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Modification by Analgesics of Lesion Development in the Urinary Tract and Various Other Organs of Rats Pretreated with Dihydroxy-di-N-propylnitrosamine and Uracil

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Effects of the analgesics phenacetin, acetaminophen and antipyrine on lesion development in the urinary tract and other organs in male F344 rats were investigated. Animals were concurrently administered with 0.1% dihydroxy-di-N-propylnitrosamine (DHPN) in drinking water and 3.0% uracil in the diet for 4 weeks and then, starting 1 week after the cessation of this treatment, received basal diet or diet containing phenacetin, acetaminophen or antipyrine for 35 weeks. The occurrences of renal cell tumors were increased in the groups given phenacetin or antipyrine, as compared with the DHPN+uracil alone controls. Antipyrine, but not the two other compounds, also enhanced development of hyperplastic lesions in the renal pelvis and ureter. In the urinary bladder, phenacetin and antipyrine treatments were both associated with increased incidence of preneoplastic or neoplastic lesions. Furthermore, phenacetin alone, without the initiating agent pretreatments, induced simple hyperplasias of the urinary bladder at high incidence. Antipyrine enhanced induction of hyperplastic lesions in the ureter and was also found to increase the incidences of preneoplastic and neoplastic lesions in the liver. Although decreased incidences of tumor development of lung and thyroid were observed for the group given phenacetin, this might have been linked to the decreased weight gain. The results confirmed that combination treatment with DHPN+uracil is effective for wide-spectrum initiation of carcinogenesis in the urological tract and demonstrated significant modification potential for both phenacetin and antipyrine.

Key words: Analgesics — Uracil — Urinary tract — Carcinogenesis — Rat

Abuse of analgesics containing phenacetin, antipyrine and acetaminophen has been linked with the development of urological tumors in man.[1, 2] Phenacetin can induce renal cell and urinary bladder tumors in rats and mice.[3, 4] Antipyrine was also reported to have carcinogenic potential for the rat urinary tract[5, 6] and has been withdrawn from clinical use. With respect to acetaminophen, its carcinogenicity is equivocal in animal experiments.[7, 8]

Uracil (a component of RNA) treatment for a short period is associated with calculi formation and papillomatosis in the urinary bladder of rodents as well as renal pelvic hyperplasia and hydronephrosis.[9–11] Calculi mechanically stimulate cell-proliferation not only in the urinary bladder epithelium but also the renal pelvic epithelium.[12] This is of significance because, recently, it was reported that a bladder tumor promoter Na_3PO_4[13] causes neoplastic lesions in the renal pelvis in rats pretreated with uracil.[12] Although even whole-life exposure to the potent bladder carcinogen N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) in normal rats does not normally cause renal pelvic tumors,[14] such lesions arise, in addition to urinary bladder tumors, under special conditions such as surgical ligation of the ureter[15] or in mutant strains of rodents having congenital hydronephrosis.[16, 17] Thus, urine stagnation might be very important for induction of renal pelvic tumors by BBN.

Dihydroxy-di-N-propylnitrosamine (DHPN) is a carcinogen which is known to induce tumors of the renal pelvis, renal tubular cells and urinary bladder in addition to the lung, liver, thyroid and esophagus of rats.[18, 19] In this study, therefore, we tested the hypothesis that DHPN administration would effectively initiate kidney, urinary bladder and ureter carcinogenesis under the conditions of hydronephrosis and increased cell proliferation of the urothelium stimulated by uracil-induced calculi. Using a combination uracil and DHPN treatment model, the effects of three analgesics (phenacetin, antipyrine and acetaminophen) were also investigated with this wide-spectrum urological tract initiation approach.

MATERIALS AND METHODS

Animals A total of 110 male F344 rats, purchased at 6 weeks of age (Charles River Japan, Inc., Kanagawa) were used. The animals were housed 5 to a plastic cage with hardwood chips for bedding and were given free

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access to water and food under conditions of controlled temperature (22±2°C), humidity (60±10%) and lighting (12 h–12 h light-dark cycle).

Chemicals DHPN was purchased from Nacalai Tesque, Inc. (Kyoto). Uracil was from Yamasa Shoyu Co. (Choshi). Antipyrine, acetaminophen and phenacetin were from Wako Pure Chemical Industries Ltd. (Osaka).

