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Original Article

The concern for uterine carcinogenesis in safety assessments for a new pharmaceutical

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ABSTRACT — Acotiamide hydrochloride hydrate (acotiamide-HH) has been newly developed as an indication for functional dyspepsia, which is characterized by digestive symptoms such as postprandial fullness, abdominal bloating, or early satiation, and is now being prescribed in Japan. As part of a safety assessment, 2-year long-term carcinogenicity studies using rats and mice were conducted. In the mouse carcinogenicity study, no evidence of carcinogenicity was obtained, even in the high-dose-treated group (up to 2000 mg/kg/day). In the rat carcinogenicity study, acotiamide-HH was administered at 200, 600, and 2000 mg/kg/day. Detailed histopathological examination revealed that the incidence of endometrial adenocarcinoma significantly increased in the 600 mg/kg/day treated group. There was no trend of this incidence and no accompanying increase in pre-neoplastic lesions or related histological changes in the genital tissues, suggesting the absence of abnormalities in the sexual endocrine system. Results of genotoxicity and reproductive/developmental studies showed that acotiamide-HH is a non-genotoxic substance and did not affect sexual balance. Acotiamide-HH did not induce an estrogen-dominant hormonal imbalance that could cause the incidence of uterine cancer and did not have initiation activity. Therefore, the proliferation of endometrial adenocarcinoma in this middle dose group in the rat carcinogenesis study was considered an accidental event of naturally occurring tumors. However, the incidence of endometrial adenocarcinoma in this group deviated from the background data collected in the same laboratory during the study period. Therefore, it is considered necessary to conduct another pre-clinical study in order to obtain data that would dispel any concerns of safety.

Key words: Acotiamide, Endometrial adenocarcinoma, Rat, Carcinogenicity

INTRODUCTION

The generic drug acotiamide hydrochloride hydrate (acotiamide-HH), marketed as Acofide® 100-mg tablets, is an acetylcholine esterase (AChE) inhibitor developed for the treatment of functional dyspepsia (FD) by Zeria Pharmaceutical Co., Ltd. in 1995. To date, no product has demonstrated efficacy or obtained marketing approval for the treatment of patients with FD diagnosed by the Rome III criteria, which is the latest version of the international classification and diagnostic criteria for functional gastrointestinal (GI) disorders. According to these criteria, FD is a GI disease characterized by subjective symptoms including postprandial fullness, early satiation, and epigastric pain, without any organic abnormalities in the GI tract. Although the etiology of FD is unclear, it has been shown that delayed gastric emptying is closely associated with this disease. Acetylcholine (ACh) is released from the cholinergic nerves located in the myenteric plexus and binds to muscarinic (mainly M3) receptors in the GI smooth muscles so as to induce contractions of the digestive tract. Furthermore, the released ACh is rapidly degraded by AChE, which regulates GI motility. Acotiamide-HH causes an increase in ACh levels at

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the cholinergic nerve terminals, which is mediated by its selective AChE inhibitory activity to produce a gastrokinetic effect and to improve the decreased gastric motility in the gastric antrum. In pre-clinical studies, acotiamide-HH was determined to be clinically safe and efficacious and was approved for clinical use in Japan in March 2013. In this study, we report the results of a long-term carcinogenicity study of acotiamide-HH in rats, which was conducted based on the concerns that were raised during the development of the drug. In particular, we report details on the development of uterine cancer, which was frequently observed in rats in the acotiamide-HH-treated groups.

MATERIAL AND METHODS

Animals and housing conditions

Four-week-old F344/DuCrj (Fischer) rats were purchased from Charles River Laboratories Japan, Inc. (Atsugi City, Kanagawa, Japan). The rats were acclimatized and quarantined for 8 days. Then, 218 male and 218 female, healthy rats were selected. They were housed in a standard specific pathogen-free environment and allowed free access to standard CRF-1 powder feed (Oriental Yeast Co., Ltd., Itabashi, Tokyo, Japan) and tap water. The animals were stratified in advance, based on body weight, and divided into 4 groups of 50 males and 50 females each by random assignment. This study was approved and conducted in accordance with the procedures for the handling and care of the animals by the Animal Care Committee of the Biosafety Research Center (BSRC) as well as the Guidelines on Carcinogenicity Tests of Drugs (Pharmaceutical Affairs Bureau Notification No. 1607); the Guidance on Toxicokinetics (Evaluation of Systemic Exposure in Toxicity Studies, July 2, 1996, Evaluation and Licensing Division Notification No. 443); and the Ministerial Ordinance for Standards for Implementing Nonclinical Study of Drug Safety (GLP standards, March 26, 1997, Ministerial Ordinance No. 21, the Ministry of Health and Welfare).

