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The effect of Korean ginseng on diethylnitrosamine-initiated hepatic altered foci in a mid-term induction system

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고려인삼이 diethylnitrosamine에 의해 유도되는 preneoplastic hepatic altered foci의 형성에 미치는 효과

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초록 : 홍삼 및 수삼이 랫트의 간조직에서 diethylnitrosamine(DEN)에 의해 유도되는 preneoplastic altered foci 형성에 미치는 영향을 관찰한 바 다음과 같은 결과를 얻었다.

Altered foci의 지표로 사용되는 glutathione S-transferase (GST-P)-positive foci의 숫자는 DEN 단독투여군(9.07±5.69)에 비하여 수삼병행 투여군(4.77±3.23)에서, 면적은 DEN 단독투여군(0.93±0.65)에 비하여 홍삼병행투여군(0.50±0.31)에서 각각 현저한 감소를 나타냈다.

이러한 결과는 홍삼 및 수삼이 간암 발생을 억제하는 작용이 있음을 암시하였다.

Key words: placental form of glutathione S-transferase, liver altered foci, diethylnitrosamine, ginseng

Introduction

Korean ginseng has been used as a traditional medicine for the past several thousand years among Oriental people and recently a number of scholars have paid attention to elucidate the efficacy of Korean ginseng scientifically.

Today we have a considerable amount of information about the chemistry[1~5] and pharmacological effects of Korean ginseng[6~7]. The potential effects of ginseng extract on transplantable tumor cell growth and cell multiplication have been studied by many investigators[8~12] and there is some experimental evidence to demonstrate anticarcinogenic action in animals[13~15]. But the antitumor and anticarcinogenic activity of ginseng has not been fully accepted and especially, no paper dealing with the effect on hepatocarcinogenesis is available.

The occurrence of histochemically detectable altered hepatocyte foci that precede tumor formation in carcinogen-treated rats is widely used as an indicator of incipient hepatic neoplasia[16~19]. Among various enzyme-histochemical markers characterizing preneoplastic liver lesions in rodents, gamma-glutamyltransferase(GGT) has been widely used. More recently, the placental form of glutathione S-transferase (GST-P) was recommended as a suitable immunohistochemical marker[20~22].

In this study we investigated the ability of Korean ginseng to modify diethylnitrosamine(DEN)-initiated hepatocarcinogenesis in a mid-term induction system.

Materials and Methods

Animals: Six-week-old male Sprague-Dawley rats

of our institute colony were housed in polycarbonate cages. They were fed on a standard animal diet (NIH-7-open formula ration) and given tap water *ad libitum*.

Chemicals and Ginseng: DEN was obtained from Wako Pure Chemical Co., Ltd., Japan and rabbit anti GST-P, used in immunohistochemical studies, was kindly provided by Prof. Kiyomi Sato, Medical school of Hirosaki University, Japan. Affinity-purified biotin-labeled goat anti-rabbit immunoglobulin IgG and avidin-biotin-peroxidase complex (ABC, Vectastain ABC Kit, PK 4001) were obtained from Vector Laboratories Inc. Korean red ginseng extract powder, spray-dried, was obtained from the Office of Monopoly (Seoul, Republic of Korea) and raw ginseng was commercial produce.

Experimental Design: The experimental schedule followed is shown in Figure 1. Five groups of 20 rats each were given a single intraperitoneal injection of DEN at 200mg/5ml saline/kg bw or the solvent alone. Two weeks later, group 2 and 3 were given drinking water containing red ginseng (1mg/ml) and group 4 and 5 were given drinking water containing raw ginseng (12.85mg/ml) for 6 weeks. Group 1 was given DEN alone. Three weeks after the beginning of the experiment two-thirds partial hepatectomy was performed on all animals. All animals were killed under ether anesthesia for examination at week 8.

