MODIFYING EFFECT OF KYN-54, A NEWLY SYNTHESIZED RETINOIDAL BUTENOLIDE, ON 2-AMINO-3-METHYLMIDAZO[4,5-*F*]QUINOLINE (IQ)-INDUCED CARCINOGENESIS IN MALE F344 RATS

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Abstract: Modifying effects of KYN-54, a newly synthesized retinoidal butenolide, were investigated on 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced carcinogenesis in rats. One hundred and twenty male F344 rats were divided into 4 groups. Starting at 5 weeks of age, rats were fed the basal diet (Groups 1 and 4) or the experimental diet (0.02% KYN-54) (Groups 2 and 3). At 7 weeks of age, Groups 1 and 2 were given IQ (0.35 m mol/kg body weight, once weekly for 12 weeks) by a intragastric tube. One week after the final IQ intubation, the experimental diet for Group 2 was switched to basal diet. Group 4 was treated as the vehicle control. All rats were sacrificed at the termination (48 weeks after the start of experiment) and 5 rats from each group, which were sacrificed at 12 and 24 weeks. At the termination, sporadic tumors were recognized only in Group 1 (IQ alone) and Group 2 (IQ+KYN-54). But no significant differences for each tumor were available between the 2 groups. Furthermore, no statistical differences were recognized for the incidences of aberrant crypt foci in the 2 groups. However, the density of enzyme altered foci of Group 2 was significantly smaller than of Group 1 ($3.21 \pm 4.49/\text{cm}^2$ vs. $10.71 \pm 9.76/\text{cm}^2$, $P < 0.05$). These results imply that the synthesized butenolide compound has a weakly inhibitory effect on hepatocarcinogenesis in rats. (*J Toxicol Pathol* 9: 323~327, 1996)

Key words: 2-amino-3-methylimidazo[4,5-*f*]quinoline, Carcinogenesis, KYN-54, Chemoprevention, GST-P positive hepatocellular foci

Introduction

Among a number of environmental factors, dietary habits have been regarded as the most important determinant for cancer development in human¹⁻³. Epidemiological data appear to show that individuals eating fried or broiled meat have a significantly elevated risk for intestinal cancer^{4,5}. From cooked foods, a number of carcinogenic heterocyclic amines have been isolated. For instance, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), has attracted considerable attention for strong mutagenicity and carcinogenic potentials in the liver and colon of rodents⁶⁻⁹.

Inhibitory effect of 5-Hydroxy-4-(2-phenyl-(E)ethyl)-2(5H)-furanone (KYN-54), a novel synthesized retinoidal butenolide, on azoxymethane (AOM)-induced intestinal carcinogenesis was demonstrated by us with its exposure during initiation phase¹⁰. Subsequently, it was found that this compound is also effective by exposure during post-initiation phase and expression of aberrant crypt foci (ACF) recognized as a precursor lesion in the large bowel carcinogenesis^{11,12}. Meanwhile, liver cell foci with altered phenotypic expression are also regarded as preneoplastic lesions in hepatocarcinogenesis¹³⁻¹⁷, and such lesions are known to be inducible by IQ⁸. Presently, we investigated the effect of KYN-54 on IQ-induced carcinogenesis in rats. In this study, analysis for expression of 2 precursor lesions in carcinogenesis of liver and colon was also performed.

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Materials and Methods

Animals, diet, water, and carcinogen

Weanling male F344 rats, which were purchased from Shizuoka SLC, Co., Shizuoka, Japan. CE-2 (CLEA Japan Inc., Tokyo, Japan) was used as a basal diet. IQ was kindly gifted from Dr. Wakabayashi, K., Division of Biochemistry, National Cancer Center Research Institute. KYN-54 was donated by Kura-ray. Co. Ltd., Osaka, Japan.

Experimental procedure

A total of 120 rats, 5 weeks of age, were divided into 4 groups: Group 1, 30 rats for IQ alone; Group 2, 30 rats for IQ and 0.02% KYN-54 in initiation phase; Group 3, 30 rats for KYN-54 alone; Group 4, 30 rats for vehicle control.

