The Effect of Simultaneous and Alternate Feeding of Furylfuramide and 4-Dimethylaminoazobenzene on Rats*

TORU MIYAJI

Department of Pathology, Osaka University Medical School, Osaka

MIYAJI, T. *The Effect of Simultaneous and Alternate Feeding of Furylfuramide and 4-Dimethylaminoazobenzene on Rats.* Tohoku J. exp. Med., 1971, 103 (4), 371-379 — In view of the remarkable effect of Furylfuramide to induce hypertrophy of liver cells, its possible action as a co-carcinogen or accelerator in hepatic carcinogenesis was examined. However, simultaneous feeding of Furylfuramide and 4-dimethylaminoazobenzene (DAB) inhibited carcinogenesis in the liver by DAB. Protein-bound azo-dye in the liver was reduced in quantity. No inhibitory effect against DAB carcinogenesis was observed when Furylfuramide was fed prior to or after the administration of DAB for various length of time. ——— furylfuramide; 4-dimethylaminoazobenzene; carcinogenesis

As rats showed enlargement and hypertrophy of liver cells when fed on diet with Furylfuramide in high and low concentrations for a short period of time (Miyaji 1971), it was expected that hypertrophy of the liver cell might lead to neoplastic changes of the liver. Therefore, a long-term feeding for as long as two years was carried out as described already, and possible co-carcinogenic or accelerative effect was tested by simultaneous and alternate feeding with 4-dimethylaminoazobenzene (Miyaji 1948).

MATERIALS AND METHODS

1) Simultaneous feeding of Furylfuramide and 4-dimethylaminoazobenzene

One hundred two 4-month-old hybrid male rats weighing 135 to 155 g were divided into three groups; 41 rats were given 0.045% DAB alone (Group 1), 20 rats 0.2% Furylfuramide alone (Group 2) and 41 rats 0.045% DAB and 0.2% Furylfuramide simultaneously (Group 3).

A semisynthetic diet consisting of 73% starch, 12% casein, 10% vegetable oil, 4% salt mixture and 1% vitamin mixture was used as the basal diet. As the amount of riboflavin was important in azo-dye carcinogenesis, it was added at a concentration of 0.2% to the vitamin mixture together with choline chloride at 0.3%.

At the end of 24 weeks, surviving rats were all sacrificed for macroscopical and

Received for publication, May 20, 1970.

* Pathological Study on the Toxicity of Furylfuramide, II.

This work was presented at the 23rd Annual Meeting of Japan Cancer Association held at Tokyo in 1964.

histological examinations. In the meantime, 3 rats each out of Groups 1 and 3 were sacrificed every week from the first to the 7th week, and the liver was subjected to protein-bound azo-dye determination and histological study.

2) *Repeated experiment of simultaneous feeding of Furylfuramide and DAB*

Using the same basal diet, 40 4-month-old male rats weighing 100 to 125g were divided into two groups; one group was fed with DAB alone and the other fed with DAB plus Furylfuramide in the concentration as described above for twenty-four weeks.

3) *Experiment on the effect of Furylfuramide feeding on carcinogenesis due to preceding DAB administration*

Using the same basal diet as described previously, two experiments of Furylfuramide feeding for various length of time were carried out following the administration of DAB for various length of time.

In the first experiment, 98 hybrid rats (Donryu) weighing 120 to 140 g were divided into 7 groups and fed on DAB diet for 0, 4, 8, 12, 16, 20 and 24 weeks. Those fed with DAB for 4, 8, 12 and 16 weeks were further divided into two groups; one fed with Furylfuramide after two weeks' feeding of basal diet, and the other fed with basal diet exclusively till the end of the 24-weeks' experiment.

4) *Experiment on the effect prior feeding of Furylfuramide on carcinogenesis of DAB administration*

Thirty-six 4-month-old male rats weighing 120 to 140 g were divided into two groups (I and II); one group was given 0.2% Furylfuramide diet and another group basal diet of the same composition as previously mentioned³ (Miyaji 1971) for four weeks and then fed 0.045% DAB for the following 16 weeks.

After 4 weeks' experiment, 3 rats in each group were sacrificed, and the remaining 15 rats were given DAB. In the meantime, 2 rats of Group I and 3 of Group II died before the end of the experiment. The amount of DAB given per rat was 695 mg in Group I and 633 mg in Group II.