Histopathological Observations: The livers were excised and cut into 2~3mm thick sections with a razor blade. Some sections were fixed in 10% neutral formalin solution for routine staining with hematoxylin and eosin(H&E), and other sections were fixed in ice-cold acetone for immunohistochemical examination of GST-P. The numbers and areas of GST-P positive foci of over 0.2mm in diameter were measured using a color video image processor (IBAS I, Zeiss, West Germany).

The ABC method was used to determine the location of GST-P in the liver. Paraffin section were routinely passed through petroleum benzene and a graded alcohol series and then treated sequentially with normal goat serum, rabbit anti GST-P(1 : 5,000), biotin-labelled goat anti-rabbit IgG(1 : 400), an avdidin-biosin-peroxidase complex (ABC). The site of peroxidase binding was detected by the diaminobenzidine method of Graham and Karnofsky.[23] The location of GST-P positive site and hematoxylin and eosin staining lesions were examined in successive serial sections.

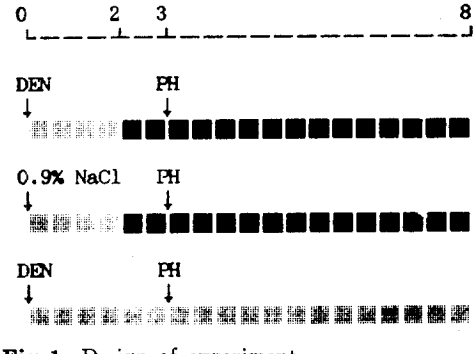

Fig 1. Design of experiment.
↓, i.p. injection of 200mg of DEN per kg body weight or 0.9% NaCl solution; ↓ PH, partial hepatectomy; ▪: red or raw ginseng in drinking water.

Results

The liver weights and their percentages of the body weight of animals treated with raw ginseng, with or without DEN initiation were lower than that of animals treated with DEN alone whereas red ginseng-treated groups with or without DEN treatment did not demonstrate significant intra-group liver weight difference(Table 1).

Grossly, small foci were recognized on the surface of the liver in some animals, but in general, the livers were smooth and no cirrhotic changes are seen. Microscopically, the altered foci were demarcated from the surrounding parenchimal tissue (Fig 2, 3) but it was difficult to distinguish foci from surrounding hepatocyte in the H & E stained tissue (Fig 2).

Areas of the foci showed strong activity of GST-P (Fig 3).

The average total numbers and total areas of foci per cm^2 of liver tissue in the each group are shown in Table 2.

The numbers and total areas of foci in groups of rats treated with raw or red ginseng showed significantly differences after induction with DEN. The

Table 1. Body and liver weights

Treatment*	Number of rats	body weight average	Average liver weight	
			g	g/100g B.W.
DEN	13	356.2±44.5	15.1±2.1	4.4±1.0
DEN→red ginseng	17	378.1±38.5	15.6±2.1	4.1±0.5
DEN→raw ginseng	13	325.4±42.9	13.2±1.6*	4.0±0.6
Red ginseng	14	375.6±31.3	14.7±2.2	3.9±0.6
Raw ginseng	13	383.4±38.1	14.3±2.4	3.7±0.4**

* All rats were subjected to partial hepatectomy 3 weeks after the beginning of the experiment.
** $p<0.05$ as compared with the carcinogen control group.

Table 2. Induction of GST-P positive foci in rats treated with DEN followed by red ginseng or raw ginseng

Treatment	Number of rats	GST-P positive foci	
		Number/cm²	Area(mm²)/cm²
DEN	13	9.07±5.69	0.93±0.65
DEN→red ginseng	17	6.17±3.54	0.50±0.31*
DEN→raw ginseng	13	4.77±3.28*	0.48±0.46
Red ginseng	14	0	0
Raw ginseng	13	0	0

* $p<0.05$ as compared with the carcinogen control group.

