Subchronic and Chronic Effects of Feeding of Large Amounts of Acetaminophen in B6C3F1 Mice

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N-Acetyl-*p*-aminophenol (acetaminophen) is one of the main metabolites of acetophenetidine (phenacetin), which was proved to be a carcinogen^{1,2)}. Ishidate and Odashima have found that acetaminophen itself induced chromosomal aberrations in cultured Chinese hamster cells³⁾. The repair test by the rec-assay also gave a positive result (Kada et al., unpublished data). These results prompted us to perform an experiment of long-term administration of acetaminophen in B6C3F1 mice.

During the preparation of this article Flaks and Flaks have reported that acetaminophen is tumorigenic in mice of the IF strain⁴⁾. The difference between results of the present study and the results obtained by Flaks and Flaks is briefly discussed.

MATERIALS AND METHODS

Six-week-old (C57BL/6×C3H/He)F1 (B6C3F1) mice of both sexes, weighing 17-25g, were purchased from Charles River Japan Inc., Atsugi and were maintained on the basal diet (CE-2, CLEA Japan Inc., Tokyo) and tap water, until they were 8-9 weeks old when tests were started. Ten or 11 mice were housed in an aluminum cage and kept in an air-conditioned room at 24-25°C with 45-55% humidity. Acetaminophen (reagent grade, more than 98% pure) was mixed with the basal diet and was processed into pellets. Acetaminophen in the pellets proved to be stable after being processed and was stored for several months.

Subchronic toxicity test

Mice of 5 experimental groups, each consisting of 10 male and 10 female mice, were fed a diet containing 0.3, 0.6, 1.25, 2.5 or 5% of acetaminophen. Ten male and 10 female mice were fed with the basal diet as a control group. All the animals were given tap water freely. They were weighed once a week during the 1st month and later once a month, and the average daily food consumption was recorded throughout the experimental period. The survivors were killed 41 weeks following the commencement of the experiment.

Chronic toxicity test

One hundred and five male and 105 female B6C3F1 mice were maintained on a diet containing 0.3 or 0.6% acetaminophen. Fifty male and 50 female mice were maintained on the basal diet. All the animals were given tap water freely. The survivors were killed after 134 weeks. All dead or sacrificed animals were necropsied. The liver, lungs, spleen, pancreas, kidneys, stomach, small and large intestines, heart, urinary bladder, thymus, adrenals, thyroid, brain, pituitary, testes, ovaries, and tumors were fixed in 10% formalin solution, embedded in paraffin, sectioned at 5 μ , and stained routinely with hematoxylin

and eosin for histologic examination.

RESULTS

Subchronic toxicity test

In the groups with 5, 2.5, and 1.25% diets, mice showed marked weight loss immediately after the commencement of the treatment, but mice of the groups with 0.6 and 0.3% diets had normal weight gain just as those of the control group during the experiment (Fig. 1). All the mice of the 5% diet group died within 2 weeks and almost all the mice of the 2.5% diet group died within 10 weeks of myocardial infarction and/or hemorrhagic enteritis (Fig. 2). All mice, except 1, of the groups with the 1.25, 0.6, and 0.3% diets survived for 13 weeks, but all the mice of the 1.25% diet group developed liver fibrosis 41

Fig. 1 Average body weight in male (A) and female (B) mice, which were fed a diet containing 0.3, 0.6, 1.25, 2.5 or 5% acetaminophen or the basal diet.

Fig. 2 Survival rates in male (A) and female (B) mice, which were fed a diet containing 0.3, 0.6, 1.25, 2.5 or 5% acetaminophen or the basal diet.

weeks after commencement (Fig. 3). The livers of the latter group of mice weighed around 0.9 g in the males and around 0.8 g in the females (compared to around 2.3 g in the control males and around 1.8 g in the control females). Histologically, necrosis and necrobiosis of parenchymal cells in the peripheral zones of the lobules were prominent. Moderate fibrosis was found in the portal triads associated with

Fig. 3 Livers of male and female mice treated with 1.25% diet of acetaminophen, showing coarsely granular surface.

