DIFFERENT EFFECTS OF BUDR ON THE INDUCTION OF CHROMATID ABERRATIONS BY MEANS OF TEM AND MALEIC HYDRAZIDE

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Received September 17, 1963

Replacement of thymidine by incorporation of 5-bromodeoxyuridine (BUDR) into DNA has been shown to enhance the radiation sensivity of phages (Kozinsky and Szybalski 1959; Stahl et al. 1961), bacteria (Lorkiewicz and Szybalski 1960; Kaplan et al. 1961, 1962), mammalian cell lines (Djordjevic and Szybalski 1960; Somers and Humphrey 1963; Humphrey et al. 1963) and higher plants (Kihlman 1962). According to Szybalski (1962) the most plausible explanation for the increased radiation sensitivity is a labilization of the phosphat ester bonds between the 5-bromodeoxyuridylic acid moiety and the adjoining nucleotide on the same DNA strand, making the phosphat ester bond more sensitive to breakage by irradiation.

Experimental: The experimental material was main roots of Vicia faba var. minuta (Dornburger Ackerbohne) which have been pretreated with various concentrations of BUDR as stated in the tables at temperatures of 24° C [+]. Following pretreatment the root tip meristems were treated with the alkylating agent triethylenemelamine (TEM) or with maleic hydrazide (MAL) in both cases for 30 min. at 24° C. All agents were solved in destilled water. After various recovery times in tap water (24° C) the roots were treated with colchicine (0.05 %, 2 h) before fixation (ethyl alcohol acetic acid 3 : 1). Slides were prepared according to the Feulgen-squash technique and metaphases analyzed for induced chromatid aberrations both in the pretreatment series and in the controls

[+] We are greatly indepted to Dr. W. Szybalski for a stimulating discussion of our results.

with either TEM or MAL. Both TEM and MAL are inducing aberrations in interphase nuclei which are exclusively on chromatid basis (Ockey 1957; McLeish 1953). The aberration types scored were: Isolocus breaks (B"), chromatid translocations (T'), triradials (Tri), duplication-deficiencies (DD) and deletions (D)(for a more detailed description of these types see Rieger and Michaelis 1962).

Results: Although BUDR has been shown by Hsu and Somers (1961) to induce chromosomal aberrations in mammalian cell strains the concentrations and treatment times of BUDR used in our experiments did not produce any aberrations in Vicia faba. This is in full accordance with Kihlman (1962). As shown in table 1 pretreatment of Vicia main root meristems with 10^{-4}M BUDR for 24 h is clearly sensitizing against the radiomimetic effectivity of TEM. The percentage of metaphases with chromatid aberrations is significantly higher after BUDR pretreatment for all recovery times tested. The aberration peak is found after 24 hrs. recovery both in the control and in the pretreatment series.

Table 1 . Effects of BUDR pretreatment on the production of chromatid aberrations by means of triethylenemelamine (TEM). 400 metaphases analysed for each fixation.

Treatment	Recovery time	Metaphases with Aberrations %	Aberration types +) B"	T'	Tri	DD	% D
0.5 h 10^{-4}M TEM	18	24.5	13.5	10.5	1.0	0.5	0.5
(control)	24	28.2	17.5	12.2		0.5	1.2
	30	19.5	10.5	6.2	0.2	2.0	1.2
	36	5.7	3.5	2.2		0.2	
24 h 10^{-4}M BUDR	18	40.0	29.0	11.5		4.0	2.5
0.5 h 10^{-4}M TEM	24	51.5	28.5	22.5	1.0	4.2	1.0
	30	31.5	14.7	14.0	2.2	1.7	1.0
	36	11.2	6.5	4.2	0.5		0.7
24 h 10^{-4}M BUDR + 10^{-8}M AMET + 10^{-6}M HYP	18	15.5	10.0	4.0		1.5	0.5
	24	29.0	16.5	14.5	0.5	2.5	0.5
0.5 h 10^{-4}M TEM	30	21.5	11.5	9.0	1.0	1.5	
	36	7.0	4.5	2.0		1.0	

+) B" = Isolocusbreaks; T' = chromatid translocations; Tri = Triradials; DD = Duplication deficiencies; D = Deletions.

Since selective inhibition of de novo synthesis of thymidylic acid is expected to result in a more extensive incorporation of BUDR instead of thymidine into DNA (Hakala 1959; Szybalski 1962) we used the metabolic antagonist amethopterin (10^{-8}M, AMET) binding folic acid reductase.

This compound was given together with BUDR and hypoxanthine (10^{-6}M, HYP) for 24 hrs. The last mentioned compound should be able to compensate the concomitant inhibition of purine synthesis provoked by amethopterin. The rationale of this experiment was the hope to find a still higher sensitization-effect against TEM (10^{-4}M, 30 min.) . In contrast to expectation the experimental results show that combined treatment with BUDR, AMET and HYP gives no longer sensitization and for this AMET alone seems to be responsible (table 2). The effect of BUDR is clearly concentration-dependent. Maximal sensitization was found with 10^{-4}M, the effect decreased with 10^{-5}M and 10^{-6}M did not sensitize any longer with treatment times of 24 h.

