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Effects of menadione on 1,2-dimethylhydrazine-induced mouse colon adenocarcinoma

BI Sheng, GENG Bao-Qin, YONG Ding-Guo
(*Department of Pharmacology, Zhejiang Medical University, Hangzhou 310006, China*)

ABSTRACT The effects of menadione (Vit K_3) administered at 20 or 40 mg·kg^{-1} ig 3 times a week for both 24 and 28 wk on 1,2-dimethylhydrazine (DMH)-induced mouse colon adenocarcinomas were investigated. At the 24th wk, the number of colon tumors in Vit K_3 20 or 40 mg·kg^{-1} group (0.3± 0.5 and 0.5± 0.8, respectively) was less than that of DMH controls (2.1± 2.5, $P<0.05$), but the difference in incidence of colon tumors in these 3 groups was not significant ($P>0.05$). After 28 wk, the tumor incidence of both Vit K_3 groups (each 8 of 13) was lower than that of DMH controls (13 of 13, $P<0.05$); the number of colon tumors of Vit K_3 40 mg·kg^{-1} group (1.3± 1.3, $P<0.05$) was decreased, whereas the Vit K_3 20 mg·kg^{-1} group (3.0± 5.1, $P>0.05$) was not different from the DMH controls (7.3± 9.3). Determination of the nuclear DNA content of cells from DMH-induced mouse colon mucosa (24 wk) indicated that Vit K_3 20 or 40 mg·kg^{-1} group showed lower DNA content (1.92 ± 0.12 C and 1.91± 0.10 C, respectively) decreased values of percent-over-3C and -4C and narrow distribution range. Besides, the colon mucosa of DMH-treated mice (28 wk) showed higher superoxide dismutase (SOD) activity (70± 28 U/mg protein, $P<0.05$) than the normal controls (30± 20 U/mg protein). Vit K_3 40 mg·kg^{-1} reduced the elevated SOD activity markedly (44± 23 U/mg protein, $P<0.05$).

KEY WORDS vitamin K; methylhydrazines; colonic neoplasms; DNA; superoxide dismutase; mucous membrane

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Recently, a great deal of interest has been focused on the cancer prevention and treatment by vitamins. Investigators discovered that the menadione (Vit K_3) had antitumor activity *in vitro* comparable to the toxic anthracyclic quinones doxorubicin and daunorubicin[1]. Vit K_3 enhanced the anti-neoplastic activity of 5-fluorouracil in Friend murine erythroleukemia cells *in vitro*[2], and of methotrexate in tumor-bearing animals[3]. Combined administration of vitamin C, K_3,

and antineosplatic agent produced synergistic effect on mouse transplantable liver tumor and did not increase the systemic and organ toxicity of chemotherapy[4]. We found that the growth rate of HR-8348 and HL-60 cells was inhibited significantly *in vitro* by Vit K_3, and showed dose-dependent[5].

This study was performed on the model of 1,2-dimethylhydrazine (DMH)-induced mouse colon carcinomas for testing antitumor activity of Vit K_3, and the mechanism of its action was also analysed.

MATERIALS AND METHODS

Vit K_3 (menadion sodium bisulfite) was obtained from Wuxi Pharmaceutical Factory. 1,2-Dimethylhydrazine (DMH) and superoxide dismutase (SOD) were from Sigma, and luminol was from Merck-Schuchardt. Microspectrophotometer (Ⅲ -MPMOIK) was made by Opton.

Female ICR mice weighing 20± *s* 4 g were supplied by the Shanghai Institute of Family Planning.

Tumor model and drug administration Among 92 mice kept on a standard diet and water *ad lib*, 78 were given sc weekly of DMH 20 mg· kg^{-1} for 20 wk for inducing colon adenocarcinomas[6], 14 were used as normal controls. At the 1st wk of sc DMH, 78 mice were randomly divided into 3 groups: DMH control, Vit K_3 20, and 40 mg· kg^{-1} (ig 3 times a week for 24 and 28 wk, respectively). At the 24th wk, half of mice (each group) were sacrificed for pathological examination and DNA measurement; after 28 wk, the remainder mice were killed for numbering the macroscopic colon tumors and assaying SOD activity.

