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1-(2-Tetrahydrofuryl)-5-fluorouracil in combination with uracil suppresses mammary carcinogenesis and growth of tumors induced with 7,12-dimethylbenz[a]anthracene in rats

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The effects of 1-(2-tetrahydrofuryl)-5-fluorouracil in combination with uracil (UFT) on mammary carcinogenesis and growth of tumors induced with 7,12-dimethylbenz-[a]anthracene (DMBA) were investigated in rats. Daily oral administration of UFT reduced the incidence and number of mammary tumors compared with those of the DMBA control group, resulting in lower activities in DNA synthesizing enzymes, thymidylate synthetase and thymidine kinase, and a reduction of bromodeoxyuridine-immunoreactive (S-phase) cells in mammary tumors of UFT-treated rats.

Key words: Bromodeoxyuridine immunohystochemistry, DMBA, mammary tumor, thymidine kinase, thymidylate synthetase, UFT,

Introduction

The anticancer action of 5-fluorouracil (5-FU) and its derivatives has been ascribed to three major mechanisms:[1] (1) metabolism to 5-fluoro-2-deoxyuridine-5-monophosphate (FdUMP), which is a potent suicide inhibitor of thymidylate synthetase (TS; EC 2.1.1.45); (2) incorporation of the ribonucleoside triphosphate of 5-FU into some species of RNA; and (3) incorporation into DNA. Previously we reported that one of the 5-FU derivatives, 1-(2-tetrahydrofuryl)-5-fluorouracil, in combination with uracil (UFT) suppressed colonic[2] and hepatic[3] carcinogenesis induced with 1,2-dimethylhydrazine and 3′-methyl-4-dimethylaminoazo-benzene, respectively, in rats.

In the present study, we investigated the effects of UFT on mammary carcinogenesis and growth of mammary tumors induced with 7,12-dimethylbenz-[*a*]anthracene (DMBA) in rats.

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Materials and methods

Animals and treatment

Sprague-Dawley female rats (Sankyo Laboratory Service, Tokyo, Japan) were given a single i.v. injection of 5 mg of DMBA (special 15% fat emulsion with DMBA; Upjohn, Kalamazoo, USA; a gift from Professor Dr CB Huggins) at 48 days of age. Simultaneously, laboratory standard diet (CE-2; CLEA Japan, Tokyo, Japan) with or without UFT [500 mg of 1-(2-tetrahydrofuryl)-5-FU and 1.12 g of uracil in 1 kg of diet; Taiho Pharmaceutical, Tokyo, Japan] was given to animals throughout the experiment. Each rat was weighed, and the appearance of palpable mammary tumor and the tumor size in each rat were recorded once a week. Three animals in the control and experimental groups, 15 animals each, were given single i.v. injections of bromodeoxyuridine (BrdU; 10 mg/kg body weight; Cell Proliferation Kit, RPN 20LR(5), Amersham, UK) in the tail vein 6 h before sacrifice at 30 weeks of age. Mammary tumors removed from rats given BrdU were immediately fixed in 10% formaldehyde buffer solution (pH. 7.2.). At autopsy at 30 weeks of age, the remaining 12 animals without BrdU injection in each group were bled by cardiac puncture under deep anaesthesia with urethane (1.5 g/kg body weight; Merck, Darmstadt, Germany) and the mammary tumors, anterior pituitary, adrenals, ovaries and uterus were removed and weighed. Tumors and separated plasma were stored at − 80°C.

Plasma levels of hormones and 5-FU

Plasma levels of prolactin (PRL), estradiol (E_2) and progesterone (PRG) were determined by radioimmunoasssay kits (PRL, a gift from the National Insti-

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tute of Arthritis, Metabolism and Digestive Diseases [NIAMDD] Rat Pituitary Hormone Distribution Program and Dr AF Parlow; E_2 and PRG, Diagnostic Products, Los Angeles, CA). Plasma levels of 5-FU, 1-(2-tetrahydrofuryl)-5-FU and uracil were measured by the method of Marunaka and Umeno[4] using HPLC in the laboratory of Taiho Pharmaceutical (Tokushima, Japan).