Rats in Groups 2 and 3 were given 0.02% KYN-54 containing diet from the start of experiment and animals in the other groups were kept on the basal diet. Animals of Groups 1 and 2 were given IQ (0.35 mg/kg body weight) once a week for 12 weeks after the start of experiment. Rats of Groups 1 and 4 were fed the basal diet alone throughout the experiment (48 weeks). For Groups 2 and 3, experimental diet was changed to the basal diet and during the experiment. All animals were housed in wire cages (3 rats/cage). They had free access to water and diets under controlled environmental conditions of humidity ($50 \pm 10\%$), lighting (12 h light/dark cycle), and temperature ($23 \pm 2^\circ\text{C}$). The experimental diet was prepared weekly and stored in a cold room. Five rats from each group were sacrificed 12 and 24 weeks after the start of experiment. At the termination of the experiment, complete autopsies on all animals were performed after sacrifice by ether anesthesia. At

autopsy, the location, number, and size of liver tumors were recorded. Liver tissues were sliced from each lobe and fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for routine histological observation with hematoxylin and eosin stain and for glutathione *S*-transferase placental form (GST-P) stain. An immunohistochemical staining for GST-P was carried out using avidin-biotin-peroxidase complex method (Vectastain ABC kit, Vector Lab. Inc., Burlingame, CA, USA). Anti-GST-P antibody was kindly provided by Dr. K. Satoh, Second Department of Biochemistry, Hirosaki University School of Medicine. The numbers of foci/cm² were measured. GST-P positive lesions composed of more than 11 cells were recognized as the altered liver cell foci.

Statistical analysis

Differences of incidence or density of pathological lesions between groups were analyzed by the χ^2 -test, Fisher's exact probability test, or Student's *t*-test.

Results

Table 1 shows the body, liver and relative liver weights of rats in each group at the termination of experiment. Relative liver weight of rats in Group 1 (IQ alone) was significantly larger than that of rats in Group 2 (IQ+KYN-54) or 4 (control) ($P < 0.05$).

At the end of the experiment, hepatocellular carcinoma, cutaneous squamous cell carcinoma, and testicular Leydig cell tumor were found respectively in one rat, and leukemia were seen in 2 rats of Group 1. In Group 2, hepatocellular carcinoma, colonic adenocarcinoma, cecal adenocarcinoma, small intestinal adenocarcinoma, and ear duct squamous cell carcinoma were seen respectively in one rat, and 2 rats

Table 1. Body, Liver, and Relative Liver Weights in Each Group

Group	Treatment	No. of rats	Body weight (g)	Liver weight (g)	Relative liver weight (%)
1	IQ alone	13	375 $\pm$ 51 ^a	14.4 $\pm$ 3.4	3.89 $\pm$ 0.94
2	IQ+KYN-54	17	386 $\pm$ 50	12.9 $\pm$ 2.5	3.33 $\pm$ 0.39 ^b
3	KYN-54 alone	20	415 $\pm$ 22	13.5 $\pm$ 1.2	3.25 $\pm$ 2.19
4	No treatment	20	402 $\pm$ 38	13.7 $\pm$ 1.1	3.41 $\pm$ 0.29 ^b

^a: Mean $\pm$ SD.

^b: Significantly different from rats treated with IQ alone by Student's *t*-test ($P < 0.05$).

developed testicular Leydig cell tumors (Table 2).

A few ACF were recognized in animals of Group 1, 2, and 4, which were sacrificed at 12 and 24 weeks. But no significant differences in incidence and multiplicity of ACF were found among the groups (Table 3).

GST-P positive hepatocellular foci were detected in rats of Groups 1 and 2. The incidence (% animals) of GST-P positive foci in Group 2 was rather lower than in Group 1, without statistical difference (59% vs. 82%). However, the density (no. of foci/cm²

area of liver section) of the enzyme altered foci of Group 2 ($3.21 \pm 4.49/\text{cm}^2$) was significantly smaller than of Group 1 ($10.71 \pm 9.76/\text{cm}^2$).

