SENSITIZATION EFFECTS OF ACRIDINE ORANGE ON THE RADIOMIMETIC ACTIVITY OF NITROGEN MUSTARD

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Acridine orange (2,8-bis-dimethylamino-5-phenylacridine = AO) or other acridine dyes have been shown to induce mutations in viruses (Freese 1959, Brenner et al. 1958, 1961), bacteria (Witkin 1947), Drosophila (Demerec 1949) and to convert F^+ clones of E. coli into stable F^- clones (Hirota 1960). AO induced structural chromosomal changes were reported after treatment of Allium cepa- (D'Amato 1950; Nuti and D'Amato 1961; Nuti 1961) and Vicia faba roots (Kihlman 1959a, b; 1961a, b). With Allium AO proved effective both in the dark and with illumination. In Vicia faba chromosomal aberrations were only found after AO-treatment in combination with oxygen, NO, sodium nitrite, diphenylnitrosamine or N-methylphenylnitrosamine and light but not in the dark. According to Kihlman (1961a) probably oxygen and NO react with radicals produced in the cell by the radiation. Complex salts are formed with AO by nucleotides, nucleic acid and polyadenylic acid (Morthland et al. 1954; Beers et al. 1958; Leerman 1961; Luzatti et al. 1961) and nucleic acid containing cell particles are stained by AO.

The starting point of the following experiments was the finding of Read (1961) that AO-pretreatment counteracts the growth retardation of Vicia faba-roots induced by triethylene-

melamine (TEM) although our results differ from those of Read and are in fact not comparable. Instead of TEM we used nitrogen mustard (di-(2-chloroethyl)methylamine, HN2) and studied the effect of AO-pretreatment of Vicia faba primary roots on the radiomimetic effectivity of HN2. Duration of pretreatment with AO at 24°C in the dark was 1, 3, 4 and 20 hours with various concentrations as stated in the table. 50 metaphases per slide were scored and analyzed for chromosome aberrations. The preparation technique was as described by Michaelis and Rieger (1961). Two or three repeat experiments were done with identical results and therefore the repetitions have been combined in the table.

All aberrations induced by treatment of primary roots with 10^{-6}M nitrogen mustard for 30 min. were of the chromatid type and preferentially localized in heterochromatin. Isolocus-breaks, chromatid translocations, triradials, duplication-deletions and deletions have been used for the analysis. In conformity with Kihlman (l.c.) AO alone proved unable to induce aberrations without illumination in V. faba-roots.

Pretreatment with 5×10^{-5}, 5×10^{-6}, 5×10^{-7}M AO for 1 h was without effect on the radiomimetic activity of nitrogen mustard but with 3 and 4 h pretreatment the highest AO-concentrations used - 5×10^{-5} and 10^{-6}M resp. - increased the number of metaphases with structural chromosomal changes and the number of aberrations per damaged cell significantly in comparison with the controls (nitrogen mustard 10^{-6}M, 30 min. without AO-pretreatment). As shown by the standard error of the mean (table) the variability between the slides was relatively high when AO-pretreatment times were short. This was much less so with 20 h pretreatment. In this case a sensitization against. HN2 is evident from the table over the whole range of tested

The effect of AO-pretreatment in the dark (a = 3 h, b = 4 h, c = 20 h) on the radiomimetic activity of nitrogen mustard (30 min., 10^{-6}M, p_H 7, 24°C) with 24 h recovery time

	No. of cells scored	Concentration of AO in M	Metaphase with aberrations abs.	%	Number of aberrations	Aberrations per damaged cell
a)	400	-	84	21,0 ± 2,0+)	91	1,08
	400	5×10^{-7}	98	24,5 ± 4,8	117	1,19
	400	5×10^{-6}	88	22,0 ± 5,0	100	1,14
	400	5×10^{-5}	215	53,8 ± 6,5	303	1,41
b)	400	-	77	19,3 ± 1,1	82	1,06
	400	5×10^{-7}	67	16,8 ± 1,0	68	1,01
	400	5×10^{-6}	231	57,8 ± 4,9	279	1,21
c)	600	-	100	16,7 ± 1,1	104	1,04
	200	5×10^{-9}	111	55,5 ± 8,1	137	1,23
	600	5×10^{-8}	406	67,7 ± 3,4	562	1,38
	600	5×10^{-7}	390	65,0 ± 3,9	514	1,32
	600	5×10^{-6}	368	61,3 ± 3,3	499	1,36

+)standard error of the mean

AO-concentrations down to 5×10^{-9}M AO. The number of cells with aberrations was approximately three times higher than in the controls and the number of aberrations per damaged cell was increased too. Methylene blue which is structurally analogous to AO proved unable to sensitize against HN2. Ethyl alcohol used instead of HN2 for the induction of chromatid aberrations in Vicia faba (Rieger and Michaelis 1960a, 1962) was not potentiated in its activity by AO-pretreatment, showing once more differences

in the mode of action of these agents (Rieger and Michaelis 1960b, 1962).

Lerman (1961) has suggested that AO-molecules become reversibly intercalated between adjacent nucleotides of the DNA-helix causing a backbone extension and an increase in stiffness. The experiments on AO-sensitization against HN2 in Vicia faba may be of some interest in connection with these findings because one might think of a causal relationship between sensitization against HN2 and incorporation of AO into DNA of the chromosomes although definite experimental evidence on a mechanism of this kind is still lacking. Further work in this direction is being done now and a full account of this will be given elsewhere.

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