Absence of chromosome damage in human lymphocytes exposed to allopurinol and oxipurinol

In vivo and *in vitro* studies

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Stevenson, A. C., Silcock, S. R., and Scott, J. T. (1976). *Annals of the Rheumatic Diseases*, 35, 143–147. Absence of chromosome damage in human lymphocytes exposed to allopurinol and oxipurinol. *In vivo* and *in vitro* studies. The biochemical evidence against incorporation of the purine analogues, allopurinol and oxipurinol, into nucleic acids is reviewed. Cytological experiments have been carried out to investigate the possibility that chromosomal damage might result from exposure of human lymphocytes to these drugs. Lymphocytes from 19 patients receiving allopurinol and one receiving oxipurinol were examined for the presence of chromatid aberrations during metaphase, and lymphocytes from untreated subjects were similarly studied during *in vitro* exposure to the drugs. The low frequency of aberrations observed was well within normal limits, and it is concluded that allopurinol and oxipurinol have no deleterious effects on chromosome structure.

Allopurinol is used extensively in the management of conditions associated with excess body urate, e.g. gouty arthritis. It reduces urate formation by inhibiting xanthine oxidase, and hence blocking the conversion of hypoxanthine and xanthine to urate/uric acid, *i.e.* the final steps in the purine catabolic pathway in man. It also reduces *de novo* purine synthesis by a feedback mechanism. The pharmacology of allopurinol and its therapeutically active metabolite, oxipurinol, has been described in depth by Rundles and others (1963), Elion (1966), Emmer-son (1966), Hitchings (1966, 1969), Chalmers and others (1968), and Kelley and Wyngaarden (1972).

Allopurinol and oxipurinol are structural analogues of the purines xanthine and hypoxanthine (Figure). The theoretical possibility has therefore been considered that allopurinol or its metabolites might be incorporated into nucleic acids, or interfere with

FIGURE Structure of allopurinol and oxipurinol

nucleic acid replication, and that long-term therapy with allopurinol could have genetic repercussions.

Nucleic acids are polymers of nucleoside monophosphates, *i.e.* base + sugar + one phosphate group. To permit incorporation of purine or pyrimidine bases, these must first be converted into the corresponding nucleoside triphosphates (Bell, Davidson, and Emslie-Smith, 1972). This is an essential step in the synthesis of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In order to verify incorporation of an analogue base, it would be necessary to identify the analogue in formed nucleic acids, and also to demonstrate formation of the corresponding precursors—in this case, nucleoside mono-, di-, and triphosphate derivatives of allopurinol or its metabolites. Failure to find such evidence would make the possibility of incorporation extremely remote.

Previous *in vitro* and *in vivo* studies showed no incorporation of 6-¹⁴C allopurinol into the nucleic acids of cultured human fibroblasts (Kelley and Wyngaarden, 1970) or livers of mice given 1 mg intraperitoneally (Elion and others, 1966). The nucleoside monophosphates of allopurinol and oxipurinol have been synthesized enzymatically *in vitro* (Krenitsky and others, 1967; Fyfe, Miller, and Krenitsky, 1973). They were shown in micromolar quantities ($<10^{-9}$ – 10^{-6} mol/l) in the liver and kidneys of rats given large (50 mg/kg) intravenous doses of 6-¹⁴C allopurinol and oxipurinol, but there was no evidence for the presence of the corresponding nucleoside di- and triphosphates (Nelson and others, 1973).

Nelson and Elion (1975) have recently provided virtually conclusive evidence against the incorporation of allopurinol or its metabolites into nucleic acids. They calculated that, had incorporation occurred, the amounts involved must have been less than 1 molecule of analogue base per 5×10^6 normal bases in RNA, and less than 1 molecule per 10^7 normal bases in DNA. This is below biochemically detectable limits.

Any instability in nucleic acids resulting from the exposure of cells to purine or pyrimidine analogues might be manifested as disturbed chromosomal behaviour, *e.g.* an increased frequency of chromosome breaks during the mitotic cycle. In the absence of any reports regarding the effects of allopurinol and oxipurinol on chromosomes, either in the replicating or nonreplicating phases of cell division, the following study was undertaken.

Material and methods

The study was designed to investigate the effect of allopurinol and oxipurinol on human chromosomes. For practical reasons explained in the discussion, the experiments were conducted on peripheral lymphocytes. These cells divide infrequently *in vivo*, but can be stimulated to

enter mitosis in culture by phytohaemagglutinins (PHA). The first experiments (*in vivo* studies) were done on lymphocytes taken from patients treated with allopurinol or oxipurinol, and thus exposed to these drugs *in vivo*. The second series (*in vitro* studies) was conducted on lymphocytes from untreated subjects, stimulated to divide in the presence of allopurinol and/or oxipurinol. A microculture technique, similar to that of Arakaki and Sparkes (1963), was used for both *in vivo* and *in vitro* experiments, in which 0.5 ml whole blood and 1 ml plasma were added to 4 ml Eagle's Minimal Essential Medium with PHA. Colcemid was added 3 hours before harvesting to arrest cells in metaphase for preparing chromosome spreads.

