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Sister chromatid exchange induced by anti-herpes drugs

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Abstract

The rate of sister chromatid exchange induced by several anti-herpes agents was measured to assess their potential mutagenicity. The agents—5-iodo-deoxyuridine (IDU), 5-trifluoromethyl-deoxyuridine (TFT), and [E]-5-(2-bromovinyl)-deoxyuridine (BVDU)—were incubated at various concentrations with human lymphocytes and fibroblasts, and the rate of sister chromatid exchanges was measured. In lymphocytes and fibroblasts BVDU and IDU did not induce exchange except at concentrations of 50 mg/l, while TFT increased the rate of exchange at a concentration of 0.5 mg/l.

The rate of sister chromatid exchange is a sensitive index of chromosomal damage, and these findings provide information on the safety of some of the anti-herpes agents tested. TFT increased the rate of exchange at a concentration that coincides with its minimal antiviral concentration, but BVDU did not induce exchange at therapeutic concentrations.

Introduction

Various 5-substituted 2'-deoxyuridines, such as 5-iodo-deoxyuridine (IDU), 5-trifluoromethyl-deoxyuridine (TFT), and [E]-5-(2-bromovinyl)-deoxyuridine (BVDU), are being used or considered for use in the chemotherapy of herpes simplex and

zoster virus infections. There are also several other 5-substituted deoxyuridine derivatives, such as 5-vinyl-deoxyuridine (VDU), which show significant anti-herpes activity, but these compounds do not appear to be sufficiently selective in their antiviral action. From comparative studies of the potency and cytotoxicity of these compounds in cell culture, BVDU has emerged as the most potent and most selective anti-herpes agent.¹

Since IDU, TFT, and BVDU are all thymidine analogues, they might act as mutagens and hence induce permanent changes in the cellular genome. Sister chromatid exchange is a sensitive indicator of mutagenesis.² We therefore examined IDU, TFT, and BVDU for their capacity to induce sister chromatid exchange in human lymphocytes and fibroblasts.

Methods

Sister chromatid exchange was scored as described previously.³ IDU was from Ludeco (Brussels) and TFT from Sigma Chemical Co, and BVDU was synthesised as described.¹ VDU, 5-vinyluridine, and 5-vinyluracil were included as reference materials.¹

The nucleoside analogues were incubated for 48 hours with human (peripheral blood) lymphocytes and 72 hours with human (fetal lung) fibroblasts at concentrations ranging from 0.05 to 50 mg/l in the presence of 3 mg/l bromo-deoxyuridine. Ethylmethane sulphonate (Sigma Chemical Co) served as a positive control: it was added at 60 mg/l, a concentration known to induce a significant increase in the number of sister chromatid exchanges.³

Results

In lymphocytes BVDU and IDU did not increase the number of sister chromatid exchanges until the concentration was raised to 50 mg/l (see table). TFT, however, caused a significant increase in the exchange rate at a concentration of 0.5 mg/l; and at 5 µg/ml it proved even more effective in inducing exchange than the standard mutagen ethylmethane sulphonate.

While not very effective in lymphocytes, VDU turned out to be an exquisitely potent inducer of exchange in fibroblasts, where it caused a significant increase in exchange frequency at concentrations of 0.5 and 5 mg/l, while BVDU and IDU failed to do so. BVDU and IDU increased the frequency of exchange in fibroblasts only at a concentration of 50 mg/l.

The level of significance was assessed by Student's *t* test, and, since the individual values were not distributed normally, significance was also monitored by the Poisson distribution.

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Induction of sister chromatid exchange in human lymphocytes and fibroblasts

Concentration (mg/l)	Lymphocytes						Fibroblasts					
	BVDU	IDU	TFT	VDU	5-vinyl-uridine	5-vinyl-uracil	BVDU	IDU	TFT	VDU	5-vinyl-uridine	5-vinyl-uracil
0	8.5	6.5	7.04	6.75	6.35	7.6	5.45	6.7	5.75	5.55	6.25	6.90
0.05	NT	NT	9.55	6.45	NT	NT	NT	NT	6.35	9.25†	NT	NT
0.5	7.4	8.3	9.85§	8.25	7.1	5.6	7.4	6.2	8.0†	22.7*†	4.95	5.15
5	6.95	7.3	21.75*†	9.4†	6.1	6.65	7.45	7.8	12.1†	39.25*†	5.75	6.55
50	12.45†	14.33*†	NT	NT	8.7	6.90	12.55*†	10.75	NT	NT	6.55	8.25
0 + Ethylmethane sulphate (60 mg/l)	16.6*†	15.75*†	18.4*†	17.80*†	16.45*†	15.75*†	13.25*†	14.65*†	12.85*†	13.75*†	16.75*†	15.3*†

At least 20 metaphases were examined in each case. In lymphocytes only four metaphases were scored for 50 mg/l IDU because of the presence of most cells in third mitosis. In fibroblasts only four metaphases were scored for 50 mg/l IDU and 5 mg/l VDU because of cellular toxicity of the compounds at these concentrations.