Fig. 4 Micrograph of a liver of a male mouse which was fed a diet containing 1.25% acetaminophen, showing fibrosis in the portal triads and pseudolobules. H-E. x 90

moderate infiltration of lymphocytes and granulocytes, forming pseudolobules (Fig. 4). Many hemosiderin-laden macrophages were noticed in the triads. From these results the maximum tolerated dose by oral feeding to young adult B6C3F1 mice on the chronic toxicity test was determined to be the 0.6% diet.

Chronic toxicity test

There was no remarkable difference in food intake between the experimental and control groups. Calculated total intakes of acetaminophen were 338 and 272 g/kg, respectively, in male and female mice of the 0.6% diet group at 52 weeks, and 863 and 675 g/kg, respectively, in male and female mice at 133 weeks. The 2 male experimental groups had slightly lower body-weight curves than the control group in the latter two-thirds of the test time period, but all the female groups had almost the same body-weight curves (Fig. 5). No difference was found in the mortality rates in the 3 groups for either of the sexes (Fig. 6). There was no difference in the rates of tumor-bearing mice for the male groups, but those for the 2 female experimental groups were slightly lower than that of the control group (Fig. 7). Tumors were found in the mice of the control and experimental groups, and in various organs: the hematopoietic tissues (bone marrows, thymus, spleen, and lymph nodes), lungs, liver, pituitary, digestive tract, uterus, ovaries, breasts, adrenals, and skin were involved (Table 1), with no statistical difference in the incidences. These tumors observed were regarded as spontaneous tumors of B6C3F1 mice⁵. No tumors were

Fig. 5 Average body weight in male (A) and female (B) mice, which were maintained on a diet containing 0.3 or 0.6% acetaminophen or the basal diet.

Fig. 6 Mortality rates in male (A) and female (B) mice, which were maintained on a diet containing 0.3 or 0.6% acetaminophen or the basal diet.

Fig. 7 Rates of animals bearing tumors in all sites in male (A) and female (B) mice, which were maintained on a diet containing 0.3 or 0.6% acetaminophen or the basal diet.

Table 1 Effects of chronic acetaminophen feeding in young adult B6C3F1 mice.

Sex	Treat- ment	No. of mice at start	Effective no. of mice*	No. (&%) of mice with					No. of mice with				
				Tumors of all sites	Leu- kemia	Lung tumors †	Liver tumors ††	Pitui- tary adenoma	Other tumors**	Pneu- monia	Ente- ritis	Miscel- laneous diseases [#]	
M	Basal diet	50	43	27(63)	5(12)	5(12)	13(30)	0(0)	1 fsh 1 fsb 1 pha 1 fsh				

* Survived more than 51 weeks, when the 1st neoplasm was found. † Contained lung adenoma and carcinoma. †† Contained liver adenoma and carcinoma. ** fsh = fibrosarcoma of hip, fih = fibroma of hip, msh = myosarcoma of hip, fsb = fibrosarcoma of back, msb = myosarcoma of back, hem = hemangioma, pha = pheochromocytoma of adrenal, osd = osteosarcoma of duodenum, cbs = carcinoma of back skin, bsu = botryoid sarcoma of uterus, amg = adenoma of mammary gland, ova = ovarian adenoma, gct = granulosa cell tumor of ovary, msc = myosarcoma of chest wall, ada = adrenal adenoma, oss = osteogenic sarcoma of spinal column, skc = skin carcinoma, aac = adenoma of adrenal cortex, hes = hemangiosarcoma, fsc = fibrosarcoma of chest wall, tha = thyroid adenoma, osc = osteosarcoma of chest wall, cmg = carcinoma of mammary gland, aro = adrenal rest tumor of ovary.

Including urethritis, cystitis, gastrointestinal bleeding, liver abscess, pyometra, and skin ulcer.

found in the nasal cavity or urinary passage. Some of the mice in each group died of pneumonia, enteritis and other miscellaneous diseases (Table 1).

