Inositol hexaphosphate-induced enhancement of natural killer cell activity correlates with suppression of colon carcinogenesis in rats

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**Abstract**

**AIM:** To investigate the anti-neoplastic effect of inositol hexaphosphate (InsP$_6$ or phytic acid) on dimethylhydrazine (DMH)-induced colon tumor in rats and its effect on blood natural killer (NK) cell activity.

**METHODS:** Healthy Wistar rats, 4 wk old, were divided into control group (fed with common food) and InsP$_6$ group (fed with common food+2% sodium inositol hexaphosphate in the drinking water), 15 rats in each group. Both groups were injected with 1,2-dimethylhydrazine subcutaneously (20 mg/kg body weight) once a week for 20 wk. Rats were killed after 21 wk. The whole large intestine was isolated to determine the general condition of tumors and to test blood NK cell activity by lactate-dehydrogenase-release assay.

**RESULTS:** Administration of InsP$_6$ significantly increased blood NK cell activity in DMH-induced colorectal tumor in rats. InsP$_6$ group had a smaller tumor size on average and a smaller number of tumors than the control group. Its mortality was also higher than that in control. However, the variables of body weight and tumor incidence were not significantly different between the two groups.

**CONCLUSION:** InsP$_6$ can increase blood NK cell activity in DMH-induced colon tumor in rats and inhibit tumor growth and metastasis in rats.

**Key words:** Inositol hexaphosphate; Phytic acid; Natural killer cell activity; Colon cancer

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Inositol hexaphosphate (InsP$_6$) is a naturally occurring compound that has various chemical properties and biological activities$[1]$. It is rich in matured plant seeds, particularly in cereals and legumes, and exists in nature as a salt with monovalent and divalent cations (Ca$^{2+}$, Mg$^{2+}$, and K$^+$. It has the ability to chelate minerals such as iron, copper, zinc, cobalt, and manganese, most efficiently at neutral pH$[2,3]$. InsP$_6$ has anti-neoplastic activity on a variety of experimental models of carcinogenesis, decreases serum cholesterol level, inhibits renal stone formation, and may find use in controlling myocardial damage following ischemia. Among these biological activities, anti-neoplastic activity is one of the most intriguing properties of InsP$_6$$[4]$. The above facts need clinical trials in human colorectal cancer. It has been reported that intestinal lipodystrophy can be prevented by InsP$_6$ treatment$[7]$. Recent studies demonstrate that InsP$_6$ inhibits experimental colon carcinogenesis in rats$[8,9]$. There is a correlation between neoplastic diseases and depressed natural killer (NK) activity$[11]$. There is evidence that NK cells are involved in the destruction and growth inhibition of tumor cells in vivo. This study aimed to study the effect of InsP$_6$ on blood NK cell activity in dimethylhydrazine (DMH)-induced colon tumor in rats.

**INTRODUCTION**

**MATERIALS AND METHODS**

**Animals and chemicals**

Thirty-four-weeks old male Wistar rats (70-110 g) were purchased from Animal Center of Henan Medical University. After acclimatization for 1 wk, the experimental animals were randomly divided into control group and InsP$_6$ group (15 rats/group). Animals in the control group were fed with the basal diet and had regular access to drinking water. Rats in InsP$_6$ group were fed with the basal diet and had access to 2% sodium inositol hexaphosphate (purchased from Guangdong Qingyun Chemical Factory) solution. Basal diet was made by American Institute of Nutrition method.

Animals in both groups were given subcutaneous injections of DMH (from Sigma) dissolved in normal saline solution (20 mg/kg body wt) once a week for 20 wk. Body weight was measured and food consumption was recorded once a week. All surviving animals were killed under 4.3% trichloraldehyde hydrate anesthesia after 21 wk.
**Tissue processing**

All animals (including rats that died before the end of experiment) were autopsied. The colons were removed, flushed with saline, opened along the longitudinal median axis. Macroscopically, the number of tumors in each colon was counted. Tumor width and length were measured with clippers. Simultaneously, peripheral blood was obtained from the abdominal aorta for testing NK cell activity.