Experimental procedure Animals were randomly divided into 7 groups of 10 or 20 rats each. For the first 4 weeks, those in groups 1 to 4 were concomitantly treated with 0.1% DHPN in drinking water and 3.0% uracil in the diet and then, 1 week after cessation of this treatment, received basal diet (group 4) (Oriental Yeast Co., Tokyo) or diet containing 2.0% phenacetin (group 1), 0.8% acetaminophen (group 2) or 1.0% antipyrine (group 3) for 35 weeks. The rats of groups 5–7 were given analgesics as in groups 1 to 3 without the initial combination treatment with DHPN and uracil. The dose levels of DHPN, uracil and analgesics were determined on the basis of previous study results.[9, 17, 19–21]

Animals were observed daily to assess their general health, and body weight and food and water consumption were measured weekly for the first 4 weeks and then biweekly until 12 weeks and once every 4 weeks thereafter.

The experiment was terminated and all surviving animals were killed by exsanguination under ether anesthesia at week 40. The kidneys were resected, weighed, and immersed in fixative, the right one being sagitally divided and the left one horizontally cut into two, then embedded in paraffin and routinely prepared for histopathological assessment (stained with hematoxylin and eosin). Urinary bladders were ligated at the neck and inflated by intraluminal injection of 10% phosphate-buffered formalin after which they were removed quickly and immersed in the fixative. Following fixation, the bladders were bisected longitudinally and excess moisture was absorbed with filter papers. After being weighed, they were cut into eight strips and processed for routine histopathological examination. Ureters, lung, liver and thyroids were also removed and processed for histopathology.

Statistical analyses The significance of differences between groups in body weights, urinalysis and quantitative histopathology data was analyzed by using Student's *t* test via the method of Welch (in the case of insufficient homogeneity of variance). The incidences of lesions were analyzed by means of Fisher's exact test.

RESULTS

General In the groups pretreated with DHPN+uracil, two rats were found dead during the experimental period. One at week 15 in the basal diet group died of leukemia, and the other at week 39 in the acetaminophen group had a kidney tumor. Data for intakes of DHPN, uracil and analgesics are presented in Table I. There were no apparent differences in average DHPN and uracil intakes among groups 1 to 4, indicating all to have been equally initiated with DHPN and uracil. Average analgesic intake was calculated from food consumption values, which were comparable among the groups. Water consumption values showed a tendency for increase in the groups given antipyrine in comparison with the DHPN+uracil alone control group. Final body weights, and urinary bladder and kidney weights are summarized in Table II. Decrease in final body weights was observed for the phenacetin and antipyrine groups independent of prior DHPN+uracil treatment. Relative kidney weights were significantly increased in the phenacetin group given pretreatment. Absolute and relative bladder weights were significantly increased in the phenacetin and antipyrine groups given pretreatment.

Histopathology

Kidney (Table III): In the DHPN+uracil-treated groups, phenacetin significantly increased hyperplasias

Table I. Average Chemical Intake and Food and Water Consumption Data for Rats Treated with DHPN+Uracil Followed by Analgesics

Treatment		No. of rats	Average chemical intake (mg/kg/day)					Food consumption (g/rat/day)	Water consumption (g/rat/day)
DHPN + Uracil	Chemical		DHPN	Uracil	Phenacetin	Acetaminophen	Antipyrine		
+	Phenacetin	20	152	1657	1145	—	—	15.8	20.7
+	Acetaminophen	20	157	1782	—	359	—	14.5	19.9
+	Antipyrine	20	153	1607	—	—	523	16.0	30.3
+	Basal diet	19	153	1737	—	—	—	13.7	19.1
—	Phenacetin	10	—	—	1068	—	—	16.7	23.4
—	Acetaminophen	10	—	—	—	376	—	17.1	23.4
—	Antipyrine	10	—	—	—	—	510	17.5	38.2

Table II. Average Final Body Weights, and Kidney and Urinary Bladder Weights for Rats Treated with DHPN+Uracil Followed by Analgesics

