Table 2. Suppressive effects of nobiletin against genotoxicity of NNK in the lung of male *gpt* delta mice

Group number*	Animal I.D.	Total colonies	No. of mutants	*gpt* MF ($\times 10^{-6}$)	Average ±S.D.†	*P*-value‡
1	M001	960,000	21	21.9		
NNK alone	M002	987,000	32	32.4		
	M003	1,320,000	57	43.2		
	M004	876,000	20	22.8		
	M005	1,892,000	23	12.2		
		6,035,000	153	25.4	26.5±11.8	
2	M007	1,156,000	16	13.8		
NNK	M008	991,000	19	19.2		
+Nobiletin	M009	828,000	20	24.2		
(100 ppm)	M010	828,000	23	27.8		
	M011	840,000	12	14.3		
		4,643,000	90	19.4	19.9±6.1	0.147
3	M013	700,000	16	22.9		
NNK	M014	1,404,000	11	7.8		
+Nobiletin	M015	1,052,000	14	13.3		
(500 ppm)	M016	760,000	10	13.2		
	M017	1,000,000	15	15.0		
		4,916,000	66	13.4	14.4±5.4	0.035‖
4	M019	1,028,000	4	3.9		
Nobiletin	M020§	388,000	4	10.3		
(500 ppm)	M021	1,640,000	6	3.7		
alone	M022	708,000	3	4.2		
	M023	972,000	2	2.1		
		4,348,000	15	3.5	3.5±1.0	0.003
5	M024§	705,000	14	19.9		
No treatments	M025	1,410,000	8	5.7		
	M026	1,410,000	5	3.6		
	M027	1,928,000	3	1.6		
	M028	2,032,000	3	1.5		
		6,780,000	19	2.8	3.1±2.0	0.003

*Group 1, mice treated with NNK (2 mg/mouse/day × 4 days) alone; Group 2, mice treated with NNK plus nobiletin at a dose of 100 ppm in diet; Group 3, mice treated with NNK plus nobiletin at a dose of 500 ppm in diet; Group 4, mice fed nobiletin at a dose of 500 ppm in diet without NNK treatments; Group 5, mice without treatments with NNK or nobiletin. The Group No. corresponds to Group No. in Fig. 1.

†Average ± standard deviation of *gpt* MF of four or five mice.

‡Differences between *gpt* MF of each group and that of Group 1 were tested for statistical significance using a Student's *t*-test.

§Two unusually high *gpt* MF of M020 and M024 were excluded for the calculation of average by the Smirnov-Grubb's outlier test.

‖Statistically significant ($P<0.05$) against Group 1. The values in Groups 4 and 5 are also statistically significant. But the mice in Groups 4 and 5 are not treated with NNK so that the values are not marked with ‖.

against genotoxicity of NNK in the lung of *gpt* delta mice. NNK exposure significantly enhanced the *gpt* MFs in the lung of mice (Tables 1, 2). There was a marked sex difference in the genotoxicity of NNK where females exhibited about twice higher sensitivity than males. This may be due to gender-related differences in the metabolic activation enzymes for NNK (31). The high sensitivity in female than in male mice may be relevant in humans because women are more sensitive to the genotoxic effects of NNK than men (32). Interestingly, dietary administration of nobiletin substantially reduced the *gpt* MFs in both sexes, and the reduction at a dose of 100 ppm in females and 500 ppm in males was statistically significant ($P<0.05$). Administration of nobiletin at 500 ppm also reduced the genotoxicity in females at a similar extent to that observed with nobiletin at 100 ppm. Ikeda *et al.* reported that NNK induces G:C-to-A:T, G:C-to-T:A, A:T-to-T:A, A:T-to-G:C in the lung of *gpt* delta mice (unpublished obervations). Since G:C-to-A:T can activate Ki-*ras* oncogene, the reduction of *gpt* MF may correlate with the reduction of lung tumors (5). Thus, we suggest that nobiletin may be a

chemopreventive agent against NNK-induced lung tumorigenesis in mice. Nobiletin inhibits metastasis (20,21) and suppresses inflammation and promotion (18,33–36). Hence, it may prevent events that occur in multi-step of lung carcinogenesis, i.e., initiation, promotion and progression/metastasis, induced by cigarette smoke. However, certain compounds that can reduce NNK-induced tumors do not necessarily reduce lung tumors in smoke-exposed animals (37). Thus, further examination is needed to evaluate the chemopreventive efficacy of nobiletin against lung tumors induced by cigarette smoke.

In addition to *in vivo* results, we observed reduction of NNK-induced mutations by nobiletin in the present of S9 activation enzymes *in vitro*. Interestingly, nobiletin exhibited a specificity inhibiting the genotoxicity of chemicals in *S. typhimurium*. Although nobiletin inhibited the genotoxicity of NNK, it inhibited the genotoxicity of BP with S9 activation only slightly and did not inhibit the genotoxicity of MNNG without S9 activation. Since MNNG induces O^6-methylguanine leading to G:C-to-A:T mutations (38), we suggest that nobiletin may not enhance the repair activity against O^6-methylguanine or promote error-free translesion bypass across the lesion. Instead, we suggest that nobiletin may suppress the genotoxicity of NNK by inhibiting the activity of CYP (P-450) enzymes involved in the metabolic activation of NNK (39–41). In fact, 8-methoxypsoralen, a specific-inhibitor of CYP2A, similarly suppressed the genotoxicity of NNK in the presence of S9 enzymes (23). The inhibitory effect of nobiletin may be specific to certain CYP enzymes including CYP2A because the genotoxicity of BP, which is activated *via* CYP1A1 (42), was weakly inhibited by nobiletin. However, since both nobiletin and 8-methoxypsoralen inhibited the genotoxicity of NNK only by 50%, we suggest that other CYP enzymes may be responsible for the remaining genotoxicity of NNK in the S9 enzymes. Although nobiletin did not effectively affect the genotoxicity of BP in the present study, Conney *et al.* (43) observed that nobiletin stimulates human liver microsomes and activates both the hydroxylation of BP and the metabolism of aflatoxin B_1 to mutagens. Nobiletin also stimulates oxidative metabolism of zoxazolamine by rat liver microsomes (44) and acetaminophen by human liver microsome (45). These reports suggest that nobiletin has a potential to modulate CYP enzyme activities.

In summary, we examined the chemopreventive efficacy of nobiletin against the genotoxicity of NNK in the lung of female and male *gpt* delta mice. Dietary administration of nobiletin significantly reduced the genotoxicity of NNK in both sexes. In addition, the chemical was able to reduce NNK-induced genotoxicity in *S. typhimurium* YG7108 in the presence of S9 activating enzymes. Our findings suggest that nobiletin could inhibit the activities of certain CYP enzymes involved in the metabolic activation of NNK, thereby suppressing the genotoxicity in the lung of mice.

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