Immunohistochemistry using BrdU

BrdU incorporated into cellular DNA was detected by a monoclonal anti-BrdU antibody by the procedure described in the protocol. Two mammary tumors removed from each rat given BrdU in each group were used. Six sections in each group were randomly chosen and BrdU-immunoreactive cells were counted in 400 cells per each section. The results were expressed in terms of BrdU-immunoreactive (S-phase) cells as a percentage of total cells.

Enzyme preparation and assay

As previously reported,[2,3] the activities of TS and thymidine kinase (TK; EC 2.7.1.21) were determined by the methods of Dunlap *et al.*[5] and Taylor *et al.*,[6] respectively. Enzyme activities were normalized to tissue contents of protein, and were expressed as fmol/mg protien/min. Values were means of duplicate assays.

Statistical analyses

The statistical significance of difference between groups was evaluated by Student's *t*-test and $p < 0.05$ was considered significant

Table 1. Body growth and organ weights (mean ± SEM)

	DMBA control ($n=12$)	UFT ($n=12$)
Body Weight (g)		
initial	165.9 ± 2.3	162.1 ± 4.9
final	278.1 ± 6.4	250.0 ± 4.9
change (%)	188.9 ± 6.3	176.6 ± 7.6
Organ wet weights (mg)		
anterior pituitary	18.9 ± 1.0	20.2 ± 1.0
adrenals	81.9 ± 2.5	84.2 ± 6.9
ovaries	84.0 ± 10.8	71.0 ± 6.4
uterus	432.8 ± 45.6	459.3 ± 18.2
spleen	556.9 ± 44.2	512.7 ± 61.6

Table 2. Plasma levels of hormones and 5-FUs (mean ± SEM)

	DMBA control ($n=12$)	UFT ($n=12$)
Hormones		
PRL (ng/ml)	74.30 ± 15.77	68.75 ± 17.03
E_2 (pg/ml)	5.79 ± 1.82	5.07 ± 1.48
PRG (ng/ml)	29.38 ± 5.01	25.81 ± 5.54
Fluorouracils (ng/ml)		
5-FU	(ND)	0.026 ± 0.002
1-(2-tetrahydrofuryl)-5-FU	(ND)	3.91 ± 0.53
uracil	0.724 ± 0.046	0.617 ± 0.056

ND, not detectable.

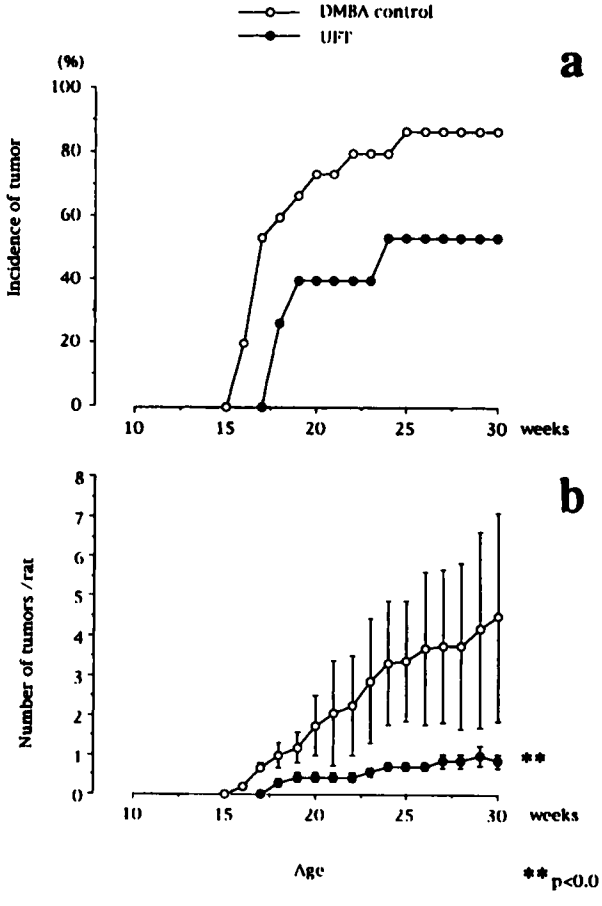

Figure 1. Incidence (a) and number (b) of mammary tumors induced with DMBA in each group of 15 rats (mean ± SEM). **Significantly different from that of the DMBA control at $p < 0.01$.