Treatment		No. of rats	Final body weights (g)	Organ weights (%)	
DHPN+Uracil	Chemical			Kidneys	Bladder
+	Phenacetin	20	306±15**	0.81±0.18*	0.04±0.01**
+	Acetaminophen	20	383±26	0.90±0.81	0.02±0.04
+	Antipyrine	20	332±17**	1.35±1.72	0.03±0.01**
+	Basal diet	19	402±34	0.64±0.24	0.02±0.01
−	Phenacetin	10	337±24	0.72±0.07	0.03±0.01
−	Acetaminophen	10	424±28	0.67±0.04	0.03±0.04
−	Antipyrine	10	360±19	0.80±0.07	0.03±0.01

* $P<0.05$, ** $P<0.01$ as compared with DHPN+uracil alone.

Table III. Incidences of Proliferative and Neoplastic Lesions of the Kidney in Rats Treated with DHPN+Uracil Followed by Analgesics

Lesion	DHPN+Uracil pretreatment				Non-pretreated		
	Phenacetin n=20	Acetaminophen n=20	Antipyrine n=20	− n=19	Phenacetin n=10	Acetaminophen n=10	Antipyrine n=10
Renal pelvis							
Hyperplasia	6 (30)	5 (25)	17 (85)**	3 (16)	0	0	0
Papilloma	0	0	1 (5)	2 (10)	0	0	0
Carcinoma	1 (5)	2 (10)	2 (10)	3 (16)	0	0	0
Renal tubules							
Hyperplasia	15 (75)**	4 (20)	3 (15)	1 (5)	0	0	0
Adenoma	5 (25)	2 (10)	7 (35)	1 (5)	0	0	0
Adenocarcinoma	4 (20)*	0	1 (5)	0	0	0	0
Tumor[a)]	9 (45)**	2 (10)	8 (40)**	1 (5)	0	0	0
Nephroblastoma	2 (10)	7 (35)	4 (20)	2 (11)	0	0	0

* $P<0.05$, ** $P<0.01$ as compared with DHPN+uracil alone.
a) Renal cell tumors refers to adenomas and adenocarcinomas in combination.

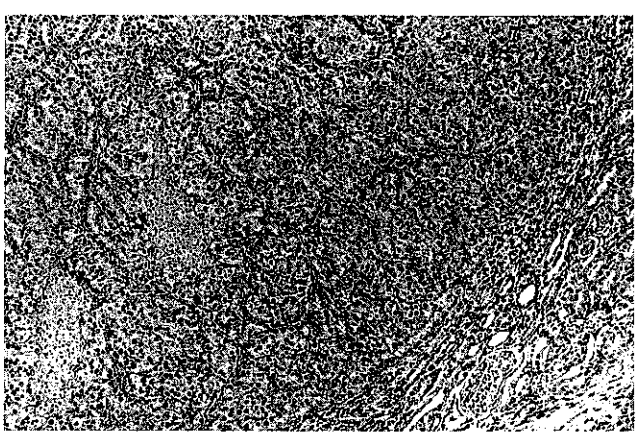

Fig. 1. A tubular adenocarcinoma of the kidney in a rat given 2.0% phenacetin after DHPN + uracil pretreatment. H & E. ×100.

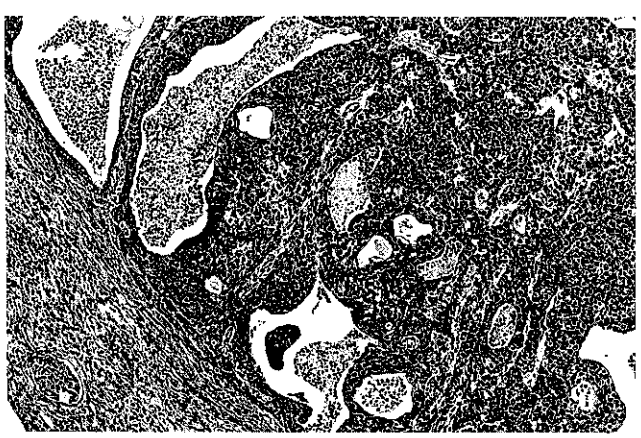

Fig. 2. A renal pelvic TCC in a rat given 1.0% antipyrine after DHPN+uracil pretreatment. H & E. ×100.