Carcinogenicity study

The preliminary carcinogenicity study was conducted in rats for 13 weeks (Shiga, 2002). Prior to the study, the rats were observed for miosis, salivation, and their general health condition. No changes were observed in their clinical laboratory parameters and body weight, and no obvious toxicity to target organs was identified even after oral administration of acotiamide-HH at 2000 mg/kg/day. From these results, this dose was selected as the high, upper limit dose for the long-term carcinogenesis study. In addition, the low dose was set at one-tenth of the high-dose (200 mg/kg/day), and the medium dose was set at 600 mg/kg/day, which is the geometric mean of the 2 extreme doses. In addition to the treated groups, a control group was included and treated with only 0.5 w/v% methylcellulose (MC, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) aqueous solution (10 mL/kg). Procedures to assess the general condition of the rats included careful palpation and measurement of body weight and food consumption and were performed according to standard methods. The organ weight measurements, as well as the hematological and pathological examinations, were conducted during the scheduled necropsy at week 104 of drug administration. All animals were subjected to detailed macroscopic observation, organ weight measurement, and histopathological examination. The following organs and tissues were paraffin-embedded according to standard methods: the brain, heart, lungs (including the bronchi), liver, kidneys, testes, spleen, ovaries, adrenal glands, thymus glands, skin, mammary glands, lymph nodes (mesentery and mandible), sublingual glands, mandibular glands, sternum, femur, bone marrow (sternum and femur), trachea, thyroid glands, parathyroid glands, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, seminal vesicle, prostate gland, epididymides, uterus, vagina, eyes (including optic nerve), harderian glands, pituitary, spinal cords (cervical, thoracic, and lumbar parts), skeletal muscle (femur), sciatic nerve, aorta, and any other macroscopically abnormal organs and tissues. Each organ or tissue was sliced to prepare hematoxylin and eosin-stained specimens for histopathological assessment. Furthermore, an authorized toxicological pathologist who was a third party not directly associated with the study, performed the pathological peer review to reconfirm the histopathological diagnosis and findings. In addition, measurement of the plasma concentrations of acotiamide-HH was performed on separate groups of rats at the time of initial drug treatment and at weeks 13 and 26 following administration. The free form concentration, maximum concentration (C_{max}), and time to reach maximum plasma drug concentration (T_{max}) were calculated and analyzed.

Statistical analysis

The statistical analysis of the body weight, food consumption, food efficiency, hematological examination results, organ weight, and relative organ weight was conducted using the Bartlett's test for equality of variance (Snedecor and Cochran, 1989) for each study group. When the equality of variance was not significant, the presence or absence of a significant difference

between the control and each drug-treated group was analyzed using the Dunnett's test for multiple comparisons (Yoshida, 1988). When equality of variance was significant, the presence or absence of a significant difference between the control group and each administration group was analyzed using the Steel test (Steel, 1959). For the comparison of the survival rate, the log-rank test (Cox and Oakes, 1984) was conducted. The 2-sided significance level was determined to be 5%. The dose-response relationship in terms of the cancerization rate, was determined using the Peto test (Peto *et al.,* 1980). The 1-sided significance level was determined to be 5%. In addition, Fisher's exact test (Wolfe, 1999) was conducted to further compare the control group with each drug-treated group. The 2-sided significance level was determined to be 5%.

RESULTS

Mortality, general condition, and food consumption

There was no difference in the mortality rate between the control group and each drug-treated group until week 104 for both the male and female rats. One male rat in the 2000 mg/kg/day-treated group, which was killed by accident, was excluded from the analysis (Table 1).

Observation of the general conditions revealed temporarily induced miosis, lacrimation, salivation, and production of reddish tears at 1 to 2 hr after drug administration in the 2000 mg/kg/day group. However, no other continuously expressed abnormalities were observed. Palpable subcutaneous and peritoneal masses were recorded (Table 2). The majority of these tumor masses were formed due to neoplastic changes. In particular, peritoneal tumor masses were found in the enlarged spleens affected by large granular lymphocyte leukemia. Other abnormal findings were mainly derived from deterioration of conditions in tumor-bearing animals (Table 2).

Administration of the test substance did not result in marked changes in the average body weight (Figs. 1 and 2) and average total food consumption (Figs. 3 and 4). There were no toxicologically meaningful changes in food efficiency in this study (data not shown).