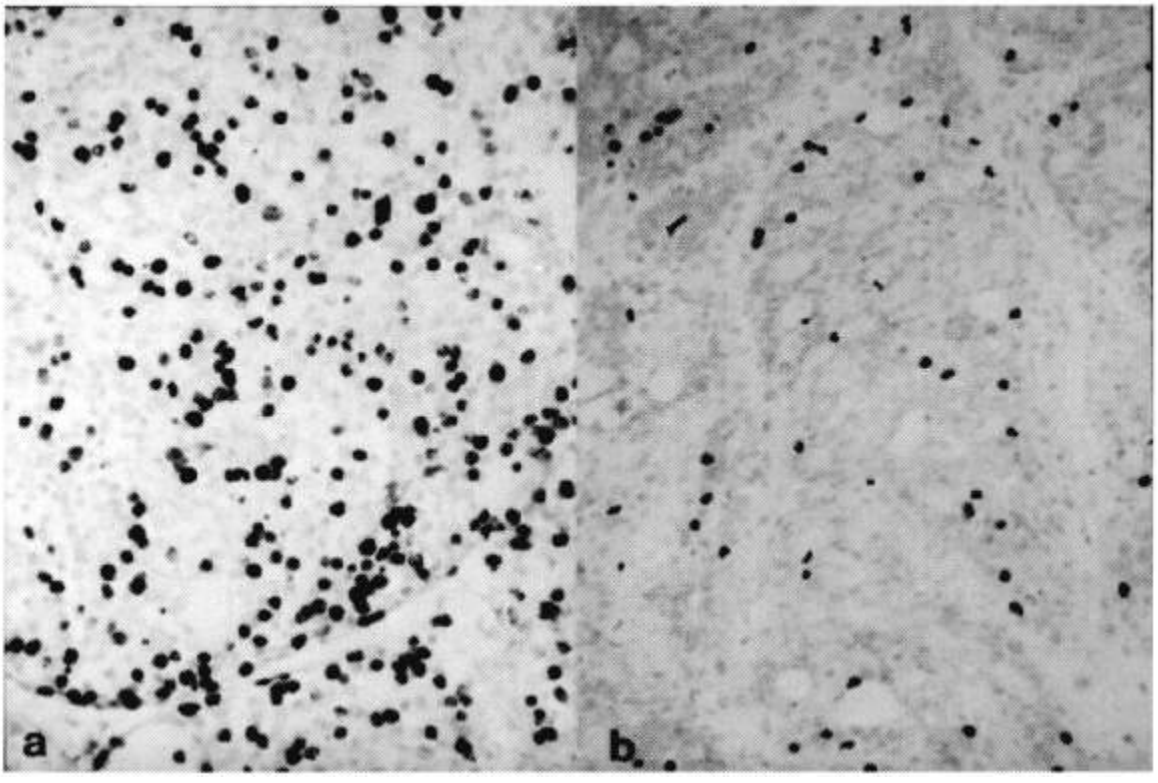

Figure 2. Representative immunohistochemical features of S-phase cells using BrdU in mammary tumors induced with DMBA: (a) DMBA control; (b) UFT group.

	DMBA control	UFT group
BrdU-immunoreactive (S-phase) cells (%)	8.09 ± 0.57	3.01 ± 0.49**

**Significantly different from that of the DMBA control at $p < 0.01$.

Results

Body growth and organ weights

Although body growth of the experimental group with the UFT diet was slightly reduced compared with that of the control, organ wet weights differed little between groups (Table 1).

Plasma levels of hormones and 5-FUs

Plasma levels of 1-(2-tetrahydrofuryl)-5-FU and 5-FU in the UFT diet group were markedly elevated, though plasma levels of hormones were little different between groups (Table 2).

Incidence and number of mammary tumors

Each mammary tumor was determined as an adenocarcinoma by microscopic observation. Mammary tumors in the UFT diet group were palpable at 18 weeks of age, 2 weeks later than in the control group. At autopsy, age 30 weeks, tumor incidences were 86.7 and 53.3% in the control and UFT diet groups, respectively (Figure 1a). Although the cumulative number of mammary tumors in the control group was simply increased as the age advanced, that of the UFT diet group was little augmented (Figure 1b).