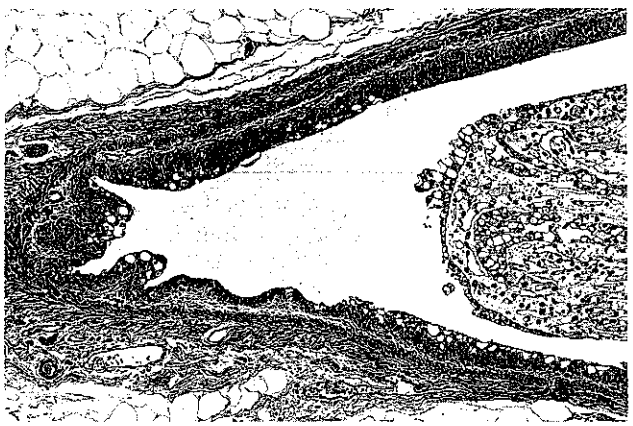

Fig. 3. A transitional hyperplasia of the renal pelvis in a rat given 1.0% antipyrine after DHPN+uracil pretreatment. Note many cytoplasmic vacuolations in the pelvic and papilla epithelia. H & E. ×100.

and adenocarcinomas (Fig. 1) arising from tubular epithelial cells. For combined renal cell tumors (adenomas +adenocarcinomas) significant increases were observed for the groups given phenacetin and antipyrine. With respect to lesions of the renal pelvis, although prior DHPN+uracil treatment caused hyperplasia, papilloma and carcinomas (Fig. 2) of transitional epithelial cell origin, none of the analgesics used in the present study enhanced renal pelvic tumor development. Antipyrine after DHPN+uracil pretreatment significantly increased only transitional cell hyperplasias (Fig. 3). In addition, vacuolation of renal pelvis epithelium was frequently observed for the antipyrine-treated groups independent of DHPN+uracil treatment (Fig. 3). In the non-DHPN +uracil-treated groups, administration of analgesics did not induce any proliferative lesions in the kidneys.

Urinary bladder and ureter (Table IV): In the DHPN +uracil-pretreated groups, the incidences of simple

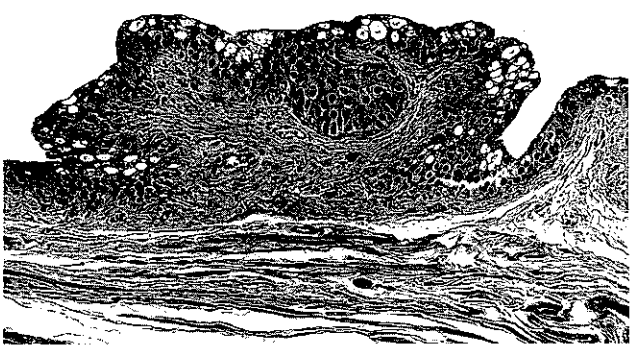

Fig. 4. A PN hyperplasia with marked cytoplasmic vacuolations, observed in the urinary bladder of a rat given 1.0% antipyrine after DHPN+uracil pretreatment. H & E. ×165.

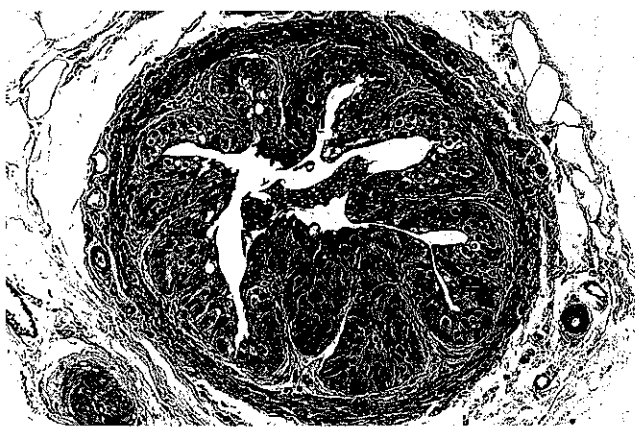

Fig. 5. A simple hyperplasia with slight cytoplasmic vacuolations in the ureter of a rat given 1.0% antipyrine after DHPN+uracil pretreatment. H & E. ×165.

