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BRIEF COMMUNICATION

Effect of dimethylsulfoxide on methylmethanesulfonate-induced chromosomal aberrations in *Crepis capillaris* cultivated *in vitro*

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Abstract

Methylmethanesulfonate induced chromosomal aberrations in callus culture of *Crepis capillaris*. The clastogenic effect was markedly decreased when calli were pretreated with dimethylsulfoxide.

Crepis capillaris is a suitable object for cytogenetic studies. The diploid cells have only three pairs of large and well distinguishable chromosomes usually marked as A, C and D group. This plant species was used as a model organism to study the ability of MMS to induce chromosomal damages and the ability of DMSO to influence clastogenic effects of MMS under conditions *in vitro*.

The callus cultures derived from diploid *Crepis capillaris* were used. The leaves were surface sterilized in 2.5 % Chloramin B (30 min), then washed three times in sterile water, placed on a modified Murashige-Skoog medium (Brossard 1977, 1979), and cultured at 25 ± 2 °C in the dark.

The medium was replaced each 3 - 4 weeks. The calli were used after 3 - 4 passages. They were shaken in a liquid medium and small pieces (*ca.* 0.5 cm in diameter) were treated with MMS dissolved in a liquid medium without saccharose at final concentrations 0, 10^{-4}, 10^{-3}, 10^{-2} and 5×10^{-2} M. Similarly, DMSO was applied at concentrations 0, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} M. The squash preparations were made immediatelly after MMS or DMSO treatment and stained with acetoorcein; colchicine was not used.

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Abbreviations: MMS - methylmethanesulfonate, DMSO - dimethylsulfoxide.
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In order to study an ability of DMSO to influence the clastogenic effect of MMS, three variants of experiments were conducted: (*1*) small pieces of calli were pretreated with DMSO (0, 10^{-4}, 10^{-3} and 10^{-2} M; 12 h) before MMS application (10^{-2} M; 12 h). After DMSO pretreatment fresh medium was used. (*2*) small pieces of calli were treated with DMSO (0, 10^{-4}, 10^{-3} and 10^{-2} M; 12 h) and MMS (10^{-2} M; 12 h) together at the same time. (*3*) small pieces of calli were treated with MMS (10^{-2} M; 12 h) and then posttreated with DMSO (0, 10^{-4}, 10^{-3}, 10^{-2} M; 12 h). After MMS treatment fresh medium was used.

In order to determine the duration of the antimutagenic effect of DMSO, small pieces of calli were treated with DMSO (0, 10^{-4} and 10^{-3} M; 12 h), then the medium was replaced and MMS (10^{-2} M; 12 h) was added 0, 12, 24, 36, 48 and 60 h after DMSO treatments. Squash preparations were made as above. Fifty metaphases were scored *per* treatment.

MMS induced chromosomal aberrations when it had been applied at concentrations higher or equal to 10^{-3} M. However, a treatment with MMS at concentration 5×10^{-2} M led to a strong mitotic inhibition and clustering of metaphasic chromosomes; thus a cytogenetic analysis became very difficult.

Both, chromosome and chromatid types of chromosomal aberrations were found but the chromatid ones occurred more often. The most frequent were chromatid breaks and isochromatid breaks. The other types of chromosomal aberrations (such as interstitial deletions, ring chromosomes, dicentric chromosomes or chromatid exchanges) were found sporadically. These findings support the assumption that monofunctional alkylating agents act mainly during S-phase of cell cycle.

Table 1. Frequency of aberrant metaphases [%] and chromosomal aberrations (*per* cell) in experiments with DMSO and MMS in callus culture of *Crepis capillaris*.

DMSO [M]	MMS [M]	DMSO before MMS		DMSO + MMS together		DMSO after MMS	
		ab. met.	chr. ab.	ab. met.	chr. ab.	ab. met.	chr. ab.
-	-	2.00	0.02	0.00	0.00	2.00	0.02
10^{-4}	-	0.00	0.00	2.00	0.02	0.00	0.00
10^{-3}	-	2.00	0.02	0.00	0.00	2.00	0.04
10^{-2}	-	6.00	0.06	6.00	0.06	6.00	0.06
-	10^{-2}	34.00	0.38	32.00	0.38	36.00	0.38
10^{-4}	10^{-2}	4.00	0.04	30.00	0.34	36.00	0.40
10^{-3}	10^{-2}	4.00	0.04	30.00	0.30	34.00	0.38
10^{-2}	10^{-2}	10.00	0.12	26.00	0.28	32.00	0.40

The induced chromosomal aberrations were distributed among the chromosomes non-randomly. About 50 % from a total number of MMS-induced chromosomal breaks was located in chromosome D. Difference in chromosomal aberrations between chromosomes A and C was not significant (χ^2-test, $P < 0.05$). A non-random distribution of chromosomal aberrations would be related to a different number of heterochromatin regions (Dimitrov 1973), because of presumption that chromosomal breaks are located predominantly in heterochromatin (Schubert *et al.* 1979).

After DMSO treatment at concentrations 10^{-2} and 10^{-1} M the increased frequencies of aberrant metaphases (6 and 12 % resp.) and chromosomal aberrations (0.06 and 0.14 *per* metaphasis) were found. The frequencies of aberrant metaphases and chromosomal aberrations did not differ as compared with control after DMSO-treatment at lower concentrations (Table 1).

Table 2. Time-effect of DMSO pretreatment on the frequency of MMS-induced chromosomal aberrations (*per* callus) in *Crepis capillaris*.

DMSO [M]	MMS [M]	Time intervals between DMSO and MMS treatments [h] 0	12	24	36	48	60
-				0.00-0.02	0.00-0.02		
10^{-4}				0.00-0.02	0.00-0.02		
10^{-3}				0.00-0.02	0.00-0.02		
-	10^{-24}			0.36-0.40	0.36-0.40		
10^{-4}	10^{-2}	0.06	0.14	0.18	0.34	0.36	0.40
10^{-3}	10^{-2}	0.04	0.20	0.24	0.40	0.40	0.38

When calli had been pretreated with DMSO before MMS, the frequency of aberrant metaphases and chromosomal aberrations progresivelly decreased, and at DMSO concentrations 10^{-4} and 10^{-3} approached control. When DMSO had been applied with MMS at the same time, the antimutagenetic effect of DMSO was low, and when DMSO had been applied after MMS treatment, then effect of DMSO was not evident (Table 1). The antimutagenic effect of DMSO (in used concentrations) manifested not longer than 24 h between DMSO and MMS treatment (Table 2). It suggests that only pretreatment of DMSO is sufficiently effective. Our results could be explained on the basis of different rate of penetration of these compounds (MMS, DMSO) into cells (Gabbay *et al.* 1970). Hypothetically, a competition between DMSO and MMS as regards reaction sites in the cell should be also taken in account.

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