Hematological examination and drug concentration determination

There were no obvious changes observed in the hematological examination. Thus, it was determined that administration of the test substance did not influence the hematological parameters examined.

Results of the measurement of the plasma concentration of the free form of acotiamide-HH showed a good correlation between the concentration and administered dose at each measurement period, and there was no significant variation in association with the administration period (Tables 3 and 4).

Pathological examination

The results of the organ weight determination revealed that both the absolute and relative weights of the kidneys and adrenal glands of female rats in the 2000 mg/kg/day acotiamide-HH-treated group were significantly higher than those of the control group. However, since the weights were only slightly different from those in the control group, it is unlikely that there is toxicological significance. In addition, the absolute or relative weights of the liver, brain, and thymus of the drug-treated groups were significantly different from those of the control group. However, there was a poor correlation between the weights and the doses; only the absolute weight varied in a dose-dependent manner. Therefore, it is unlikely that administration of the test substance caused the weight change. At the time of necropsy, various age-relat-

Table 1. Mortality rates (%) of 24 month carcinogenicity study of acotiamide-HH in rats.

Sex	Male				Female			
Dose levels (mg/kg)	0*	200	600	2000	0*	200	600	2000
Dose volume (mL/kg)	10	10	10	10	10	10	10	10
No. of animals/group	50	50	50	50[#]	50	50	50	50
Period (Week)								
13	0.0	0.0	0.0	0.0 (49)	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0 (49)	0.0	2.0	0.0	0.0
52	0.0	0.0	0.0	0.0 (49)	0.0	4.0	0.0	0.0
78	2.0	6.0	0.0	0.0 (49)	10.0	4.0	8.0	6.0
104	18.0	20.0	12.0	20.4 (49)	30.0	34.0	38.0	22.0

*: 0.5w/v% methylcellulose solution.

[#]: Numbers within parenthesis indicate total animals excluding one accidentally dead animal.

Table 2. Results of General observation.

Sex	Male				Female			
Dose Levels (mg/kg)	0	200	600	2000	0	200	600	2000
Period (Week)								
1-26	(50)	(50)	(50)	(49)	(50)	(48)	(50)	(50)
Tissue-mass/abdomen	0	0	0	0	0	1	0	0
Opacity of eyeball	0	0	0	1	1	0	1	0
Eye discharge	0	1	0	0	0	1	0	0
27-52	(50)	(50)	(50)	(49)	(50)	(48)	(50)	(50)
Tissue-mass/surface	0	0	1	0	0	0	0	0
Tissue-mass/subcutaneous site	1	0	0	1	1	0	2	0
Opacity of eyeball	1	0	0	3	2	2	3	1
Eye discharge	0	1	0	0	2	0	0	0
53-78	(50)	(50)	(50)	(49)	(50)	(48)	(50)	(50)
Tissue-mass/surface	1	0	1	0	0	0	0	0
Tissue-mass/subcutaneous site	4	1	2	1	1	2	5	1
Tissue-mass/abdomen	0	1	1	0	3	1	4	2
Opacity of eyeball	2	0	0	5	3	3	4	1
Eye discharge	1	3	1	0	6	1	4	2
79- 104	(49)	(47)	(50)	(49)	(45)	(48)	(46)	(47)
Wasting	6	2	3	1	4	5	8	3
Piloerection	1	0	0	0	3	5	1	1
Subnormal temperature	0	1	1	2	2	5	2	1
Pallor (auricle etc.)	4	5	2	4	4	9	8	5
Tissue-mass/surface	1	1	4	0	0	0	0	0
Tissue-mass/subcutaneous site	10	9	4	6	5	10	13	7
Tissue-mass/abdomen	5	6	4	7	9	10	10	6
Abdominal distention	0	0	0	0	2	0	0	1
Eye discharge	1	4	3	1	4	6	8	4
Opacity of eyeball	2	0	2	5	6	3	4	2
Urogenital hemorrhage	0	0	0	0	5	5	0	4

Table 3. Plasma concentration of free form in male rats.

