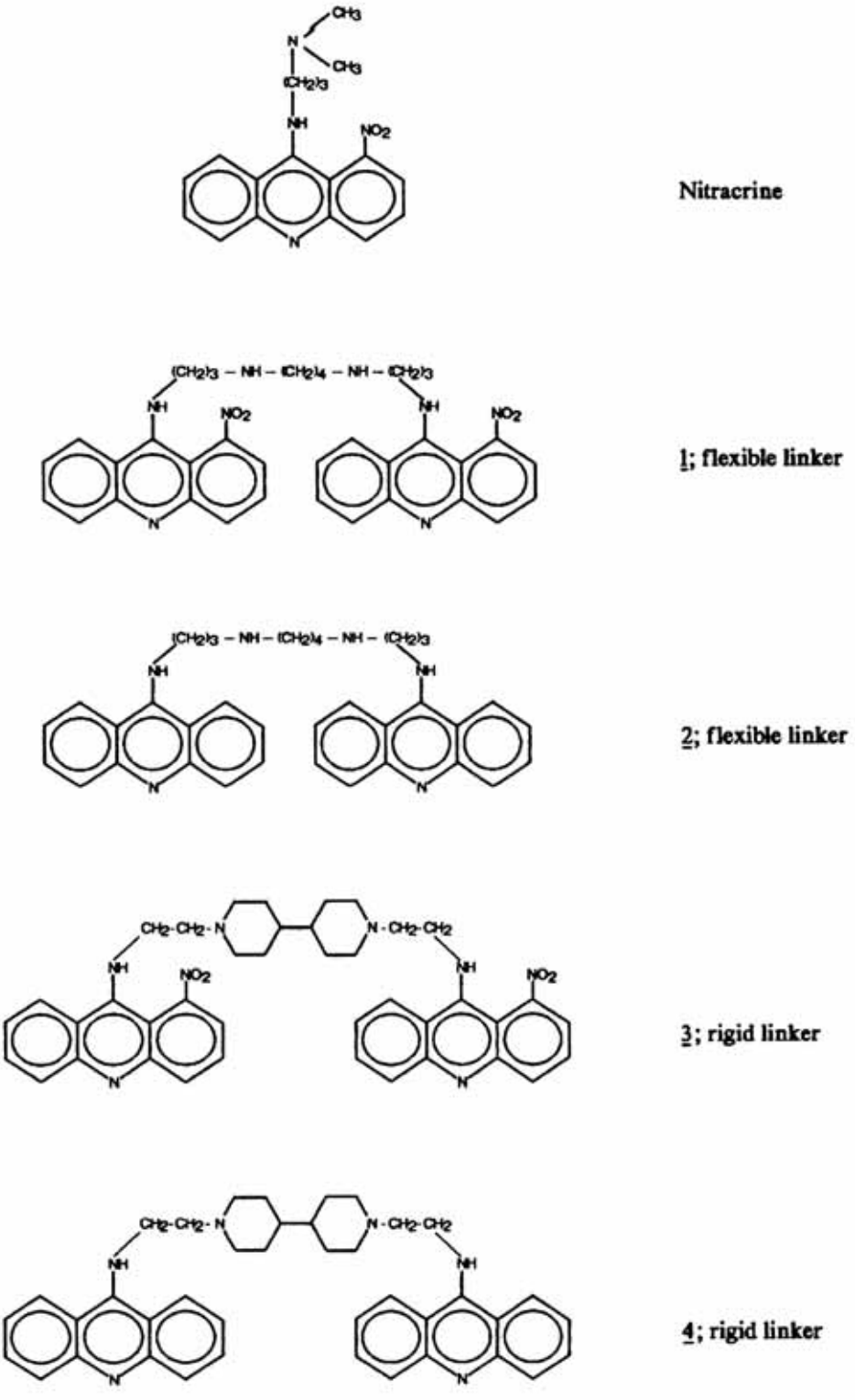

Fig. 1. Structure of acridines

totoxic activity (Figs. 2 and 3). This result agrees with the data obtained by Woynarowski *et al.* [5], who found a correlation between the ability of several 1-nitroacridines to induce DPC and their growth inhibitory potency.