Measurement of DNA content The mice were sacrificed by cervical dislocation. The colon was excised, and slit lengthwise into two equal sections; and then one part was fixed in 10% neutral formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E) for histologic examination, and the other was fixed in Carnoy's solution, and processed for DNA measurement with the Feulgen reaction.

The nuclear DNA content in the cells of each section was measured by using a microspectrophotometer[7]. As a control for each specimen, lymphocytes on the same slides were scutinized to determine the value of the DNA content corresponding to a diploid chromosome complement (2C). The mean DNA content, distribution range, and percent of nuclei with a DNA content 3 and 4 times greater than the basic C value (namely, %-over-3C, %-over-4C, respectively) were calculated.

SOD assays The mice were killed by cervical dislocation. A 7 cm segment of colon above the anus was immediately excised, opened longitudinally, and washed clean with cold phosphate buffer 0.2 mol· L^{-1} (pH 8.6, 4℃); then the colon mucosa was scraped gently by a slide for SOD assay with the alkaline dimethylsulfoxide-luminol chemiluminescence method[8].

Statistical analyses Differences in the occurrence of mice colon tumors in several groups were evaluated by means of contingency tables from which chi-square statistics was calculated. The significance of the data presented as $\bar{x}\pm s$ was determined by analysis of variance, or rank sum test.

RESULTS

Effect of Vit K_3 on occurrence of DMH-induced colon tumors in mice During the entire experimental period, mice in all 3 groups gained weight, however there were no statistic differences. Pathological examination showed that the tumors occurred mainly in colon (5 cm above anus) and involved both adenomas and adenocarcinomas. At the 24th wk, the incidence of DMH-induced colon tumors treated with Vit K_3 (20 and 40 mg· kg^{-1}, respectively) was not decreased, but the number of tumors was reduced significantly; after 28 wk, two Vit K_3 regimen decreased the incidence of colon tumors, and Vit K_3 40 mg· kg^{-1} reduced the number of tumors (Tab 1).

Effect of Vit K_3 on nuclear DNA content of DMH-induced mouse colon carcinomas The results of the nuclear DNA content of

Tab 1. Effect of Vit K_3 (20 or 40 mg·kg^{-1} ig 3 times a week for both 24 and 28 wk) on occurrence of 1,2-dimethylhydrazine(DMH)-induced mouse colon tumors. n= 13 mice. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$ *vs* **DMH control.**

	Tumor-bearing mice	Tumors per mouse
DMH control	9	2.1± 2.5
24 wk Vit K_3 20 mg·kg^{-1}	4*	0.3± 0.5**
40 mg·kg^{-1}	4*	0.5± 0.8**
DMH control	13	7.3± 9.3
28 wk Vit K_3 20 mg·kg^{-1}	8**	3.0± 5.1*
40 mg·kg^{-1}	8**	1.3± 1.3**

Tab 3. Effect of Vit K_3 (20 and 40 mg·kg^{-1} ig 3 times a week for 28 wk, respectively) on superoxide dismutase(SOD) activity in DMH-induced mouse colon carcinomas. n= 7 mice. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$ *vs* control; $^{+}P>0.05$, $^{++}P<0.05$ *vs* **DMH control.**

	SOD U / mg protein
Control	30± 20
DMH control	70± 28**
Vit K_3 20 mg·kg^{-1}	56± 25**+
40 mg·kg^{-1}	44± 23**+

cells from DMH-induced mouse colon mucosa at the 24th wk showed that Vit K_3 20 or 40 mg·kg^{-1} could reduce the mean DNA content and the values of percent over 3C and 4C and the range of DNA content distribution (Tab 2).

Effect of Vit K_3, on SOD activity of DMH- induced mouse colon carcinomas At the 28th wk, the colonic mucosa of DMH-induced mice showed higher SOD activities than those of normal controls. Vit K_3 20 mg·kg^{-1} did not influence the effect, whereas Vit K_3 40 mg·kg^{-1} decreased the elevated SOD activity (Tab 3).