Legends of figures

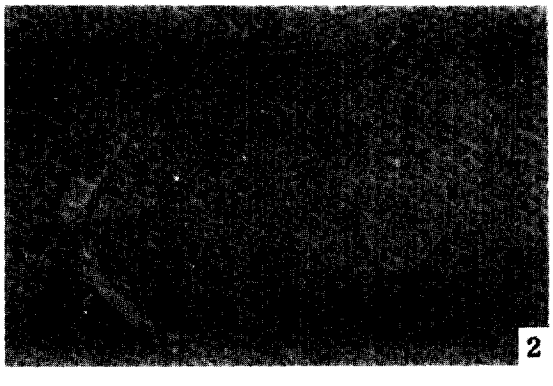

Fig 2. A neoplastic altered foci induced with DEN in hepatectomized rat. Cells of the altered foci have clear cytoplasm. H & E×20.

Fig 3. An adjacent section of the same focus as in figure 2. Positive histochemical reaction of GST-P in altered foci induced with DEN in a hepatectomized rat. H & E×20.

average total area of foci changed in a similar way to the average numbers of foci. The area of rats given DEN with red ginseng and number of rats given DEN with raw ginseng were significantly lower than those of DEN alone group ($p<0.05$). Red or raw ginseng alone could not induce the GST-P positive foci.

Discussion

We investigated the effect of Korean ginseng in induction of foci of rat liver cells containing the GST-P.

Ginseng extracts showed inhibition of the growth of certain experimental tumors, such as Sarcoma 180 and Adenocarcinoma 755, while experimental leukemia in mice was not influenced.[9,10] On the other hand, the protective activity of ginseng on cells was also demonstrated.[6] These results again seem to indicate that ginseng may contain constituents with opposite activities, namely with cytostatic as well as cell protective or cell stimulating properties. Therefore characteristics of the anticancer effect of ginseng may be summrized as follows: 1) it is observed only in slow-growing tumors such as Ehrilich and Sarcoma 180 ascites tumor; 2) it is not observed in rapid-growing tumors such as L1210, P388, and Walker carcinosarcoma 256; and 3) there is no dose-response relationship and no cumulative effect.[8-11] The anticeacer effects of ginseng support the suggestion of Brekhman and Dardymov[24] that ginseng may increase nonspecific resistance of the organism.

On the other hand, in the point of anticarcinogenic effect of ginseng, Yun et al.[13,15] and Yun et al.[14] carried out to evaluate the effect. The results indicate that the Korean red ginseng extract inhibited the incidence and also the proliferation of tumors induced by various chemical carcinogen and the anticarcinogenic effect of ginseng may be related to the augumentation of natural killer cell activity.

In this experiment, we used GST-P as a marker for preneoplastic foci, because it is more specifically distributed in these lesions than r-GT, and therefore considered a better marker than r-GT.[25]

The number of foci per cm^2 was significantly reduced in rats given DEN followed by raw ginseng ($p<0.05$) and the area of GST-P positive foci was also reduced in rats given DEN followed by red ginseng ($p<0.05$) as compared to those in the group given DES alone. These results indicate that red or raw ginseng may interact with hepatocytes directly or indirectly. Though the mechanism(s) underlying the process of inhibition are unclear, the data from these works thus suggest that red or raw ginseng may play an important role in the initiation stage of carcinogenesis including a direct enhancing or inhibitory effect via influencing carcinogen methabolism.

Since a good correlation exists between ability to inhibit in the mid-term test and in long-term experiments,[26] it might be expected that red and raw ginseng also exert long-term inhibitory potential.

Conclusion

The effects of red ginseng and raw ginseng were examined in vivo mid-term test for hepatocarcinogens in rats.

The number of placental type of glutathione S-transferase (GST-P)-positive foci of the liver was significantly reduced in rats given diethylnitrosamine (DEN) followed by raw ginseng (4.77 ± 3.23, $p<0.05$) as compared to the controls given carcinogen alone (9.07 ± 5.69).

The area of GST-P positive foci was also significantly reduced in rats given DEN followed by red ginseng (0.5 ± 0.31, $p<0.05$) as compared to the control (0.93 ± 0.65). These result suggest that red or raw ginseng are not hepatocarcinogens and rather may posses inhibitory potential for liver carcinogenesis.

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