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Sister chromatid exchanges induced *in vitro* in human lymphocytes by N-substituted phosphorodiamidic acids*

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The drug ifosfamide, a member of the oxazaphosphorine cytostatics is of interest because of its significant activity against many of malignant tumors [1]. Several new analogues of ifosfamide, with the chlorine atom replaced by another atom or group undergoing nucleophilic substitution, were synthesized [2]. Bromofosfamide which exhibited a higher therapeutic index than ifosfamide did, appeared to be the most promising analogue [2].

There are numerous data indicating that metabolic activation of ifosfamide leads to formation of *N*-mustard-phosphorodiamidic acids (isophosphoramide mustards) which are responsible for alkylation of a variety of cellular targets [3]. It is believed that the crucial event in cytotoxic action of isophosphoramide mustard is alkylation of DNA leading to interstrand crosslinking and other types of DNA lesions [4 - 6]. Interstrand crosslinks are generally recognized as lethal lesions whereas formation of monoadducts may be responsible for undesirable side effects (e.g. mutagenicity).

The alkylating metabolites of ifosfamide and its bromoanalogues have been recently tested for their cytotoxicity and DNA damaging properties [6]. Isophosphoramide mustard **2** (Fig. 1), the alkylating metabolite of bromofosfamide, exhibited the highest ratio of DNA interstrand crosslinks to total DNA lesions. Therefore it seemed that some improvement in the therapeutic efficacy of bromofosfamide *versus* ifosfamide may be related to the enhancement of interstrand crosslinking potency of the alkylating metabolite [6].

In the present work we have focused attention on genotoxicity of isophosphoramide mustard **1** and its three analogues **2** - **4** (Fig.1). Mustards were synthesized according to the procedure described earlier [7]. Sister chromatid exchanges (SCEs) analysis was employed to study genotoxic damage produced in human lymphocytes. Student's *t*-test was used to examine the differences in the frequencies of SCE. Cell cultures were prepared by incubation of 0.5 ml heparinized whole blood from 1 - 6 healthy donors (females) in 5.0 ml RPMI 1640 medium containing 10% of foetal calf serum, 1% phytohaemagglutinin and 10 μg/ml of 5-bromodeoxyuridine. After 48 h of incubation the drugs at different concentrations were added to the cultures and incubation was continued for additional 24 h in the dark. Air dried preparations were made and stained using fluorescence and Giemsa technique [8]. For each concentration of the drug 10 - 90 metaphases per donor were examined and the number of SCE per cell was determined (Table 1).

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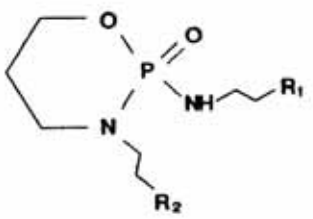
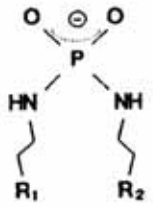


Oxazaphosphorine



Isophosphoramide mustard
(N-substituted phosphorodiamidic acid)

R_1	R_2	Oxazaphosphorine	Isophosphoramide mustard
Cl	Cl	Ifosfamide	1
Cl	Br	bromofosfamide	2
Br	Br	dibromofosfamide	3
SO_3-CH_3	SO_3-CH_3	dimetanosulphonofosfamide	4

Fig. 1. Structures of oxazaphosphorine drugs and their alkylating metabolites

No differences between phosphorodiamidic acids 1 - 4 were found in their ability to induce SCEs when the drugs were used at the concentrations not exceeding 2.5 μM. At higher concentrations (5 μM and 10 μM), compounds 1, 3 and 4 were almost equivalent whereas 2 induced a significantly higher frequency of SCE (Table 1). Enhancement of SCE levels has been used as an indicator of human genotoxic exposure to a number of therapeutic agents including the alkylating antitumour drugs [9, 10]. It is known that among some patients treated with alkylating agents, a subsequent development of a "second malignancy" may occur [11]. The mechanism of "second malignancy" is not well understood but may be related to DNA damage caused by therapeutic drugs. Therefore, high capacity to induce SCE seems to be an undesirable feature of any potentially anticancer agent. Compound 2 is believed to be an alkylating metabolite of bromofosfamide, an analogue of ifosfamide exhibiting promising anticancer activity in an experimental murine model [2]. Thus the observation that compound 2 was somewhat more potent in SCE induction in human lymphocytes than other isophosphoramide mustards tested in this work, could point to a higher risk of mutation and second malignancy for patients who would be treated with bromofosfamide. On the other hand, comparison of our results with the data of McDiarmid *et al.* [10] shows some advantage of compounds 1 - 4 *versus* phosphoramide mustard which is the alkylating metabolite of the widely used cytostatic cyclophosphamide. At the concentration of 0.25 μg/ml (1.1 μM) phosphoramide mustard induced SCEs at an almost twice as high frequency as the alkylators tested in this work. As it was mentioned earlier, 2 produces many more interstrand crosslinks in DNA of the treated cells than do the compounds 1, 3 [6] and 4 (Studzian *et al.*, unpublished). Some DNA lesions occurring in the parallel or neighbouring sites of complementary DNA strands, like double strand breaks and interstrand crosslinks, are repaired efficiently by the recombination repair mechanism [12].

