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The effect of caffeine on initiation step of diethylnitrosamine-initiated hepatic altered foci in a mid-term induction system

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Caffeine이 diethylnitrosamine에 의해 유도되는 preneoplastic hepatic altered foci 형성의 initiation 단계에 미치는 효과

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초록 : Caffeine이 랫트의 간조직에서 diethylnitrosamine(200mg/kg B.W., DEN)에 의해 유도되는 preneoplastic altered foci형성의 initiation단계에 미치는 효과를 관찰한 바 다음과 같은 결과를 얻었다. Altered foci의 지표로 사용되는 glutathione S-transferase(GST-P)-positive foci의 수는 caffeine 음수 ml당 2mg병행투여군(7.48±3.33) 및 1mg병행투여군 (7.50±3.32) 모두에서 DEN 단독투여대조군(14.08±5.16)에 비하여 현저히 낮게 나타났으며, 면적 또한 caffeine 2mg병행투여군 (0.29±0.17), 1mg병행투여군 (0.30±0.13)에서 DEN단독투여대조군(0.46±0.21)에 비하여 유의성 있는 낮은 수치가 관찰되었다. 이러한 결과는 caffeine이 간암발생의 initiation 단계에 작용하여 억제효과를 나타냄을 암시하였다.

Key Words: Caffeine, glutathion S-transferase placental form, liver altered foci, diethylnitrosamine, initiation step.

Introduction

Caffeine, a trimethylxanthine, occurs naturally in coffee, tea, and cocoa and as additive in other beverages such as soft drinks. Caffeine is also added to many medications, notably analgesics and diet aids. The pharmacological and potential pathological activities of caffeine have received considerable attention due to its daily ingestion by a large portion of the population; caffeine is the most widely consumed drug in much of the world today.[1~4]

The impact of caffeine consumption on tumorigenic processes is at present unclear; reports pertaining to the consumption of this drug and tumor development have been inconsistent and inconclusive. In *in vitro* studies, caffeine has been shown to both inhibit[5] and enhance[6,7] neoplastic mammalian cell transformation by chemical carcinogens as well as increasing mutation in mammalian cells treated with U.V. light or chemical carcinogens. *in vivo*, the alkaloid has been shown to inhibit[8~10] as well as enhance[11] tumorigenesis in a variety of organ sites. Especially, in human, caffeine consumption has been reported to be associated with an increased for the

development of urinary tract,[12] pancreatic,[13] ovarian,[14] colon,[15] and breast tumors.[16,17] Other reports have failed to demonstrate an association between caffeine ingestion and tumor development in human population.[18~20]

The occurrence of histochemically detectable altered hepatocyte foci that precede tumor formation in carcinogen-treated rats is wide used as an indicator of incipient hepatic neoplasia. 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.[21~23]

In the studies described in this communication, we investigated the modification potential of caffeine, administered via the drinking water, on the initiation step of diethylnitrosamine(DEN)-induced rat preneoplastic hepatic altered foci.

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: Caffeine was obtained from Sigma. DEN was from Wako Pure Chemical Co., Ltd., Japan and rabbit antiGST-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 G and avidin-biotin-peroxidase complex (Vectastain ABC Kit, PK 4001, ABC) were obtained from Vector Laboratories Inc.

Experimental design: The experimental schedule followed is shown in Fig 1. A total of 80 rats were divided into 4 groups. Group 1, group 2, and group 4 were given drinking water containing caffeine (2mg/ml or 1mg/ml) for 1 week before DEN or solvent treatment. Group 1, group 2, and group 3 were treated with a single intraperitoneal injection of DEN at DEN at 200mg/5ml saline/kg B.W. Two weeks later after DEN or saline injection, all of the

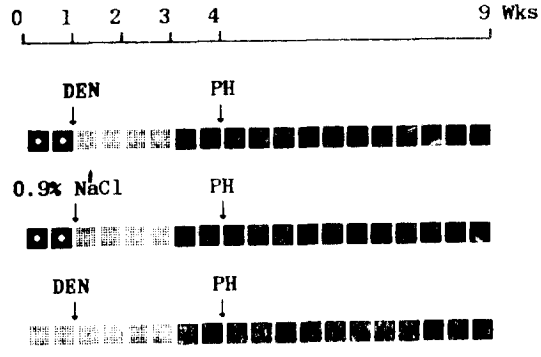

Fig. 1. Design of experiment 1.

