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Inhibition of methyl methanesulfonate-induced unscheduled DNA synthesis in spermatozoa of mice by Poly I-C

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ABSTRACT Methyl methanesulfonate (MMS 75 mg · kg^{-1})-induced unscheduled DNA synthesis (UDS) was inhibited significantly in the spermatozoa of mice injected hypodermically with Poly I-C (0.05, 0.50, and 5.00 mg · kg^{-1}) at 4 h prior to ip MMS. Radioactivity was reduced from 53 ± 3 to 50, 45 ± 5, and 34 ± 6 dpm / million sperms respectively. The effects of Poly I-C were dose-dependent ($r = 0.9498$, $P < 0.05$) in inhibition of MMS-induced UDS. The effect of serum collected from the mice injected with Poly I-C (0.50 mg · kg^{-1}) had similar effects as that of Poly I-C. Our findings suggest that both Poly I-C alone and mouse serum induced with Poly I-C may prevent the DNA damage produced by MMS.

KEY WORDS Poly I-C; methyl methanesulfonate; spermatozoa; DNA repair; inbred C57BL mice

Poly I-C, a polymer of polyinosinic polycytidylic acid, is an effective inducer for interferons[1]. It also caused strong inhibition on growth of some rodent tumors[2]. The combination of Poly I-C and indomethacin led to the greater inhibition of carcinogen-induced bladder cancer in rats[3]. It is conjectured that Poly I-C may protect DNA from damage caused by mutagens. But no related information has been available up to now. The research reported here was carried out to determine the effect of Poly I-C on methyl methanesulfonate (MMS, an alkylator which can damage DNA)-induced UDS in the spermatozoa of mice. The purpose was to investigate the protective activity of Poly I-C against DNA damage.

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MATERIALS AND METHODS

Nine wk old C57BL / 6J ♂ mice (20.9 ± *s* 1.1 g) were used. Normal saline was used as the negative control. MMS (Sigma Co, USA) 75 mg · kg^{-1} was ip as positive control group. Poly I-C (Tianjin Pharmaceutical Factory for Biochemical Medicine) was dissolved in saline. The Poly I-C was injected to the mice at the same time with MMS or at 4, 12, and 24 h before ip MMS.

At 4 h after sc Poly I-C (0.50 mg · kg^{-1}), serum 0.1 ml / mouse was taken and was injected to other mice at 1 h before MMS injection.

The experiment was done according to improved Sega's method[5]. Under general anesthesia with phenobarbital, a small incision was made in the scrotum to expose the testes. The micro-syringe needle was inserted along the longitudinal axis of the testis to its approximate center and 20 μl of [^{3}H]TdR (specific activity 1110 TBq · mol^{-1}, purchased from the Shanghai Atomic Nuclear Institute) was injected. The MMS was injected ip. No suture was done for the incisions but they healed completely within 4-5 d.

All the mice were killed on d 17 after injecting [^{3}H]TdR. Sperms taken from the caudal epididymis were suspended in saline. The suspended sperm samples were centrifuged for 10 min at 1 000 × *g*. The supernatant was removed and the residue (0.3 ml) was resuspended. Sperms per 0.1 ml of the suspension were counted with a hemacytometer. Under continuous negative pressure

(13.2–26.4 kPa), each 0.1 ml of the suspension was dropped onto glass fibre filter paper (Type 49, diameter 20 mm), which was then washed with 5 ml of saline, trichloroacetic acid, and ethanol in succession. The sperm samples of each mouse were collected on 2 filters. The radioactivities of the samples were measured with a liquid scintillation counter (LKB WALLAC 1211, which efficiency is 50 %). Each sample was counted for 1 min. Radioactivity was calculated according to the formula: (dpm of sample − dpm of background) / sperm count. The background was 16 ± 2 dpm. Data were shown as dpm / million sperms and % inhibition of Poly I–C on MMS induced UDS. A statistical analysis was carried out using t test.

RESULTS

The UDS in the mice injected with MMS (75 mg · kg^{-1}) was greater than that in the untreated ones. MMS also decreased sperms in the epididymis. Poly I–C did not significantly increase UDS in the spermatozoa and did not affect the number of sperms in the epididymis. Results indicated that Poly I–C neither damaged DNA in the germ cells nor affected the development of sperms (Tab 1).