DISCUSSION

The results showed the activity of Vit K_3 against DMH-induced mouse colon carcinoma, which coincided with the results of our previous studies *in vitro* and of some other authors[1-5]. With respect to the nuclear DNA content, the percent-over-4C and -3C values in severe colon mucosal dysplasia and carcinoma were almost identical, and significantly higher than those in mild and moderate mucosal dysplasia. The wider range of DNA content distribution was existed in severe dysplasia and carcinoma[7]. Since Vit K_3 improved the altered DNA content in DMH-induced colon carcinomas, it was suggested that the proliferation of tumor cells was inhibited and the malignant level of cancer cells was decreased. Our results demonstrated that the SOD activity in DMH-induced colon carcinomas was greatly increased, which was significantly lowered by Vit K_3 40 mg·kg^{-1}. Owing to the SOD activity in tumor cells may

Tab 2. Effect of Vit K_3 (20 and 40 mg·kg^{-1} ig 3 times a week for 24 wk, respectively) on nuclear DNA content in DMH-induced mouse colon carcinomas. *C*: the ratio of the DNA content in colon mucosal cells to that in lymphocytes. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control; $^{-}P>0.05$, $^{+}P<0.05$, $^{++}P<0.01$ *vs* **DMH control.**

	n	DNA content (C)	Range of distribution (C)	%-over-3C value	%-over-4C value
Control	7	1.87± 0.18	0.6~6.7	3.2± 3.5	0.8± 0.9
DMH control	8	2.27± 0.17**	0.7~9.3	14.9±6.7***	4.9± 4.8**
Vit K_3 20 mg·kg^{-1}	8	1.92± 0.12*+	0.7~6.5	4.0± 5.1*++	1.0± 2.9*+
40 mg·kg^{-1}	8	1.91± 0.10*+	0.7~5.5	4.2± 5.2*++	1.2± 2.6*+

closely reflect the presence of malignant transformation[9]. Vit K_3 reduce the malignant transformation of tumor cells. On the other hand, the potent carcinogenic effect of DMH was attributed to its metabolite, methylazoxymethanol(MAM) that could generate an activated alkylating radical[6]. It was reported that the induction of SOD synthesis was stimulated by increasing rates of intracellular O_2^- production[12]. It was suggested that MAM enhanced the SOD activity in colon tumor-bearing mouse, and Vit K_3 might influence the generation of the free radical of MAM.

Several hypothetical mechanisms may be involved in the antitumor action of Vit K_3. This study provided indirect evidences of the influence of alkaline DNase[10], and of the effects of carcinogenesis[11]. The model of DMH carcinogenesis is similar in many respects to the development of neoplasms in the human colon[6]. The influence of Vit K_3 at early stage of carcinogenesis was suggested owing to the fact that the colon tumor incidence of Vit K_3-treated mouse was only 4 of 13 (30.8%) at the 24th wk and 8 of 13 (61.5%) at the 28th wk. As a result, it is worth looking for the optimal dose of Vit K_3 to block the development of cancer at early stage and prevent the relapse of cancer after operation or chemotherapy.

In summary, Vit K_3 has antitumor activity *in vivo*, which may be related to the inhibition of DNA synthesis of tumor cells. However, further experiments are needed to confirm our preliminary results and possible mechanisms.

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维生素 K_3 对二甲肼诱发小鼠结肠癌的影响

毕 胜, 耿宝琴, 雍定国

(浙江医科大学药理教研室, 杭州 310006, 中国)

摘要 维生素 K_3 (Vit K_3)分别以 20 和 40 mg·kg^{-1} 灌胃, 每周三次, 共 24 和 28 周, 能使二甲肼诱发小鼠的结肠肿瘤发生率下降, 肿瘤结节数减少. Vit K_3 能降低诱癌小鼠结肠上皮细胞核 DNA 含量; 使诱癌小鼠结肠粘膜超氧化物歧化酶活力下降. 结果表明 Vit K_3 在体内有抗肿瘤作用, 该作用可能与抑制肿瘤细胞 DNA 合成有关.

关键词 维生素 K; 甲基肼类; 结肠肿瘤; 脱氧核糖核酸; 超氧化物歧化酶; 粘膜