Table IV. Incidences of Proliferative and Neoplastic Lesions of the Urinary Bladder and Ureter in Rats Treated with DHPN +Uracil Followed by Analgesics

Organ/Lesion	DHPN+Uracil pretreatment				Non-pretreated		
	Phenacetin n=20	Acetaminophen n=20	Antipyrine n=20	− n=19	Phenacetin n=10	Acetaminophen n=10	Antipyrine n=10
Urinary bladder							
Simple hyperplasia	18 (90)**	3 (15)	11 (55)*	4 (21)	8 (80)	0	0
PN hyperplasia	13 (65)**	2 (10)	7 (35)*	1 (5)	1 (10)	0	0
Papilloma	4 (20)*	1 (5)	1 (5)	0	0	0	0
Ureter							
Simple hyperplasia	0	1 (5)	6 (30)**	0	0	0	0
PN hyperplasia	0	0	2 (10)	0	0	0	0

* $P<0.05$, ** $P<0.01$ as compared with DHPN+uracil alone.

hyperplasia, papillary or nodular (PN) hyperplasia (regarded as a preneoplastic lesion) and papillomas of the urinary bladder were significantly increased by phenacetin administration. Antipyrine significantly induced simple hyperplasia and PN hyperplasias (Fig. 4) of the bladder. Phenacetin without prior DHPN+uracil treatment caused simple hyperplasia and PN hyperplasias of the bladder.

In the ureter, the incidence of simple hyperplasia (Fig. 5) was significantly increased in the antipyrine group after DHPN+uracil pretreatment and in addition, a few PN hyperplasias developed in this group. Proliferative lesions of the ureter were not observed in the non-DHPN +uracil treatment groups.

Vacuolation of epithelial cells of the urinary bladder (Fig. 4) and ureter (Fig. 5) was frequently noted in the groups given antipyrine with or without DHPN+uracil treatment.

Liver and esophagus (Table V): Incidences of foci of cellular alteration and hyperplastic nodules of the liver were significantly elevated in the antipyrine group after prior carcinogen treatment and furthermore, a few hepatocellular carcinomas developed in this group. Esophageal lesions were comparable among the groups pretreated with DHPN+uracil. No lesions of the liver or esophagus were observed in the non-DHPN+uracil treatment groups.

Lung and thyroid (Table VI): Significant decreases in development of adenomas and adenocarcinomas of the lung and thyroid were found in the phenacetin group after DHPN+uracil pretreatment. No such lesions were observed in the non-pretreatment groups.

Table V. Incidences of Proliferative and Neoplastic Lesions of the Liver and Esophagus in Rats Treated with DHPN+Uracil Followed by Analgesics

Organ/Lesion	DHPN+Uracil pretreatment				Non-pretreated		
	Phenacetin n=20	Acetaminophen n=20	Antipyrine n=20[a)]	− n=19	Phenacetin n=10	Acetaminophen n=10	Antipyrine n=10
Liver							
Foci	0	0	13 (100)**	2 (11)	1 (10)	0	0
Hyperplastic nodule	0	0	4 (31)**	0	0	0	0
HCC	0	0	2 (15)	0	0	0	0
Esophagus							
Simple hyperplasia	12 (60)	18 (90)	20 (100)	17 (89)	0	0	0
PN hyperplasia	0	2 (10)	2 (10)	0	0	0	0
Papilloma	5 (25)	8 (40)	4 (20)	5 (26)	0	0	0

** $P<0.01$ as compared with DHPN+uracil alone.
a) Numbers of livers available in the antipyrine group were only 13 because this organ was not initially intended for examination but macroscopic lesions were found on terminal examination.