DISCUSSION

The results of the present tests show that feeding the maximum tolerated dose of acetaminophen (0.6 % diet) held no carcinogenic hazard for B6C3F1 mice. Hiraga et al. also showed that 0.9 and 1.3% diets of acetaminophen administered to Fischer 344 rats did not induce tumors at statistically higher rates (unpublished data). However, Flaks and Flaks fed IF mice with the diet containing 1.0% acetaminophen and have shown that 20 out of 23 male mice which survived for 78 weeks developed liver tumors⁴⁾. It was also shown that their dose was too high for a long term test, because 32 out of 60 male mice died of liver necrosis usually within 2 days after the commencement of the treatment. On the other hand, all of the B6C3F1 mice treated with the 1.2% diet developed liver fibrosis, but not liver tumors, after 41 weeks in the present study. Currently it is not known whether the discrepancy on tumorigenesis between these studies is due to the different strain of mice used or the purity of the compound.

Phenacetin has been reported to be a carcinogen in man, rats and mice, inducing urothelial tumors of the renal pelvis and tumors in the nasal cavity^{1,2,6-9)}. On the other hand, acetaminophen, which is the major metabolite of phenacetin^{10,11)}, induced no urothelial tumors nor tumors in the nasal cavity in rats nor mice. These findings suggest that hydroxylated metabolites, not acetaminophen, of phenacetin may be proximal mutagens and carcinogens, as assumed by many studies¹²⁻¹⁴⁾.

In the chronic toxicity test, the daily dosage of acetaminophen used was about 12 to 24 times larger than human regular dosage in Japanese Pharmacopoeia. Papillary necrosis of the renal pelvis was a prominent feature in patients with phenacetin abuse for an average of 17 years⁹⁾. The papillary necrosis and papillary hyperplasia are assumed to be precursors of renal pelvic tumors^{15,16)}. However, acetaminophen induced neither papillary necrosis nor papillary hyperplasia in mice in the present tests.

Acetaminophen is rapidly conjugated with glucuronate by the liver and excreted by the kidney¹⁰⁾. Gastrointestinal hemorrhage, hepatic necrosis, renal necrosis, and cardiac myotonia were induced in man and rats, as acute toxicities of acetaminophen^{17,18)}. The present test also showed acetaminophen induced liver fibrosis in mice as a subchronic toxicity.

SUMMARY

Subchronic and chronic effects of acetaminophen were examined, using B6C3F1 mice orally administered with 0.3, 0.6, and 1.25% acetaminophen diets daily. All of the mice treated with a 1.25% diet developed liver fibrosis after 41 weeks. Mice in the experimental groups treated with 0.3 and 0.6% diets for 52-135 weeks, as well as mice in the control group treated with the basal diet, developed various tumors, but no difference in tumor incidence was detected among these groups.

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B6C3F1 マウスにおけるアセトアミノ

フェンの長期経口投与の影響

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アセトアミノフェンは解熱、鎮痛剤として広く用いられている。しかし最近フェナセチンに癌原性がみいだされたため、その主なる代謝産物であるアセトアミノフェンについても癌原性の検索が必要となった。既にアセトアミノフェンの変異原性についての検索が行われ、Ames test (－), repair test (－), chromosome test (－), Silkworm test (－)の成績が得られている。このような現状に鑑み、アセトアミノフェンの亜慢性毒性および癌原性の有無を明らかにする目的で本研究を行った。

アセトアミノフェンの5, 2.5, 1.25, 0.6および0.3%混合飼料を(C57BL/6×C3H/He) F1 (B6C3F1) マウスに経口投与したところ、5および2.5%投与群マウスの大多数は10週間以内に心筋梗塞あるいは肝臓萎縮で死亡した。1.25%投与群では41週後の屠殺時全例に肝線維症が認められた。

この亜慢性実験の結果からアセトアミノフェンの最大耐量を0.6%混合飼料と決定し、0.6%およびその半量の0.3%混合飼料を雌雄のB6C3F1マウスに自由に摂取させ、136週間飼育観察した。体重曲線、飼料摂取量、死亡率、腫瘍発生率、腫瘍発生期間のいずれについても投与群と対照群の間に有意差は認められなかった。これらの結果から、アセトアミノフェンはB6C3F1マウスに対し癌原性を持たないものと結論した。

この論文作成中に、Flaks および Flaks がアセトアミノフェンに造腫瘍性があると報告したので、彼等の実験結果と本研究の実験結果の相違について若干の考按を加えた。

Key words: Acetaminophen, Long-term feeding, Subchronic effect

アセトアミノフェン, 長期経口投与, 亜慢性効果

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