Immunohistochemistry with BrdU in tumors

The UFT diet markedly reduced the appearance of BrdU-immunoreactive (S-phase) cells in mammary tumors, compared with the control (Figure 2). The percentage of S-phase cells in the UFT diet group was decreased to less than 40% of that of the control ($p < 0.01$).

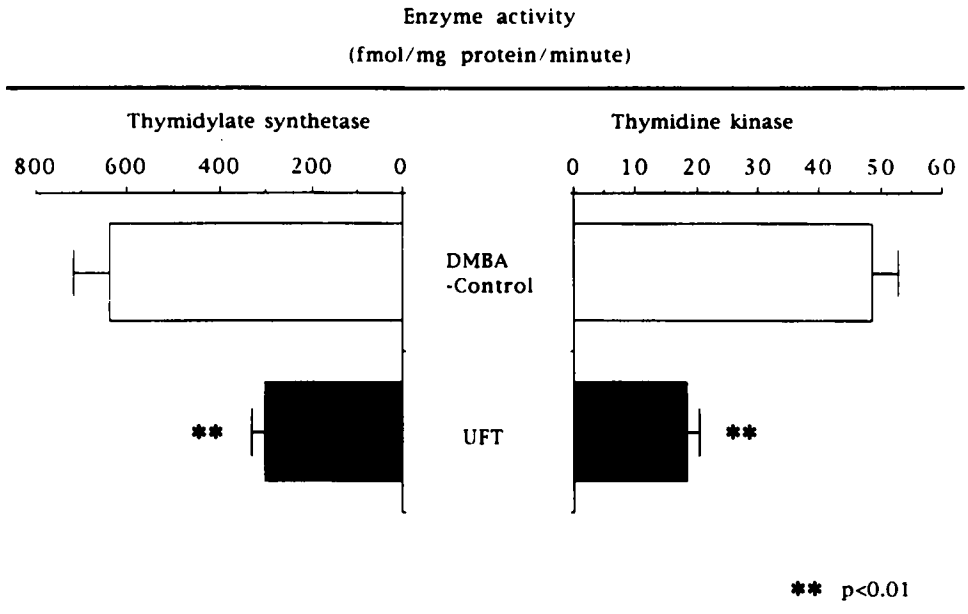

Figure 3. Activities of TS (left) and TK (right) (fmol/mg protein/min) in each group (mean ± SEM). **Significantly different from that of the DMBA control at $p < 0.01$.

DNA synthesizing enzyme activities in tumors

The mean activities of TS and TK in the UFT diet group was markedly suppressed to less than 50% of those of the control (Figure 3) ($p < 0.01$).

Discussion

TS is the enzyme responsible for the *de novo* synthesis of deoxythymidine monophosphate (dTMP) by catalyzing the methylation of deoxyuridine monophosphate (dUMP) with the concomitant conversion of N^5,N^{10}-methylenetetrahydrofolic acid to 7,8-dihydrofolic acid. TK catalyzes the formation of dTMP by the phosphorylation of thymidine via the salvage pathway. High TS and TK activities have been found in rapidly proliferating tissues of normal, fetal and neoplastic tissues.[7–11] Previously we reported that UFT suppressed chemical tumorigenesis in the colon[2] and liver[3] in rats. These tumors are known to be hormone-independent in general. However, a rat mammary tumor induced by DMBA is recognized as being a hormone-dependent tumor.[12–14] In the present study, we investigated the effects of UFT on mammary carcinogenesis and the growth of tumors induced with DMBA in rats. Chronic oral administration of UFT reduced the incidence and the number of mammary tumors and the activities of tissues TS and TK without relation to plasma hormone levels, indicating the suppression by UFT of mammary carcinogenesis and the salvage pathway as well as the *de novo* pathway for pyrimidine nucleotide synthesis in the mammary glands of rats treated with DMBA.

Conclusion

These findings suggest that chronic administration of UFT could markedly prevent mammary carcinogenesis and suppress the growth of tumors via not only the *de novo* but also the salvage pathways in DNA synthesis. Thus it may be possible to prevent mammary carcinogenesis using this attractive derivative in mammary cancer high risk families.

Acknowledgments

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