RADIOMIMETIC EFFECTS OF 1-METHYL-3-NITRO-1-NITROSOGUANIDINE IN VICIA FABA

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Kihlman (1957, 1959, 1961) has tested various nitrosamines for their ability to induce chromatid aberrations in Vicia meristems; from these cupferron (ammonium salt of N-nitroso-phenylhydroxylamine), N-methylphenylnitrosamine (MPNA) and N-nitroso-N-methylurethan (NMU) were found to have radiomimetic effectivity. We treated main root meristems of Vicia faba (for the methods used and the aberration types scored see Michaelis and Rieger (1961), Rieger et al. (1963)) with 1-methyl-3-nitro-1-nitrosoguanidine (NG), which was reported to be mutagenic in E.coli strain S (Mandell and Greenberg 1960) and to have anticancer activity in mice (Goldin et al. 1959; Green and Greenberg 1960; Skinner et al. 1960). According to Mandell and Greenberg in their experiments the NG-structure and no breakdown product of it (nitrous acid under acid conditions, diazomethan under alkaline conditions) was the effective mutagenic agent, the effect of which could be reversed by sulfhydryl compounds (Mandell et al. 1961).

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In our experiments the substance proved to be rather toxic under acid conditions (p_H 4.2 : aqua dest.; p_H 5.5 : citrate buffer) but not under alkaline conditions (p_H 8.9 : boric acid buffer). The same p_H-dependence hold true for the range of those concentrations inducing chromosomal aberrations (induction of aberrations under acid conditions, no aberrations with p_H 8.9). All aberrations induced were of the chromatid type only and preferentially localized in heterochromatin. Usually the aberration peak was obtained between 24 and 48 h after NG treatments, thus classifying the agent into the group of those with delayed effects. It seems unnecessary to summarize all the concentrations of NG and the various treatment times tested in a table, since most were either without aberrations or toxic. With the concentrations of NG showing radiomimetic effects the percentage of metaphases with chromatid aberrations was found to be rather low, generally not higher than 20 %. Using this highest aberration percentage we have tested the influence of pretreatment with 2,4-dinitrophenol (DNP) and sodium azide as well as simultaneous treatment with NG and nitrogen (p_H 4.2). As shown in the table, pretreatment with DNP was without influence on the radiomimetic effectivity of NG, but the percentage of metaphases with induced aberrations was significantly decreased by means of NaN_3 and N_2. EDTA had no influence on the effects of NG. This is in contrast with similar experiments in which triethylenemelamine and ethyl alcohol were used for the induction of aberrations (Michaelis et al. 1962; Rieger et al. 1963). The percentage of NG-induced aberrations was found to be dependent on the temperature of the treatment solution insofar as the effectivity of the agent increased from 18 to 24°C and decreased with higher temperatures (30°C).

Influences of metabolic inhibitors and ethylendiaminetetraacetic acid on the radiomimetic activity of 1-methyl-3-nitro-1-nitrosoguanidine (400 metaphases scored in each case)

Pretreatment (time, concentration, agent)	Treatment	Recovery time h	metaphases with aberrations %
	1h 5×10^{-4}M NG	24	without divisions
		48	19.0 ± 0.8
		60	13.0 ± 2.6
2h 10^{-5}M DNP	1h 5×10^{-4}M NG	48	16.5 ± 1.0
		60	15.5 ± 0.7
2h 5×10^{-4}M NaN_3	1h 5×10^{-4}M NG	48	5.5 ± 0.7
		60	5.5 ± 1.0
	24h 6×10^{-5}M NG	24	19.0 ± 3.5
		48	3.0 ± 2.0
	N_2 + 24h 6×10^{-5}M NG	24	5.75 ± 1.1
		48	5.0 ± 2.6
20h 10^{-4}M EDTA	1h 5×10^{-4}M NG	48	14.8 ± 2.9
1h 10^{-3}M EDTA	+ 5×10^{-4}M NG	48	17.75 ± 1.1
24h 10^{-5}M EDTA	+ 6×10^{-4}M NG	24	18.0 ± 2.2

Kihlman (l.c.) has brought together some evidence suggesting that NMU might become converted into the alkylating agent diazomethane whereas the reactive species being responsible for the induction of aberrations by both cupferron and MPNA are suspected to be organic peroxides and/or free radicals being produced by decomposition of these agents via enzyme-catalized oxidation. This is inferred from the influences of inhibitors and anaerobiosis on the aberration production by these agents. NMU was found to be uninfluenced by oxygen being present or

not. The induction of aberrations by means of MPNA was drastically decreased by sodium azide and cupferron and unaffected by the uncoupling agent DNP. The effect of MPNA was in contrast to cupferron independent of p_H and temperature both having been almost inactive in the absence of oxygen.

Although on a theoretical basis a similar behaviour of NG and NMU might have been expected, the experimental evidence is on the contrary. Our results are in substantial agreement with those of Kihlman for MPNA as far as the effects of inhibitors and nitrogen are concerned. The influences of p_H on NG may easily be explained by the instability of the agent above p_H 7.0 and the temperature effect may have the same base. Thus a similar mode of action for NG, MPNA and cupferron might be suggested although clearcut evidence on the nature of the agent inducing the aberrations is still lacking. In fact the mode of action of cupferron, MPNA and NG is different from that of NMU, the behaviour of which seems to be quite typical of alkylating agents with radiomimetic activity (see Kihlman 1961 b; Rieger and Michaelis 1962). Although NG might become converted into diazomethane like NMU, a decomposition of this type does not seem to be engaged in aberration production by NG under the experimental conditions used.

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