Male	Dose	Concentration (ng/mL)		C_{max}	T_{max} (hr)
	(mg/kg)	0.5 hr	4 hr		
Initiation	200	663.8 ± 125.0	211.6 ± 70.1	663.8	0.5
	600	2647.9 ± 423.0	886.5 ± 597.3	2647.9	0.5
	2000	3093.7 ± 182.9	2187.6 ± 832.2	3093.7	0.5
13week	200	584.3 ± 156.3	609.5 ± 224.1	609.5	4
	600	1370.2 ± 702.1	1195.8 ± 126.4	1370.2	0.5
	2000	2390.9 ± 1280.2	575.5 ± 60.0	2390.9	0.5
26week	200	683.5 ± 389.5	506.3 ± 54.2	683.5	0.5
	600	1196.3 ± 474.1	421.1 ± 97.9	1196.3	0.5
	2000	2544.4 ± 930.8	1202.6 ± 150.7	2544.4	0.5

ed changes were observed. However, the number of nodules of the thoracic vertebrae decreased dose-dependently in both male and female rats, suggesting an influence of the administered test substance. Histopathological examination showed that the nodes of the thoracic vertabra were formed due to exostosis, which suggests that this was not an abnormal finding. Accordingly, it was determined that the nodules of the thoracic vertebrae were not toxicologically significant.

Histopathological examination revealed that there was no difference between the control group and each acotiamide-HH-treated group regarding the total number

Table 4. Plasma concentration of free form in female rats.

Female	Dose	Concentration (ng/mL)		C_{max}	T_{max}
	(mg/kg)	0.5 hr	4 hr		(hr)
Initiation	200	480.6 ± 188.8	228.9 ± 88.6	480.6	0.5
	600	1939.9 ± 608.1	838.2 ± 714.8	1939.9	0.5
	2000	3732.3 ± 685.4	1443.7 ± 623.4	3732.3	0.5
13week	200	1028.4 ± 345.9	573.6 ± 248.5	1028.4	0.5
	600	1944.1 ± 199.9	697.9 ± 177.5	1944.1	0.5
	2000	4274.2 ± 1508.1	9087.0 ± 14810.2	9087.0	4
15week*	2000	3222.7	429.3		
26week	200	716.7 ± 81.7	257.6 ± 30.2	716.7	0.5
	600	2393.7 ± 1093.1	980.7 ± 497.1	2393.7	0.5
	2000	5668.8 ± 670.9	1146.9 ± 411.4	5668.8	0.5

* 15 week plasma concentration was retested in animal which showed high plasma concentration in 13 week.

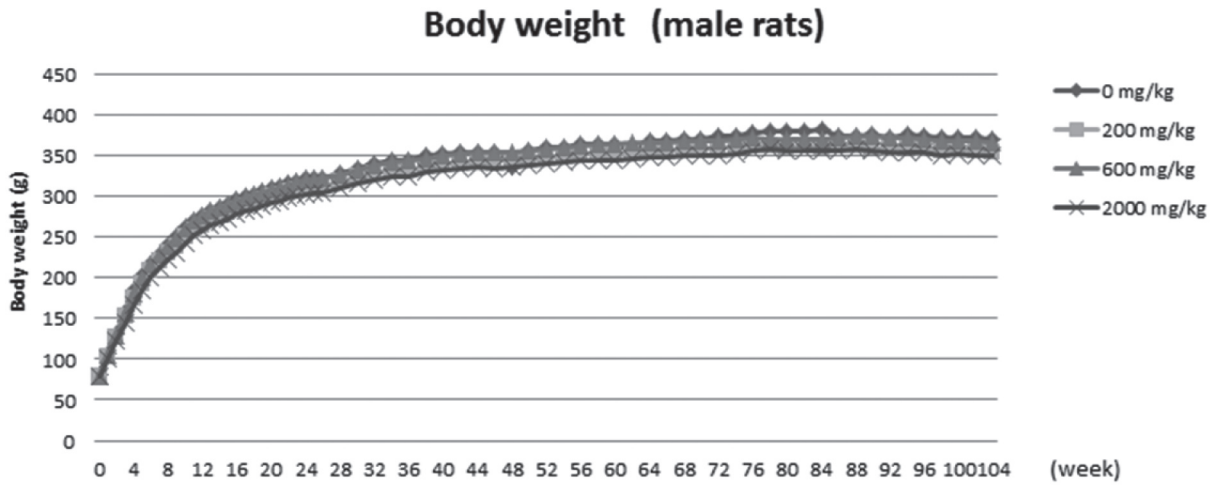

Fig. 1. Change of body weight in male rats.