Table VI. Incidences of Proliferative and Neoplastic Lesions of the Lung and Thyroid in Rats Treated with DHPN+Uracil Followed by Analgesics

Organ/Lesion	DHPN+Uracil pretreatment				Non-pretreated		
	Phenacetin n=20	Acetaminophen n=20	Antipyrine n=20	− n=19	Phenacetin n=10	Acetaminophen n=10	Antipyrine n=10
Lung							
Hyperplasia	19 (95)	20 (100)	20 (100)	19 (100)	0	0	0
Adenoma	12 (60)**	20 (100)	18 (90)	19 (100)	0	0	0
Adenocarcinoma	4 (20)**	13 (65)	10 (50)	14 (74)	0	0	0
SCC	1 (15)	2 (10)	5 (25)	4 (21)	0	0	0
Thyroid							
Hyperplasia	14 (70)	17 (85)	19 (95)	13 (68)	0	0	0
Adenoma	3 (15)*	10 (50)	7 (35)	9 (47)	0	0	0
Carcinoma	3 (15)**	10 (50)	14 (70)	11 (58)	0	0	0

* $P<0.05$, ** $P<0.01$ as compared with DHPN+uracil alone.

DISCUSSION

Phenacetin has been shown to have genotoxic properties in bacterial mutagenesis,[22] forward mutation[23] and sister chromatid exchange[24] tests. In addition, it is a carcinogen for the urinary bladder and renal tubules but not renal pelvis in rodents.[3,25] Human epidemiologic studies, on the other hand, have demonstrated an association between phenacetin abuse and tumor development in both the urinary bladder and renal pelvis.[1,5] In line with this, Anderström *et al.* have reported that phenacetin can act as a promoter for the renal pelvis in rats[26] and furthermore, can induce renal pelvic tumors when administered to a special rat strain SD/cShi which suffers from hereditary hydronephrosis.[17] The clear correlation that has been shown between renal pelvic carcinogenesis and hydronephrosis[12,14,16,17] is presumably related to stasis of urine containing proximate carcinogens, so that exposure of the renal pelvis is prolonged.

The present animal model was intended to make the renal pelvis and ureter more susceptible to the action of DHPN because of proliferation and hydronephrosis due to uracil-induced urolithiasis. The results indicated that this approach was indeed successful, at least for the renal pelvis.

Of ingested phenacetin, 70% is metabolized in the liver to acetaminophen, which is conjugated and then excreted in the urine.[27] However, this compound is presumed to be non-genotoxic[28–30] and although animal experiments using mice have demonstrated carcinogenicity[7] and promoting activity[31] in the liver rather than the urinary tract, it does not exert any carcinogenic potential in rats.[8] It was previously reported that this compound inhibits hepatocarcinogenesis, while promoting renal cell carcinogenesis in rats initiated with N-ethyl-N-hydroxyethylnitrosamine.[21] Furthermore, acetaminophen possesses no initiating activity for the rat liver.[32] Thus, data regarding acetaminophen carcinogenicity are conflicting and in the current experiment, this agent did not demonstrate any promoting activity.

In an earlier two-year study, administration of antipyrine resulted in a low incidence of urinary tract tumors in Sprague-Dawley rats.[5] However, administration to the same strain of animals pretreated with N-[4-(5-nitro-2-furyl)thiazolyl]formamide (FANFT) resulted in enhanced tumor development in the urinary bladder and renal pelvis.[6] In the present study, antipyrine also enhanced tumorigenicity in the same sites, in addition to influencing the renal tubules, ureter and liver. Cytoplasmic vacuolization of the uroepithelium (urinary bladder, renal pelvis and ureter) was frequently observed, in line with the findings and increased DNA synthesis in the urinary tract after antipyrine treatment reported by Johansson *et al.*[20] These latter authors suggested that this change is a sign of toxic effects with degeneration and cell death, possibly involving an apoptotic process, followed by regeneration, being responsible for the increment in DNA synthesis.[20] Thus, in the case of antipyrine, increased urothelial proliferation might have played a role in the observed promoting activity. It has been shown that interaction of antipyrine with nitrite leads to formation of 4-nitrosoantipyrine under acidic conditions such as those which prevail within the stomach.[33] In addition, it has been reported that antipyrine metabolites generate high levels of formaldehyde in the bile in an isolated perfused rat liver system.[34] Since these compounds have been shown to have mutagenic properties,[35] another possibility is that these might have contributed to the enhancing effects of antipyrine.

In conclusion, the present study indicated that combination treatment with DHPN+uracil is an effective initiation schedule for the entire urological tract. The experiments also provided support for carcinogenic or promoting potential for phenacetin and antipyrine in this organ system, as well as for antipyrine on the rat liver.

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