RESULTS

1) *Simultaneous feeding of Furylfuramide and DAB*

Though approximately the same amount of DAB was administered to rats in Groups 1 (DAB) and 3 (DAB and Furylfuramide), the amount of protein-bound azo-dye in the liver of Group 3 was lower compared with that of Group 1 which showed two peaks as shown in Fig. 1. The protein-bound dye in Group 1 showed a plateau in the second week and began to rise in the 4th week, reaching its highest peak in the 6th week and then gradually falling thereafter. In Group 3 the value was lower than that of Group 1. It reached its peak in the 2nd week and decreased gradually with time.

Changes of the liver. Histological examination of the liver was carried out every week until the 7th week of the experiment. Group 1 rats showed more pronounced changes compared with other groups including Group 3 during these 7 weeks. The results obtained at the end of 24 weeks are shown in Table 1. A fairly high incidence of cyst, cholangiofibrosis and hepatoma was observed in Group 1 compared with rats of Group 3. Nodular hyperplasia was observed in

Fig. 1. The amount of bound dye in the liver of rats fed DAB, and DAB+Furylfuramide.

TABLE 1. *Histological change of the liver of rats fed with Furylfuramide and DAB for 24 weeks*

Group	1 (DAB)	2 (Furylfuramide)	3 (DAB+Furylfuramide)
Total number of rats	18	20	20
Cysts	13	0	7
Cholangiofibrosis	15	0	7
Nodular hyperplasia	0	0	0
Fibrosis	10	0	0
Cirrhosis	1	0	0
Hepatoma	7	0	0
Hepatocellular type	4	0	0
Cholangiocellular type	5	0	0
Mixed type	5	0	0

none of the groups, and a mild increase of connective tissue was noted, which was possibly attributable to the low concentration of DAB (0.045%) in the diet. Hepatoma was only observed in Group 1 rats.

2) Repeated experiment of simultaneous feeding of Furylfuramide and DAB

By the end of the 24th week, two rats in the group of DAB and Furylfuramide died but none in DAB group. Histological changes of the liver at the end of the experiment are shown in Table 2.

In summary, it was evident that simultaneous feeding of Furylfuramide had a suppressive effect on hepatic carcinogenesis by DAB. At the same time, lower concentration of bound-dye in the liver was noted. The mechanism can be interpreted in various ways, and further study is desired. However, it was clear at least that Furylfuramide did not work on the liver in the same way as DAB.

TABLE 2. *Histological changes of the liver in rats fed DAB and Furylfuramide for 24 weeks*

Groups	DAB	DAB and Furylfuramide
Total number of rats	20	18
Cyst	15	6
Cholangiofibrosis	18	6
Nodular hyperplasia	3	1
Fibrosis	10	2
Cirrhosis	0	0
Hepatoma	6	0
Hepatocellular type	3	0
Cholangiocellular type	3	0
Mixed type	3	0

3) *Experiment on the effect of Furylfuramide feeding on carcinogenesis due to preceding DAB administration*

As it was found that administration of DAB from 8 to 12 weeks was critical, 152 rats were divided into 4 groups and fed on DAB diet for 8, 10 and 12 weeks, and were switched to basal diet or Furylfuramide diet after two weeks' feeding of basal diet. In two groups each consisting of 10 rats, the animals were fed DAB for 8 weeks and switched to basal or Furylfuramide diet as described above, and were sacrificed at the end of 20 weeks. Otherwise, as in the first experiment, two experiments were continued until the end of 24 weeks.

Results of alternate feeding of Furylfuramide following DAB administration

Body weight. The curves of average body weight of rats, to which DAB was administered for 8, 10 and 12 weeks in the second experiment, showed a sharp increase in body weight when switched to basal diet, but not so when switched to 0.2% Furylfuramide diet with a distinct difference between the corresponding groups.

Intake of DAB. During the experiment, food intake was measured 3 times a week, and DAB was administered in amounts as shown in Tables 3 and 4 to each rat. Almost the identical value was obtained in two experiments depending on the length of time of DAB administration.