IN VIVO STUDIES

Lymphocytes were cultured from 20 patients treated for hyperuricaemia and gout, 19 of them with allopurinol and one with oxipurinol (because of skin sensitivity to allopurinol). All were responding satisfactorily to therapy. Dosage and duration of treatment were as follows: allopurinol 200–600 mg daily for a mean period of 40 months; oxipurinol 600 mg daily for 55 months. At the time of sampling, sera of all patients concerned in the study showed a peak on chromatograms identifiable as oxipurinol.* After a culture period of 48 hours, metaphase chromosomes were examined for chromatid and isochromatid gaps and breaks.

IN VITRO STUDIES

Lymphocytes were taken from a pool of normal donors. The chromosomal behaviour of these lymphocytes was well established from previous studies. They were cultured in the presence of 10, 40, 80, and 100 µg/ml allopurinol alone; 100 µg/ml allopurinol plus 100 µg/ml oxipurinol; 100 µg/ml allopurinol plus 50 µg/ml amylobarbitone; and 200 µg/ml oxipurinol plus 50 µg/ml amylobarbitone. The barbiturate experiments were included as a follow-up to previous studies on drug-induced modifications of chromosome behaviour, and are of no immediate relevance to the present study (Stevenson, San Roman, and Patel, 1973). Allopurinol and oxipurinol were dissolved in N/15 sodium hydroxide (NaOH) before being added to the cultures. The amount of NaOH needed to dissolve the highest drug concentrations was added to control cultures. Both test and control cultures were corrected to pH 7 by gassing with carbon dioxide. After a culture period of 72 hours metaphase chromosomes were examined for chromatid and isochromatid gaps or breaks.

Results

All results were compared with control values for this laboratory: 73 normal subjects with 7900 cells analysed showed frequencies for chromatid gaps, chromatid breaks, isochromatid gaps, and isochromatid breaks of 0.62%, 0.42%, 0.42% and 0.44%, respectively (see Table III).

* The original method based on high pressure liquid chromatography (HPLC) was capable of qualitative oxipurinol determination only. A more complex HPLC method is currently being developed, and it is hoped to assign quantitative levels of both allopurinol and oxipurinol to these samples in the near future (A. Bye and M. E. Brown, personal communication, 1975).

IN VIVO STUDIES

One hundred metaphase spreads were analysed for each of the 20 patients. No chromosomal aberrations attributable to damage *in vivo* were found. There was a low frequency of chromatid type aberrations attributable to damage in culture, but this was well within the normal limits. The detailed results are set out in Tables I and III.

IN VITRO STUDIES

One hundred metaphase spreads were examined from each culture. The findings showed no damage to chromosomes over and above control values during any stage of the cell cycle in the presence of allopurinol or oxipurinol, with or without the addition of amylobarbitone. The detailed results are set out in Table II.

Discussion

Chemical substances interfering with DNA replication can do so by causing structural damage to intact DNA, by inhibiting the incorporation of normal bases into DNA, or by themselves becoming incorporated in place of normal components. Whatever the mechanism, chromosome aberrations may well result. There is no evidence to suggest that allopurinol or oxipurinol could damage intact DNA (cf. radiomimetic agents such as nitrogen mustards). However, because they are purine analogues, they might in theory inhibit the incorporation of normal

bases (cf. 6-mercaptopurine) or become incorporated as abnormal components (cf. 5-fluorodeoxyuridine). The majority of substances causing chromosomal damage probably do so during the DNA replication phase ('S' phase) of cell division. The best method for investigating such an effect is to allow cells to divide in the presence of the suspected substance(s) and then examine the chromosomes.

For *in vivo* studies, the preferred material would have been bone marrow cells, preferably sampled 12 to 24 hours after a single large dose of drug, since these cells have a rapid turnover (about once every 36 hours). However, sampling of bone marrow involves bone puncture, whereas peripheral lymphocytes are both readily accessible and easy to culture. They were therefore selected on practical grounds. Lymphocytes from peripheral blood exist almost exclusively in an interphase stage peculiar to these cells, in which there is minimum metabolic activity, conventionally termed 'G₀'. It is believed that they only divide *in vivo* about once every 3 years (Norman and others, 1965; Buckton, Smith, and Court Brown, 1967). It follows that, since the mean duration of treatment of the patients tested was about 38 months, most lymphocytes would probably have divided once during the period of treatment. In patients taking their drug regularly, any dividing cells would have been exposed to some level of oxipurinol. The plasma half-life of oxipurinol is 18–30 hours, whereas that of allopurinol is about 1 hour (Elion and others, 1966). Thus with daily administration of either drug, some