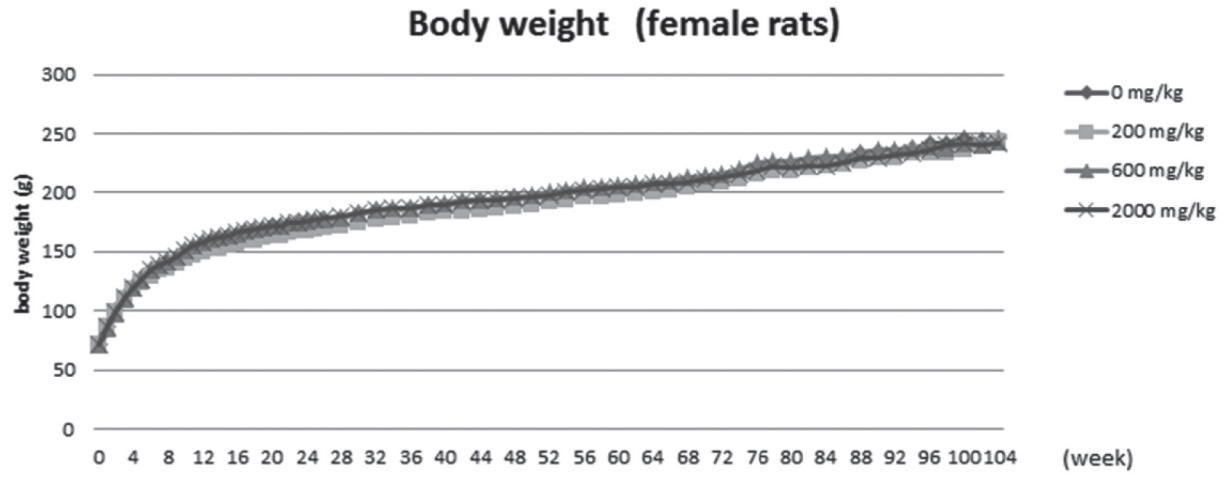

Fig. 2. Change of Body weight in female rats.

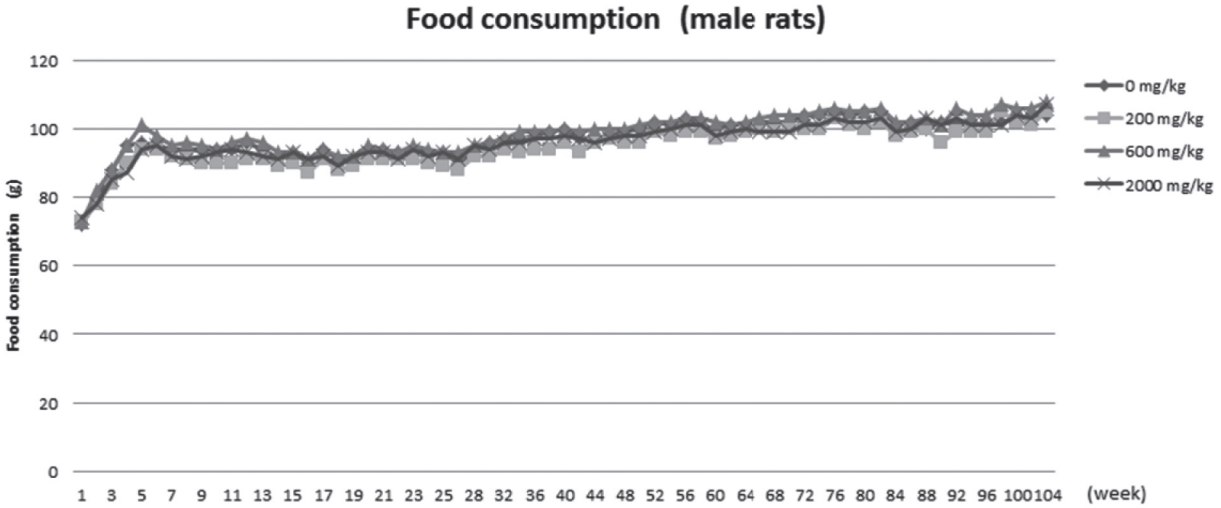

Fig. 3. Mean total amount of food consumption in male rats.