NT = Not tested.

*Significant at 0.05 level of Poisson distribution.

†Significant at †0.001, †0.01, or §0.05 level of Student's *t* test.

Discussion

The effects of the deoxyuridine derivatives in inducing sister chromatid exchange may be considered specific, since the free base (5-vinyluracil) and ribosyl counterpart (5-vinyluridine) of the most effective inducer (VDU, in fibroblasts) did not affect the rate of exchange in either lymphocytes or fibroblasts. These findings also indicate that 5-vinyluracil and 5-vinyluridine are not converted to VDU in these cells.

The efficiency of VDU as an inducer of sister chromatid exchange may be linked to its ability to be incorporated into DNA. The presence of 5-vinyluracil residues in DNA affects the γ -radiation sensitivity of phage DNA molecules,⁴ and a similar situation may occur with eukaryotic DNA. When incorporated into DNA 5-vinyluracil may participate in cross-linking reactions, as has been postulated previously for 5-hydroxymethyluracil, and these cross links may be directly responsible for the phenomenon of sister chromatid exchange. In keeping with this hypothesis is the observation that mitomycin C, being the prototype of the cross-linking agents, is also an excellent inducer of chromatid exchange.²

Our results cast some doubt on the selectivity and safety of TFT as an antiviral drug, since this compound increased the rate of sister chromatid exchange at a concentration (0.5 mg/l) that coincides with its minimal antiviral concentration.¹ This is particularly important since TFT is now generally available (as a 1% ophthalmic solution) for the topical treatment of herpetic keratitis and may supersede IDU for that purpose. In marked contrast to TFT, BVDU did not significantly alter the rate of

sister chromatid exchange until it was used at a concentration of 50 mg/l, which is approximately 5000 times higher than its minimal antiviral concentration¹ and 50 times higher than the blood drug concentrations achieved after oral administration at the recommended dose of 7.5 mg/kg/day.⁵ Since the rate of sister chromatid exchange represents a very sensitive index of chromosomal damage, it may seem wise to limit the clinical use of anti-herpes agents to doses that do not induce such exchange.

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OF MELANCHOLY.

MELANCHOLY is that state of alienation or weakness of mind which renders people incapable of enjoying the pleasures, or performing the duties of life. It is a degree of infamy, and often terminates in absolute madness.

CAUSES.—It may proceed from a hereditary disposition; intense thinking, especially where the mind is long occupied about one object; violent passions or affections of the mind, as love, fear, joy, grief, over-weening pride, and such like. It may also be occasioned by excessive venery; narcotic or stupefactive poisons; a sedentary life; solitude; the suppression of customary evacuations; acute fevers, or other diseases. Violent anger will change melancholy into madness; and excessive cold, especially of the lower extremities, will force the blood into the brain, and produce all the symptoms of madness. It may likewise proceed from the use of aliment that is hard of digestion, or which cannot be easily assimilated; from a callous state of the integuments of the brain, or a dryness of the brain itself. To all which we may add gloomy or mistaken notions of religion.

SYMPTOMS.—When persons begin to be melancholy, they

are timorous; watchful; fond of solitude; fretful; fickle; captious and inquisitive; solicitous about trifles; sometimes niggarly, and at other times prodigal. The body is generally bound; the urine thin, and in small quantity; the stomach and bowels inflated with wind; the complexion pale; the pulse slow and weak. The functions of the mind are also greatly perverted, in so much that the patient often imagines himself dead, or changed into some other animal. Some have imagined their bodies were made of glass, or other brittle substances, and were afraid to move, lest they should be broken to pieces. The unhappy patient, in this case, unless carefully watched, is apt to put an end to his own miserable life.

WHEN the disease is owing to an obstruction of customary evacuations, or any bodily disorder, it is easier cured than when it proceeds from affections of the mind, or an hereditary taint. A discharge of blood from the nose, loathings, scabby eruptions, the bleeding piles, or the *menfes*, sometimes carry off this disease.

(Buchan's *Domestic Medicine*, 1786.)