This leads to DNA segments exchange between sister DNA chains occurring during the repair process. Therefore it seems also possible that the somewhat more potent SCEs induction by compound 2 does not necessarily points to its higher mutagenicity but can reflect the repair of interstrand crosslinks induced by this drug. To clarify this problem further research

Table 1

Sister chromatid exchange frequencies in human lymphocytes exposed in vitro to isophosphoramide mustards 1 - 4

Drug	Concentration (μM)	Number of SCEs per metaphase	Number of metaphases	Number of blood donors
1	0.5	9.5 ± 0.9	239	5
	1.0	10.6 ± 0.8	193	4
	2.5	14.4 ± 1.4	119	3
	5.0	22.3 ± 2.7	39	1
	10.0	35.4 ± 1.9	271	6
2	0.5	9.1 ± 0.9	168	4
	1.0	12.1 ± 1.0	175	4
	2.5	17.4 ± 2.1	82	2
	5.0	36.0 ± 4.3	41	2
	10.0	56.4 ± 4.4	102	6
3	0.1	9.9 ± 0.8	205	4
	0.5	12.2 ± 1.1	165	4
	2.5	18.1 ± 1.6	78	2
	5.0	28.0 ± 2.4	91	1
	10.0	38.6 ± 2.9	141	5
4	0.5	9.7 ± 0.7	187	4
	1.0	11.5 ± 0.9	180	4
	2.5	15.1 ± 1.7	70	2
	5.0	23.4 ± 2.1	100	2
	10.0	37.7 ± 2.5	168	6

Data are means ± S.E. ($P < 0.001$). The means for control and solvent-control (dimethyl sulphoxide) were 5.9 ± 0.8 and 5.7 ± 1.9, respectively

including carcinogenic transformation *in vitro* and tests on animals should be undertaken.

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REFERENCES

1. Zalupski, M. & Baker, L.H. (1988) *J. Natl. Cancer. Inst.* **80**, 556 - 566.
2. Misiura, K., Kinas, R., Stec, W.J., Kuśnierczyk, H., Radzikowski, C. & Sonoda, A. (1988) *J. Med. Chem.* **31**, 226 - 230.
3. Brock, N. (1986) *J. Cancer Res. Clin. Oncol.* **111**, 1 - 12.
4. Crook, T.M., Souhami, R.L., & McLean, A.E.M. (1986) *Cancer Res.* **46**, 5029 - 5034.
5. Hauseheer, F.H., Singh, V.C., Saxe, J.D. & Colvin, O.M. (1989) *Anti-Cancer Drug Design* **4**, 281 - 194.
6. Studzian, K., Kinas, R., Ciesielska, E. & Szmigiero, L. (1992) *Biochem. Pharmacol.* **43**, 937 - 943.
7. Misiura, K., Okraszek, A., Pankiewicz, K., Stec, W.J., Czownicki, Z. & Utracka, P. (1983) *J. Med. Chem.* **26**, 674 - 679.
8. Perry, P. & Wolff, S. (1974) *Nature* **251**, 156 - 161.
9. Vyas, R.C., Adhuvaryan, S.G. & Shah, V.C. (1988) *Mutation Res.* **206**, 163 - 166.
10. McDiarmid, Z.A., Strickand, P.T., Kolodner, K., Hansen, J. & Jacobsen-Kram, D. (1990) *Mutation Res.* **241**, 273 - 278.
11. Hoover, R., & Fraumini, J.F., Jr. (1981) *Cancer* **47**, 1071 - 1080.
12. Hovard-Flanders, P. (1973) *British Medical Bulletin* **29**, 226 - 235.