**Test of NK cell activity**

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque density centrifugation from the collected blood. Lactate-dehydrogenase (LDH)-release assay was used to measure the NK cell activity. PBMCs were washed and suspended in complete RPMI-1640 medium, counted and diluted to $1.0 \times 10^6$/mL. The amount of LDH released from the lysed target cells was determined for NK cell activity measurement. The NK-sensitive cell line K562 (human erythroleukemia cell line, Shandong Medical Science Institute, Shandong, China) was used as the target cell. K562 cells were washed with complete RPMI-1640 medium, counted and finally diluted to $1.0 \times 10^5$/mL with the medium. An equal volume of K562 cells and PBMCs was added to the wells of 96-round-bottomed microwell plates (the cell ratio of effector-to-target was 10:1). Each test was repeated in three wells. To ensure contact between cells, the plate was centrifuged at a low speed for 2 min. After 2-h incubation at 37 °C in a humidified atmosphere with 50 mL/L CO$_2$, the plate was centrifuged at 1 000 r/min for 5 min. The supernatant from each well (100 µL) was transferred into the corresponding wells of a 96-flat-bottomed microwell plate. Then 100 µL of lactic acid hydrogenase substrate mixture was added to each well. After 3 min, reactions were stopped by adding 50 µL of cold medium. Finally, a microtiter plate reader (Bio-Rad, MODE-550) was used for evaluation of changes in the absorbance at a wavelength of 490 nm. The release of LDH from K562 cells was expressed as absorbance. The percentage of NK cell activity was calculated by the formula: NK cell activity = $(E-S)/(M-S) \times 100\%$, where $E$ represents the experimental release of LDH activity from target cells incubated in the presence of PBMCs, $M$ represents the maximum release of the LDH activity determined by lysing the target cells with 1% of NP40, and $S$ is the spontaneous release of the LDH activity from target cells incubated in the absence of PBMCs.

**Statistical analysis**

Results were expressed as mean±SD. Statistical analyses were performed with SPSS 9.0. The significance of differences in the average values between the two groups was analyzed using t-test. $P<0.05$ was considered statistically significant.

**RESULTS**

During the initial period of the experiment, body weight of animals increased steadily in the first 20 wk. Then both groups began to loose their body weight. In addition, the animals consumed a less amount of food. The change in two groups had no difference (Figure 1).

![Figure 1](image.png)

**DISCUSSION**

Diet composition is an important etiologic factor in colon...
carcinogenesis and has a significant impact on colon cancer occurrence. Inositol hexaphosphate (InsP$_6$) is a dietary phytochemical present in cereals, soy, legumes, and fiber-rich foods.$^{[12,13]}$. Epidemiological studies have shown that InsP$_6$ can inhibit the metastasis of tumor.$^{[14-18]}$. But the anti-tumor mechanism of InsP$_6$ awaits further investigation.

Our study demonstrated that InsP$_6$ could significantly increase blood NK cell activity in DMH-induced colorectal cancer in rats (P<0.01). The number and size of tumors were smaller in InsP$_6$ group than in control group (P<0.05), indicating that InsP$_6$ can also inhibit tumor growth and metastasis in DMH-induced colorectal cancer in rats. InsP$_6$ is degraded into lower polyphosphorylated forms of inositol (including InsP$_1$-InsP$_5$) by the enzyme meso-inositol hexaphosphate phosphohydrolase, and dephosphorylated by acid, acid phosphatase and intestinal alkaline phosphatase. When InsP$_6$ was administered to rats as a soluble form in drinking water, it is rapidly absorbed through the upper gastrointestinal tract and quickly distributed in various organs, most notably in liver, kidneys, and skeletal muscle.$^{[17,18]}$. Among the lower polyphosphorylated forms of inositol, InsP$_6$ appears to act as a second messenger and promotes intracellular free calcium (Ca$^{2+}$) release, which can induce proliferation of NK cells$^{[19]}$ as well as the release of NK cell cytotoxic factor (NKCF). NKCF can bind to target cells (tumor cells) which are subsequently lysed$^{[20]}$. Close contact between the plasma membrane of the two types of cells, affects the cytotoxic reaction. InsP$_6$ can also affect the membrane phosphatidyl inositol proteins, which may be important in attachment and subsequent fusion with the target cells, suggesting that InsP$_6$ mediates its chemopreventive and probably chemotherapeutic effect via InsP$_6$$^{[21,22]}$.

Our data indicate that DMH depresses the NK cell activity, while InsP$_6$ significantly increases the NK cell activity and inhibits tumor growth, suggesting that changes in NK cell activity are related to progressive cancer growth$^{[23]}$. Since InsP$_6$ enhances the NK cell activity in vivo, it may have potential application in therapy of cancer and other diseases associated with depressed NK cytotoxicity.

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