Tab 1. Unscheduled DNA synthesis induced by Poly I–C in the spermatozoa of mice. $n=5$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **saline control.**

Drugs	Dosage, mg · kg^{-1}	Sperm count, million · ml^{-1}	Radioactivity, dpm / million sperms
Saline		13.3 ± 1.4	16 ± 7
Poly I–C	0.05	17.2 ± 2.9*	22 ± 5*
	0.50	13.1 ± 1.6*	24 ± 4*
	5.00	14.0 ± 2.5*	20 ± 6*
MMS	75.00	7.4 ± 1.4***	53 ± 3***

The UDS in the mice with MMS and Poly I–C injected at 4 h prior to MMS were found to be lower than that in the mice injected with MMS alone. Moreover, the inhibitory effect of Poly I–C was dose–dependent ($r=0.9498$, $P<0.05$). The serum taken from mice 4 h after the Poly I–C (0.50 mg · kg^{-1}) injection also inhibited UDS induced by MMS significantly. The effect of the serum on UDS was similar with that of Poly I–C (0.50 mg · kg^{-1}) (Tab 2).

Tab 2. Effect of Poly I–C on MMS (75 mg · kg^{-1})–induced UDS. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Poly I–C, mg · kg^{-1}	n	Sperm count, million · ml^{-1}	Radioactivity dpm/million sperms	Inhibition, %
	5	7.4 ± 1.4	53 ± 3	
0.05	1	3.8*	50*	6.3
0.50	5	11.9 ± 2.3**	45 ± 5**	16.2
5.00	5	17.3 ± 1.3***	34 ± 6***	35.5
serum	5	12.4 ± 2.1**	45 ± 5**	15.3

The inhibitory effects of Poly I–C (0.50 mg · kg^{-1}) administrated at 4 or 12 h were greater than those at 0 or 24 h prior to MMS administration. The effect of Poly I–C was not significant in the groups administrated at 0 or 24 h prior to MMS administration (Tab 3).

Tab 3. Effect of Poly I–C (0.50 mg · kg^{-1}) on MMS (75 mg · kg^{-1})–induced UDS. $n=5$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **MMS control.**

Injection time of Poly I–C before MMS	Sperm count, million · ml^{-1}	Radioactivity, dpm/million sperms	Inhibition, %
	7.4 ± 1.4	53 ± 3	
0 h	22.1 ± 2.6**	51 ± 6*	3.6
4 h	11.9 ± 2.3**	45 ± 5**	16.2
12 h	18.4 ± 2.7**	45 ± 4**	15.4
24 h	14.7 ± 0.8**	51 ± 9*	4.1

DISCUSSION

UDS is an index of the excision repair of DNA primary damage. If the nuclear DNA in any meiotic or post meiotic germ cell is

damaged and then UDS is induced, the UDS can be detected by an unscheduled incorporation of [^{3}H]TdR[4-6]. The inhibited UDS may be due to the reduction of DNA primary damage or the inhibition of DNA excision repair. In our experiments, MMS (75 mg · kg^{-1}) apparently induced UDS and decreased sperms. Poly I–C inhibited the UDS induced by MMS. Our other experiments also showed that Poly I–C reduced the chromosomal aberrations and the sister chromatid exchanges in the marrow cells induced by mitomycin C (to be published). So, we conclude that Poly I–C may protect DNA from injury, but not inhibit excision repair.

In this experiment, the effect of Poly I–C administrated simultaneously with MMS was not significant, but administrated at 4 or 12 h prior to MMS were greater. The serum taken from mice 4 h after Poly I–C injection also reduced the UDS. These results indicated Poly I–C did not react directly on MMS, but acted through inducing some substance in mice serum.

It has been reported that IFN–α reduced the sister chromatid exchanges and chromosomal aberrations in human lymphocytes induced by various mutagens and suggested that IFN–α might have the property as a biostabilizer[7,8]. We found that IFN titers in the serum at 0, 4, 12, 24 h after Poly I–C injection were 49, 1340, 1340, 948 U / ml respectively (unpublished data). The effective time of Poly I–C was related to the variation of IFN titers in serum. It is possible for Poly I–C to induce IFN to protect DNA from damage.

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多聚肌苷酸–胞苷酸类(Poly I–C)对甲基磺酸甲酯所诱发小鼠精子非程序脱氧核糖核酸合成的抑制作用

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提要 在 ip 注射甲基磺酸甲酯(MMS，75 mg · kg^{-1})之前 4 h 将 Poly I–C (0.05，0.50 和 5.00 mg · kg^{-1}) sc 给小鼠，可明显抑制 MMS 诱发的小鼠精子非程序脱氧核糖核酸合成(UDS). Poly I–C sc (0.50 mg · kg^{-1}) 后 4 h 取小鼠血清，该血清具有 Poly I–C 类似的效应. 提示 Poly I–C 和经 Poly I–C 诱导的小鼠血清均可预防 MMS 对脱氧核糖核酸的损伤.

关键词 多聚肌苷酸–胞苷酸类；甲基磺酸甲酯；精子；脱氧核糖核酸修复；C57BL 近交系小鼠