Table I Chromosome findings in 48-hour cultures of lymphocytes from 19 patients taking allopurinol and one (Case 13) taking oxipurinol. All patients were male except for Case 18. 100 metaphases were scored for all patients except for Case 16 (only 97 good metaphases seen)

Case no.	Chromatid		Isochromatid		Total damaged cells	Daily dose (mg)	Duration of treatment (m)
	Gaps	Breaks	Gaps	Breaks			
1	0	0	1	2	2	200	20
2	0	0	0	0	0	200	91
3	0	0	0	0	0	400	53
4	0	0	0	0	0	400	41
5	0	0	0	3	3	400	45
6	0	0	0	0	0	600	36
7	0	0	0	0	0	400	17
8	0	0	0	0	0	400	30
9	1	0	1	2	2	400	52
10	1	0	0	1	1	400	41
11	0	0	0	1	1	400	43
12	2	0	0	0	0	400	28
13	0	1	0	0	1	600	55
14	0	0	0	1	1	400	36
15	1	0	0	0	0	400	19
16	0	2	0	0	2	400	24
17	5	0	1	1	1	600	68
18	1	2	2	0	2	300	2
19	0	0	0	0	0	200	57
20	0	2	0	0	2	400	10
Total	11	7	5	11	18		

Table II Chromatid type aberrations in metaphases of lymphocytes from 72-hour cultures. The drugs shown were present throughout culture

Experiment	Drug and concentration ($\mu\text{g/ml}$)	No. of metaphases analysed	Aberrations				No. of cells with any damage (breaks)
			Chromatid		Isochromatid		
			Gaps	Breaks	Gaps	Breaks	
1	Allopurinol						
	10	100	0	0	0	1	1
	40	100	0	1	0	0	1
	80	100	0	0	0	0	0
	100	100	0	0	1	0	0
2	Allopurinol						
	100	100	0	0	0	0	0
	100	100	0	1	0	0	1
	Oxipurinol						
	200	100	0	0	0	0	0
	200	100	1	0	1	0	0
3	Allopurinol	200	1	1	0	0	1
	100 +amylobarbitone 50						
	Oxipurinol	200	0	1	0	0	1
	200 +amylobarbitone 50						

Table III Frequency of chromatid type aberrations in lymphocytes of 19 patients taking allopurinol and one patient taking oxipurinol: comparison with control cells (values expressed as percentages)

	Frequency of aberrations (%)				No. of cells analysed
	Chromatid		Isochromatid		
	Gaps	Breaks	Gaps	Breaks	
Patients' lymphocytes	0.55	0.25	0.25	0.55	2000
Control lymphocytes from 73 subjects*	0.62	0.42	0.42	0.44	7900

* Laboratory reference standard.

oxipurinol would always be present in the plasma. As noted above, allopurinol or oxipurinol would not be expected to damage intact, nondividing DNA, hence chromosomal damage would be unlikely. Such damage would occur during the 'S' phase of cell division and be manifested as chromatid type damage. Unless it were sufficiently severe to cause cell death, most of it would be recognized in the first metaphase after culture. However, small deletions where a break had occurred during the *in vivo* division could well be missed in the first metaphase in culture if the acentric fragment had been absorbed. The 48-hour culture period ensured that the lymphocytes were in their first metaphase after leaving the body. For practical purposes, they can be considered to have divided in the absence of allopurinol or oxipurinol, except for

small amounts of these drugs or their metabolites that might theoretically have been present intracellularly or carried over in the patients' sera. The absence of chromosome aberrations attributable to allopurinol or oxipurinol in these preparations substantiated the concept that neither drug can cause structural damage to intact DNA.

The *in vitro* study was designed to examine the effects of known concentrations of allopurinol and oxipurinol on dividing cells, although all but the lowest concentrations in these cultures were considerably higher than the maximum plasma concentrations likely to be achieved during therapy. Any chromosomal aberrations appearing after PHA-stimulated cell division in culture would reflect effects exerted during DNA replication. The 72-hour

culture period ensured that 70% of the lymphocytes examined were in their second metaphase and 30% in their first metaphase, i.e. they had undergone two 'S' phases and one 'S' phase, respectively. The absence of aberrations in these metaphase preparations indicated that allopurinol and oxipurinol have no deleterious effects on DNA replication.

Conclusions

The evidence from biochemical and cytological investigations strongly suggests that allopurinol and

oxipurinol have no deleterious effects on DNA at any stage of the cell cycle. There appears to be no demonstrable grounds for anxiety regarding the mutagenic effects of allopurinol or oxipurinol.

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