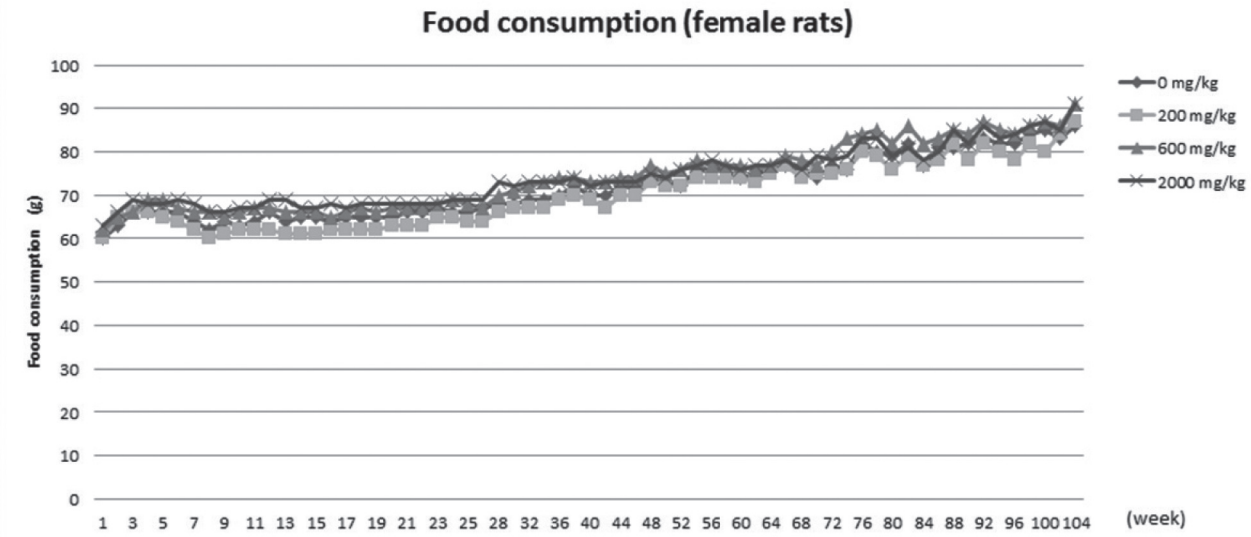

Fig. 4. Mean total amount of food consumption in female rats.

Table 5. The number of tumors and number of animals bearing tumors.

	Male				Female			
Dose levels (mg/kg)	0	200	600	2000	0	200	600	2000
No. of animals examined	50	50	50	50	50	50	50	50
No. of benign tumor	117	93	107	96	59	61	54	59
No. of malignant tumor	16	18	11	17	22	23	23	21
Total No. of tumor	133	111	118	113	81	84	77	80
No. of animals bearing benign tumor	50	47	50	48	35	36	36	35
No. of animals bearing malignant tumor	16	16	10	16	21	20	22	19

Table 6. The major tumors.

	Male				Female			
Dose levels (mg/kg)	0	200	600	2000	0	200	600	2000
No. of animals examined	50	50	50	49	50	50	50	50
Spleen								
LGL leukemia	5	4	2	11	9	10	7	8
Lung								
Alveolar/bronchiolar adenoma	3	2	4	2	2	3	3	2
Liver								
Hepatocellular adenoma	2	2	3	0	2	2	0	2
Testis								
Interstitial cell tumor	43	41	46	41	-	-	-	-
Mammary gland								
Fibroadenoma	-	-	-	-	6	8	8	5
Pituitary gland								
Adenoma, pars distalis	18	15	15	20	17	17	17	18
Thyroid gland								
C-cell adenoma	16	8	13	9	9	11	10	10
C-cell carcinoma	3	2	2	1	0#	1	1	4
Adrenal gland								
Pheochromocytoma, benign	4	4	4	4	1	1	0	1
Pancreatic islet								
Adenoma	18	9	14	12	1	1	1	2
Subcutaneous tissue								
Fibroma	7	3	1	4	0	0	0	2

Significantly different from control group: (Peto's test) : # $P < 0.05$.
The tumor which found more than three animals at least one treatment group were extracted.

Table 7. Uterine proliferation lesions.

	0	200	600	2000
No. of animals examined	50	50	50	50
Cystic endometrial hyperpalsia	6	14	10	10
Endmetrial hyperplasia	4	7	4	8
Endmetrial adenoma	1	0	1	0
Endmetrial adenocarcinoma	1	5	8*	5

Fisher's exact test: *$P < 0.05$.