Pathology and summary. There was no macroscopical difference between the liver of rats fed Furylfuramide or basal diet after DAB administration except for the color of the liver of Furylfuramide rats, which appeared dark red compared with basal diet group. All the animals in both experimental groups were sacrificed after 24 weeks' experiment. Histological examination of the liver was made for such changes as cyst, cholangiofibrosis, nodular hyperplasia, fibrosis, cirrhosis, and hepatocellular tumors. The results are summarized in Tables 3 and 4. Statistically, no significant difference was found between those given Furylfuramide diet and basal diet after the administration of DAB for various period

TABLE 3. *Average total intake of DAB by rat and lesions of the liver (Experiment I)*

Experiment	Initial number of rat	Amount of DAB	Final number of rat	Liver cell tumor	Cirrhosis
Furylfuramide 4 wks	7	120.9 mg	4	0	0
DAB 4 wks + Furylfuramide 18 wks	7	120.9	5	0	0
DAB 4 wks + Basal diet	7	118.5	5	0	1
DAB 8 wks + Furylfuramide 14 wks	7	318.8	5	0	0
DAB 12 wks + Furylfuramide 10 wks	7	499.0	5	3	3
DAB 12 wks + Basal diet 10 wks	7	494.3	5	5	2
DAB 16 wks + Furylfuramide 6 wks	7	610.5	5	3	1
DAB 16 wks + Basal diet 6 wks	7	609.4	5	5	2
DAB 20 wks + Furylfuramide 2 wks	7	719.3	5	5	4
DAB 20 wks + Basal diet 2 wks	7	722.0	5	5	3
DAB 24 wks	7	789.9	5	5	5

of time, though simultaneous feeding of Furylfuramide and DAB inhibited the change of the liver caused by DAB. It was also found that Furylfuramide did not have any effect of promoting carcinogenesis with DAB.

4) *Experiment on the effect of prior feeding of Furylfuramide on carcinogenesis of DAB administration*

Macroscopical and histological examination revealed no difference in the organs between the groups as summarized in Table 5. Although the results obtained were not confirmed by repeated experiments as in the preceding experiments, it was considered that Furylfuramide did not have an initiating effect on carcinogenesis by DAB.

It was also considered that the inhibitory action of simultaneous feeding of Furylfuramide on DAB was only effective on the process of metabolizing DAB in the liver.

DISCUSSION

Simultaneous feeding of Furylfuramide with DAB to rats suppressed the development of carcinoma in the liver remarkably as shown in Tables 1 and 2. However, it was not clear whether the suppression was due to enhanced metabolism of DAB in the liver (Pitot and Heidelberger 1963), or to delay of synthesis of protein to be bound to DAB, or Furylfuramide competed with DAB for special protein such as 'slow h_2 ' (Sorof *et al.* 1963). The amount of protein-bound azo dye in the liver has been considered to be the good measure of the relation of the liver with DAB and the process of carcinogenesis in the liver, as the substance does not appear in the liver of the animal which does not develop carcinoma of the liver with DAB, and it does decrease gradually with malignant change of the liver in

TABLE 4. *Average total intake of DAB by rat*

Experiment	Number of rats	Amount of DAB	Number of rats	Cyst
DAB 8 wks+Furylfuramide 10 wks	10	326.3	10	8
DAB 8 wks+Basal diet 10 wks	10	329.8	10	8
DAB 8 wks+Furylfuramide 14 wks	22	330.8	10	17
DAB 8 wks+Basal diet 14 wks	22	329.9	20	17
DAB 10 wks+Furylfuramide 12 wks	22	387.8	20	18
DAB 10 wks+Basal diet 12 wks	22	389.6	20	18
DAB 12 wks+Furylfuramide 10 wks	22	497.1	21	21
DAB 12 wks+Basal diet 10 wks	22	498.2	21	20

TABLE 5. *Liver lesions of rats fed DAB for 16 weeks following four weeks' administration of 0.2% Furylfuramide diet or basal diet*

Lesions	Furylfuramide and DAB	Basal diet and DAB
Total number of rats	12	12
Cysts	13	12
Bile duct proliferation	13	10
Cholangiofibrosis	7	7
Nodular hyperplasia	9	9
Fibrosis	4	1
Cirrhosis	9	11
Hepatoma	5	5
Hepatocellular type	2	3
Cholangiocellular type	3	3

rats (Miller and Miller 1947, Ueda 1967 a, b, c, Terayama and Sasada 1968). In the present experiment, the protein-bound azo-dye in the rat fed DAB alone reached a plateau in the 2nd week, and then to its highest peak in the 6th week to fall off gradually with time. In the rats simultaneously fed Furylfuramide and DAB, the lower peak was attained in the 2nd week and the concentration fell gradually thereafter.

The suppressive effect of Furylfuramide in simultaneous administration with DAB was confirmed by Miyaki and his associates repeatedly (Miyaki *et al.* 1968, 1969 a, b). Studying the metabolic fate of DAB in the rat fed DAB and Furylfuramide, Miyaki and his associates noted a decrease of DAB and its metabolites in the liver and blood, and an increased activity of the liver to metabolize DAB, when DAB and Furylfuramide were administered to the rat for more than 3 to 4 months.