↓ : i.p. injection of 200μg of DEN/g body weight or 0.9% NaCl solution, ↓PH : partial hepatectomy, ◧ : caffeine 1mg or 2mg/ml of drinking water, ▦: basal diet, ■ : phenobarbital 0.05% in drinking water.

rats were given drinking water containing phenobarbital (Hong Sung Pharmaceutical Co., Korea) at a concentration of 0.05% for 6 weeks. Four weeks after the beginning of the experiment two-thirds partial hepatectomy was performed on all animals. The animals were killed under ether anesthesia for examination at week 9.

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(HE), 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 (Kontron Ltd, 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 antiGST-P(1 : 6,000), biotin-labelled goat anti-rabbit IgG(1 : 400), an avidin-biotin-peroxidase complex (ABC). The site of peroxidase binding was detected by the diaminobenzidine. The location of GST-P positive site and HE staining lesions were examined in successive serial sections.

Results

The body and liver weights showed no difference between groups. But the relative (% body weight) liver weights of animals treated with caffeine were higher than that of animals in group 3(Table 1). Grossly, livers were smooth and no cirrhotic changes were seen. Microscopically, the altered foci were demarcated from the surrounding parenchimal tissue (Fig 2,3) but it was difficult to distinguish focus from surrounding hepatocyte in the HE stained tissue(Fig 2). Areas of the focus showed strong

Table 1. Body and liver weight

Group	No. of rat	Body weight (mean±SD)	Liver weight(mean±SD)	
			g	mg/g body weight
1. Caffeine(2mg) →DEN→PB	17	368.3±29.3	18.3±1.9	49.7±5.0
2. Caffeine(1mg) →DEN→PB	20	369.7±22.7	17.9±2.2	48.7±5.1
3. DEN→PB	19	369.5±28.9	17.0±2.0	45.9±2.4[a,b]
4. Caffeine(2mg) →saline→PB	20	361.2±22.5	17.6±3.0	48.5±6.9

DEN: diethylnitrosamine, PB: phenobarbital.
[a] $p<0.01$ as compared with group 1.
[b] $p<0.05$ as compared with group 2.

Table 2. Quantitative value of GST-P positive foci in the liver

Group	No. of rat	GST-P positive foci (mean±SD)		
		No./cm²	Area(mm²)/cm²	Maximum diameter (mm)
1. Caffeine(2mg) →DEN→PB	17	7.48±3.33[a]	0.29±0.17[a]	0.30±0.02
2. Caffeine(1mg) →DEN→PB	20	7.50±3.32[a]	0.30±0.13[b]	0.31±0.02
3. DEN→PB	19	14.08±5.16	0.46±0.21	0.29±0.02
4. Caffeine(2mg) →saline→PB	20	—	—	—

DEN: diethylnitrosamine, PB: phenobarbital.
[a] $p<0.025$ as compared with group 3.
[b] $p<0.01$ as compared with group 3.