of tumors, benign tumors, and malignant tumors (Table 5). Furthermore, there was no difference between the groups with respect to the number of tumors per organ and tissue, except for the uterus (Table 6). Uterine adenocarcinoma was observed in 5 out of 50, 8 out of 50, and 5 out of 50 rats in the 200, 600, and 2000 mg/kg/day groups, respectively, compared with 1 of 50 rats in the control group. Therefore, the incidence was higher in the acotiamide-HH-treated groups than it was in the controls. However, this increase was statistically significant only in the 600 mg/kg/day acotiamide-HH-treated group (Table 7). The incidence of uterine adenocarcinoma was even higher than the background level (2-4%, 2002 to 2005, n = 300) obtained during the study period in the same institution. The morphological observation revealed that the uterine adenocarcinoma had infiltrated and proliferated to the level of the serosal surface of the uterus in a scattered manner; however, each uterine adenocarcinoma was locally formed with no observable distant metastasis (Fig. 5). The variation in the incidence between the groups did not correlate with the dose of acotiamide-HH administered. In addition, incidences of endometrial cystic hyperplasia, which is a pre-neoplastic lesion formed by a proliferative change in the endometrium,

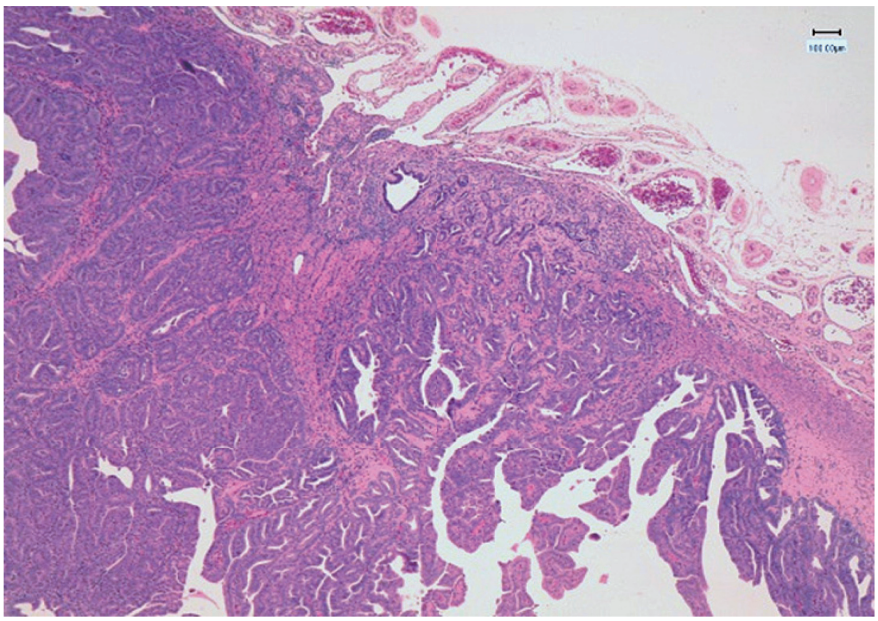

Fig. 5. Endometrial adenocarcinoma found in acotiamide-HH treatment group. The tumor cell showed invasive growth beneath of subserous layer in uterus. HE staining x 40.

Table 8. Histopathological finding of uterine, vagina, ovary, and mammary gland exclude the animals bearing endometrial tumor.

Dose levels (mg/kg)		0		200		600		2000	
No. of animals examined		48		45		41		45	
Findings	Grade	No. of animals (%)							
Mammary gland	-	8	(17)	9	(20)	7	(17)	9	(20)
hyperplasia	+	28	(58)	24	(53)	32	(78)	32	(71)
	++	9	(19)	11	(24)	1	(2)	4	(9)
	+++	3	(6)	1	(2)	1	(2)	0	(0)
	total	40	(83)	36	(80)	34	(83)	36	(80)
Ovary	-	45	(93)	40	(89)	35	(85)	41	(91)
atrophy	+	3	(6)	5	(11)	6	(15)	4	(9)
Uterus	-	16	(33)	21	(47)	15	(37)	13	(29)
endometrial proliferation	+	20	(42)	15	(33)	20	(49)	24	(53)
	++	8	(17)	4	(9)	5	(12)	7	(16)
	+++	4	(8)	5	(11)	1	(2)	1	(2)
	total	32	(67)	24	(53)	26	(63)	32	(71)
Vagina: mucosa	squamous epithelium	5	(10)	15	(33)	5	(12)	14	(31)
	mucosal epithelium	43	(90)	30	(67)	36	(88)	31	(67)

-: no change, +: mild, ++: moderate, +++: severe

endometrial hyperplasia, and benign endometrial adenoma were not influenced by acotiamide-HH administration (Table 7). In consideration of these results, all female rats other than the endometrial tumor-bearing animals in the control group and each drug-treated group were subjected to histopathological examinations of the uterus, ovaries, vagina, and mammary glands. There was no significant difference between the control group and each drug-treated group with respect to the state of the estrus cycle, degree of proliferation of the mammary gland and endometrium, and changes in the ovary and vaginal mucosa. Furthermore, there was no significant dif-

ference between the control group and each drug-treated group with respect to the occurrence of ovarian atrophy (Table 8).