The point to be made clear, however, was whether Furylfuramide could work as a co-carcinogen or initiator of carcinogenesis. Although the simultaneous feeding suppressed the development of malignant change in the liver markedly, neither previous feeding of Furylfuramide followed by DAB administration, nor following administration of Furylfuramide after DAB feeding for various length of time suppressed or accelerated the carcinogenesis by DAB.

and lesions of the liver (Experiment II)

Cholan- giofi- brosis	Nodular hyper- plasia	Fibrosis	Cirrhosis	Carci- noma of liver	Hepato- cellular type	Cholangio- cellular type	Mixed type
7	1	1	1	0	—	—	—
6	2	3	0	0	—	—	—
13	2	3	0	1	0	1	0
13	3	3	0	1	0	1	0
14	1	6	0	3	1	1	1
15	3	3	2	2	1	3	0
18	10	10	3	12	4	5	5
19	4	10	4	13	8	6	1

References

- 1) Miller, E.C. & Miller, J.A. The presence and significance of bound aminoazo-protein in livers of rats fed p-dimethylaminoazobenzene. *Cancer Res.*, 1947, **7**, 468-480.
- 2) Miyaji, T. Experimental hepatoma caused by azo-dye derivatives. Nanjo Publ., Tokyo, 1948.
- 3) Miyaji, T. Acute and chronic toxicity of Furfylfuranide in rats and mice. *Tohoku J. exp. Med.*, 1971, **103**, 331-369.
- 4) Miyaki, K., Akao, M., Kuroda, K. & Terao, K. Mode of action of inhibitors on DAB carcinogenesis. Study on the metabolic fate of DAB in rats. Proc. 27th Ann. Meeting Japan Cancer Ass., 1968, pp. 70-71.
- 5) Miyaki, K., Akao, M., Kuroda, K. & Kanisawa, M. (a) Effect of nitrofurans on induction of hepatic tumors in rats fed 4-(dimethylamino)-azobenzene. Proc. 28th Ann. Meeting Japan Cancer Ass., 1969, pp. 65-65.
- 6) Miyaki, K., Akao, M., Terao, K. & Kuroda, K. (b) Modes of action of three bacteriostatic agents in their inhibitory effect on induction of hepatoma in rats fed 4-(dimethylamino)-azobenzene. I. Effect on metabolic fate of 4-(dimethylamino)-azobenzene. *Gann*, 1969, **60**, 169-179.
- 7) Pitot, H.C. & Heidelberger, C. Metabolic regulatory circuits and carcinogenesis. *Cancer Res.*, 1963, **23**, 1694-1700.
- 8) Sorof, S., Young, E.M., McCue, M.M. & Fetterman, P.L. Zonal electrophoresis of the soluble proteins of liver and tumor in azo dye carcinogenesis. *Cancer Res.*, 1963, **23**, 864-882.
- 9) Terayama, H. & Sasada, M. Activity of the cell-sap factor inhibiting DNA synthesis of rat ascites hepatoma cells in normal tissues, various malignant and minimal deviation hepatomas, as well as livers during the course of carcinogenesis. *Gann*, 1968, **59**, 51-59.
- 10) Ueda, Y. (a) The relation between experimental liver carcinoma and liver cirrhosis induced by simultaneous administration of p-dimethylaminoazobenzene and carbon tetrachloride. *Tohoku J. exp. Med.*, 1967, **92**, 83-107.
- 11) Ueda, Y. (b) The relation between experimental liver carcinoma and liver cirrhosis induced by successive administration of p-dimethylaminoazobenzene and carbon tetrachloride. *Tohoku J. exp. Med.*, 1967, **92**, 109-140.
- 12) Ueda, Y. (c) Experimental liver carcinoma and liver cirrhosis induced by p-dimethylaminoazobenzene after preliminary carbon tetrachloride injections. *Tohoku J. exp. Med.*, 1967, **92**, 175-206.

Fig. 2. The liver of rat fed Furfurylamine and DAB simultaneously for 24 weeks. No macroscopical lesion of the liver was noted.

Fig. 3. Histology of the liver shown in Fig. 2. Hypertrophic liver cells with clear cytoplasm are noted, but there was no deterioration of lobular structure.

Fig. 4. The liver of rat fed DAB for 24 weeks. It shows nodular appearance of the surface due to development of hepatocellular carcinoma.

Fig. 5. Histology of the liver shown in Fig. 2. Typical hepatocellular carcinoma caused by DAB administration.