The results presented in this report show that the mode of action of bis-1-nitroacridines is similar to that of nitracrine, as the same chromatin lesion was found in the cells treated with nitracrine or with its dimers. Our results are in

Table 1

Cytotoxicity and DNA lesions induced in HeLa cells by nitracrine and bis-acridines

Cells were treated with the drugs at the concentration indicated for 1 h at 37°C and immediately subjected to alkaline elution. ED_{50} is the dose which reduces the relative cell number to 50%; DNA-protein crosslink (DPC) frequencies expressed in arbitary units "rad-equivants" e.g. the dose of gamma radiation producing an equivalent effect on DNA [8]

Drug	ED_{50} (μM)	DPC frequency		
		1 μM	2 μM	4 μM
Nitracrine	0.15	174 ± 36	276 ± 10	not assayed
1	0.25	15 ± 17	46 ± 20	151 ± 39
2	2.15	N.D.*	N.D.*	N.D.*
3	0.21	17 ± 21	81 ± 30	152 ± 24
4	1.88	N.D.*	N.D.*	not assayed

Data are means of 3 - 4 independent determinations ± S.D.
*N.D., not detected.

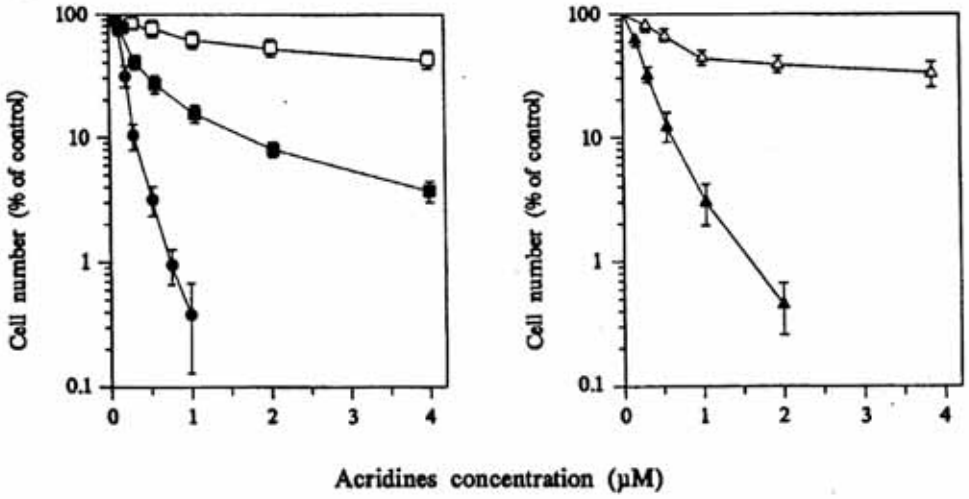

Fig. 2. Inhibition of HeLa cells proliferation by acridines.
Cells were treated with the drugs for 1 h at 37°C. Nitracrine (●), 1 (■), 2 (□), 3 (▲), 4 (Δ)

agreement with the hypothesis that DNA-protein crosslinks are specific cellular lesions which may be responsible for the cell killing effect of nitracrine and other 1-nitro substituted acridines [4 - 6].

REFERENCES

1. Gniazdowski, M., Filipski, J. & Chorąży, M. (1979) in *Antibiotics, Mechanism of Action of Antieukaryotic and Antiviral Compounds* (Hahn, F.E., ed.) vol. 5, pp. 275 - 297, Springer Verlag, Berlin.
2. Gniazdowski, M., Ciesielska, E. & Szmigiero, L. (1981) *Chem.-Biol. Interact.* **34**, 355 - 366.
3. Konopa, J., Pawlak, J.W. & Pawlak, K. (1983) *Chem.-Biol. Interact.* **43**, 175 - 197.
4. Szmigiero, L. & Studzian, K. (1989) *Biochim. Biophys. Acta* **1008**, 339 - 345.
5. Woynarowski, J.M., McNamee, H., Szmigiero, L., Beerman, T.A. & Konopa, J. (1989) *Biochem. Pharmacol.* **38**, 4095 - 4101.
6. Ciesielska, E., Walicka, M., Pastwa, E. & Szmigiero, L. (1992) *Acta Biochim. Polon.* **39**, 53 - 57.
7. Markovits, J., Wilmaska, D., Lescot, E., Studzian, K., Szmigiero, L. & Gniazdowski, M. (1989) *Chem.-Biol. Interact.* **70**, 73 - 87.
8. Kohn, K.W., Ewig, R.A.G., Erickson, L.C. & Zwelling, L.A. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E.C. & Hanawalt, P.C., eds.) vol. 1, Part B, pp. 379 - 401, Marcel Dekker, New York, 1981.