Discussion

In this study, only a few hepatocellular neoplasms were available in IQ treated rats. Furthermore, no intestinal tumors were recognized in rats of Group 1 (IQ alone) and not many ACF developed in rats treated with IQ. This suggests that the total

Table 2. Tumor Incidence in Rats Treated with IQ

Treatment	No. of rats	Liver		Intestine						Ear duct		Skin		Testis (Leydig cell tumor)	Leu- kemia	Total	
		HCC ^a	Ad ^b	Total	Colon		Cecum		Sm int ^d		SCC ^e	Pap ^f	SCC				Pap
					Ca ^c	Ad	Ca	Ad	Ca	Ad							
IQ alone	13	1 (8)	0	0	0	0	0	0	0	0	0	0	1 (8)	0	1 (8)	2 (15)	4 (31)
IQ+ KYN-54	17	1 (6)	0	3 (18)	1 (6)	0	1 (6)	0	1 (6)	0	1 (6)	0	0	0	2 (12)	0	7 (41)

(): %.

^a: Hepatocellular carcinoma

^b: Adenoma

^c: Adenocarcinoma

^d: Small intestine

^e: Squamous cell carcinoma

^f: Papilloma

Table 3. Aberrant Crypt Foci of Rats in Each Group

Treatment	14 weeks			24 weeks		
	Incidence	No. of ACF /colon	No. of aberrant crypt /focus	Incidence	No. of ACF /colon	No. of aberrant crypt /focus
IQ alone	3/5	1.4 ± 0.8^a	1.2 ± 0.7	3/5	0.8 ± 0.8	1.8 ± 1.9
IQ/KYN-54	4/5	1.0 ± 1.1	1.5 ± 1.2	5/5	1.2 ± 0.4	4.6 ± 2.2
KYN-54 alone	0/5	0	0	0/5	0	0
No treatment	0/5	0	0	1/5	0.2 ± 0.4	0.4 ± 0.8

a: Mean $\pm$ SD.

Table 4. Results of Hepatocellular Lesions in IQ Treated Animals

Treatment	No. of rats	Average no. of GST-P positive foci/area (/cm ²)	Hepatocellular adenoma (No. of rats)	Hepatocellular carcinoma (No. of rats)
IQ alone	13	10.71 ± 9.76^a	0	1 (5%)
IQ+KYN-54	17	3.21 ± 4.49^b	0	1 (5%)

^a: Mean $\pm$ SD.

^b: Significantly different from rats treated with IQ alone by Student's *t*-test ($P < 0.05$).

volume of IQ used in this study was not enough. Accordingly, further experiments would be necessary to examine the modifying effects of this butenolide compound on IQ-induced carcinogenesis.

Nevertheless, in this study, dietary exposure of KYN-54 suppressed the occurrence of IQ-induced GST-P positive foci in rats. Liver cell foci have been regarded as preneoplastic lesions in the hepatocarcinogenesis¹³⁻¹⁷. GST-P can detect a single initiated cell by positive reactivity and GST-P positive foci are used as reliable biomarkers in short- and medium-term tests to detect the carcinogens and carcinogenesis modifiers¹⁸. It is suggested that KYN-54 weakly suppresses IQ-induced hepatocarcinogenesis.

Recently, KYN-54 was proved to have both blocking and suppressive effects against intestinal carcinogenesis¹⁰⁻¹². In these studies, ODC activity, AgNORs scores, and colonic mucosal polyamine concentration were decreased by the administration of KYN-54^{11,12}. The results seem to suggest that KYN-54 control AOM-induced hyper cell proliferation. This point may be related to the fact that KYN-54 suppressed IQ induced expression of precursor lesions in hepatocarcinogenesis.

Considering that retinoids are regarded as promising agents for the prevention of different human neoplasms, the present results seem to deserve further attention to the possibility that this retinoidal butenolide is useful for protection of liver neoplasms.

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