Based on the fact that DNA is double stranded and replicating semiconservatively we tried to test possible effects of "unifilar" and "bifilar" (Szybalski 1961) incorporation of BUDR. With a mitotic cycle lasting 22.9 hrs. at 25° C in Vicia faba (Evans and Savage 1959) pretreatment with BUDR for 48 h should enable a certain fraction of the meristem cells to incorporate the halogenated thymidine analogue for two rounds of DNA replication. In this case TEM concentrations of 5×10^{-5}M and BUDR concentrations of 5×10^{-5}M as well as 10^{-4}M have been used (table 2). Pretreatment of meristems with these BUDR concentrations for 24 h is sensitizing against TEM, the amount of sensitization being higher with 10^{-4}M BUDR. When the pretreatment with 5×10^{-5}M BUDR was prolonged to 48 h (change of solution after 24 h) the percentage of metaphases with aberrations is increased from 20.0 to 34.0 whereas 28 % damaged cells have been scored after 24 h pretreatment with the double BUDR concentration of 10^{-4}M. Although the effect is not drastic incorporation of BUDR "bifilarly" seems to have some influence on the sensitivity of the meristem cell population against TEM.

Our experiments with BUDR and TEM in Vicia root tip meristems show a significant sensitization against the effect of the alkylating agent TEM which seems to be in good accordance with the results in case of irradiation. It is tempting to interprete this effect in terms of BUDR incorporation into DNA of chromosomes. The finding that amethopterin fully compensated the sensitizing effect of BUDR is somewhat unexpected

Table 2 . Effects of different BUDR concentrations and treatment times on the production of chromatid aberrations by means of TEM. (400 metaphases analysed for each fixation; recovery time 24 hrs.).

Treatment	Metaphases with Aberrations %	Aberration types %				
		B"	T'	Tri	DD	D
0.5 h 10^{-4}M TEM (control)	32.5	16.5	13.0	1.5	3.5	1.5
24 h 10^{-4}M BUDR						
0.5 h 10^{-4}M TEM	62.5	37.0	29.0	1.5	6.5	3.0
24 h 10^{-5}M BUDR						
0.5 h 10^{-4}M TEM	47.0	26.5	21.0	1.0	2.5	3.0
24 h 10^{-6}M BUDR						
0.5 h 10^{-4}M TEM 10^{-8}M AMET +	34.5	23.0	9.5	1.5	2.5	2.0
24 h 10^{-4}M BUDR						
0.5 h 10^{-4}M TEM 10^{-6}M HYP +	29.0	20.5	7.0		2.5	3.5
24 h 10^{-4}M BUDR						
0.5 h 10^{-4}M TEM	54.0	36.0	18.5	2.0	3.5	2.0
0.5 h 5 x 10^{-5}M TEM (control)	14.5	10.0	4.0	0.5		
24 h 5 x 10^{-5}M BUDR						
0.5 h 5 x 10^{-5}M TEM	20.0	14.0	4.5	0.5	1.0	0.5
24 h 10^{-4}M BUDR						
0.5 h 5 x 10^{-5}M TEM	28.0	16.5	10.0		1.5	0.5
24 h 5 x 10^{-5}M BUDR						
24 h 5 x 10^{-5}M BUDR						
0.5 h 5 x 10^{-5}M TEM	34.0	16.5	12.0	1.0	3.0	2.5

and needs further investigation. We are just about to test quantitatively whether BUDR is incorporated into DNA in the presence of amethopterin or not.

When maleic hydrazide (MAL; 5 x 10^{-4}M, 30 min.) was substituted for TEM it was impossible to increase its effectivity in the production of chromatid aberrations by means of BUDR pretreatment. Table 3 summarizes the results of one experiment (24 hrs. recovery

time). Other experiments with different recovery times gave identical results and it seems fairly clear that there is no sensitization effect. This finding points into the same direction as some other experimental results (no interaction of chromatid breaks induced by MAL and TEM (Michaelis and Rieger 1963), different times during which breaks induced by MAL and TEM are staying open (Rieger and Michaelis 1963)) giving evidence for different modes of action of both substances.

Table 3 . The induction of chromatid aberrations by maleic hydrazide with and without pretreatment by BUDR (400 metaphases analysed for each fixation, recovery time 24 hrs.)

Treatment	Metaphases with Aberrations %	Types of aberrations %				
		B"	T'	Tri	DD	D
0.5 h 5 x 10^{-4}M MAL	10.25	6.7	3.5	0.2	0.2	
24 h 10^{-4}M BUDR						
0.5 h 5 x 10^{-4}M MAL	10.25	5.2	4.2		0.7	0.5

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