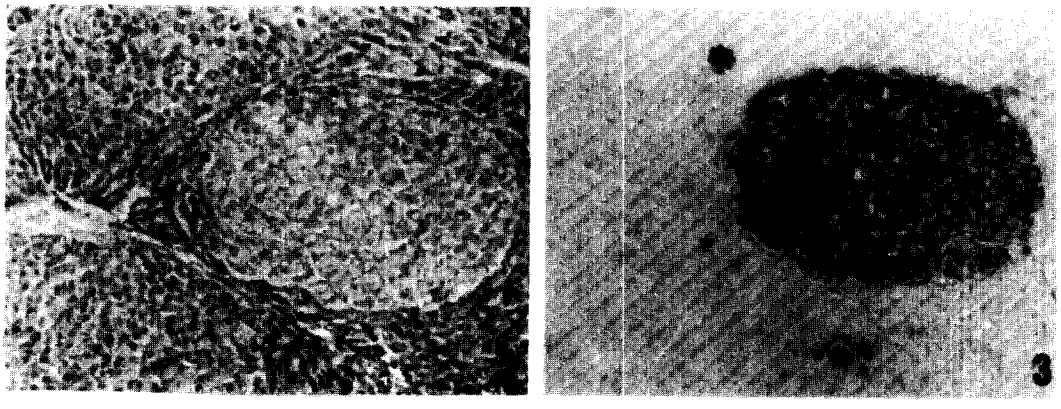

Fig 2. A preneoplastic altered focus induced with DEN in hepatectomized rat. Cells of the altered focus have clear cytoplasm. HE ×20.

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

activity of GST-P(Fig 3).

The average total numbers, total areas of foci per cm^2 and maximum diameters of the foci in the each group are shown in Table 2. The numbers and areas of rat treated with caffeine and DEN were significantly lower than those of DEN alone group. But the average maximum diameter showed no difference between groups. The average total area of foci changed in a similar way to the average numbers of foci. Caffeine alone could not induced the GST-P positive foci.

Discussion

We investigated the effect of caffeine on the initiation step of DEN-induced rat preneoplastic hepatic altered foci.

Hepatocellular carcinoma is among the top ten most common cancers in the world. It has been estimated that more than 250,000 people die of hepatocellular carcinoma each year.[24] Therefore, the liver has been studied intensively as a site for cancer development. In experimental animals, rat chemical hepatocarcinogenesis has been most extensively studied and sequential analysis of the process involved has played important role in generating the hypothesis that chemical carcinogenesis comprises at least two or three stages; initiation and promotion, together with further progression stages. Initiation is generally considered to result from an irreversible genetic alteration, which is insuffcient in itself to induce tumorigenesis.[25~28]

There might be three actions in the mechanism of antiinitiative effect. The first possible explanation is that caffeine alters the binding affinity of a certain chemical to DNA, probably by competing fat binding sites of the DNA, thus, reducing the concentration gradient driving the diffusion process causing the intracellular chemical accumulation.[29~31] The second, molecular complex formation of caffeine and chemical which may by transported through cell membrane at a reduced rate and/or show reduced activity of chemical at the cellular targets.[32] The third, caffeine apparently stimulates the detoxifying enzyme of the aryl hydrocarbon hydroxylase group.[33]

In this study, we used DEN,[34] non-hydrocarbon carcinogen, as initiator and caffeine was treated for one week till 24 hours before DEN injection, therefore, considering the plasma clearance and half-life of caffeine,[2] we can confirm indirectly that the anti-initiative effect of caffeine might be caused by the inhibition of the intracellular carcinogen accumulation. The initiated tissue might be irreversible because the maximum diameter of altered foci showed no difference between groups.

Since a good correlation exists between ability to inhibit in the mid-term test and in long-term experiments,[23,35] it might be expected that caffeine also exert long-term inhibitory potential.

Conclusion

The modification potentialls of caffeine on the development of preneoplastic hepatic enzyme altered foci were examined in an in vivo mid-term assay system. The number and area of glutathione S-transferase placental form(GST-P) positive foci of the liver was significantly reduced in rats given caffeine (2mg or 1mg/ml of drinking water) followed by DEN (200mg/kg B.W. I.P.) as compared with the controls given carcinogen alone. These results suggest that the antiiniative effect of caffeine may be caused by the inhibition of the intracellular carcinogen accumulation and caffeine may posses inhibitory potential for liver carcinogenesis.

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