DISCUSSION

Acotiamide-HH causes an increase in the amount of ACh at the cholinergic nerve terminals, which is mediated by selectively inhibiting AChE to produce a gastrokinetic effect and improve the decreased gastric motility in the gastric antrum in rats (Kawachi *et al*., 2011) and in dogs (Nagahama *et al*., 2012).

In the carcinogenicity study in rats, miosis, lacrimation, salivation, and reddish tears were temporarily observed immediately after administration in association with these pharmacological effects. No other adverse effects were observed over the course of exposure to the study drug throughout the animals' lives for any of the examined factors. However, a high incidence of uterine adenocarcinoma was observed in the medium-dose group (600 mg/kg/day) in this present carcinogenicity study using rats, which might be attributable to the administration of acotiamide-HH.

To evaluate the genotoxicity of acotiamide-HH, an *in vitro* chromosome aberration study was conducted using Chinese hamster lung cells (CHL), which showed an increase in cells with abnormal chromosomal structures (data not shown). However, negative results were obtained in an Ames test and a micronucleus study in rats. Additionally, negative results were obtained in a chromosome aberration study using human peripheral lymphocytes and an unscheduled DNA synthesis (UDS) study using rat hepatic cells. Accordingly, these results are indicative of the fact that acotiamide-HH is a non-genotoxic substance (Review Report, 2013), based on comprehensive judgment.

In general, estrogen is known to be a contributing factor to endometrial proliferation and a tumorgenetic factor in the uterus and cervix (Fox, 1984; Nagaoka *et al*., 1990; Nagaoka *et al*., 1994; Kitamura *et al*., 1999). With the exception of galantamine, the influence of other AChE inhibitors with similar drug efficacy on endometrial adenocarcinoma has not been confirmed, regardless of the strength of AChE inhibition. Mechanisms underlying the development of endometrial or endocervical cancer induced by galantamine have been proposed to involve the following processes. First, an increase in ACh concentration in the brain stimulates the release of dopamine, which inhibits the release of prolactin. Subsequently, a decrease in the prolactin level causes suppression of progesterone production, which results in a relatively estrogen-dominant hormonal imbalance that, in turn, induces endometrial cancer (Reminyl tablet 4-mg, Review Report, 2010). It is thought that the increase in ACh concentration in the brain would stimulate dopamine release, which would, in turn, influence other hormones, thereby leading to endometrial hyperplasia, pseudopregnancy, and vaginal cornification as has been confirmed in the case of galantamine. These results show that hormonal imbalance is an important factor in the induction of endometrial adenocarcinoma, and it influences the related tissues and organs. However, unlike galantamine, the central transitivity of acotiamide-HH is presumed to be low; therefore, its concentration in the brain is unlikely to increase (Yoshii *et al*., 2011). Thus, it is considered unlikely that a hormonal imbalance would be mediated by the action of acotiamide-HH in the brain. In fact, the various toxicological effects observed with galantamine were not confirmed with acotiamide-HH. For example, histopathological examination did not suggest any hormonal imbalance in the female genital organs and tissues including the mammary glands.

In a long-term carcinogenicity study of acotiamide-HH in mice, no carcinogenic activity was observed, and there was no effect on the sexual glands or endocrine system. Acotiamide-HH administration did not show any effects on the endocrine system in various toxicity studies in rats and dogs (Acofide tablets 100-mg, Review Report, 2013). In addition, several reproductive and developmental toxicity studies of acotiamide-HH have been conducted in rats including evaluations of fertility and early embryonic development until implantation, embryonic and fetal development, and prenatal and postnatal development and maternal function; however, no influence on the endocrine system or genital system was observed (Acofide tablets 100-mg, Review Report, 2013). Similarly, no influence of acotiamide-HH was observed in a study on embryonic and fetal development using rabbits. Thus, no influence of acotiamide-HH administration on sex hormones has been confirmed in numerous toxicity studies. The results of the present carcinogenicity study in rats strongly suggest that the naturally occurring endometrial adenocarcinoma was an accidental bias. However, the high value of the incidence of endometrial adenocarcinoma observed in the 600 mg/kg/day group cannot be explained in this present study. In addition, the background data obtained in the testing facility of this study supports this notion. In recent years, the incidence of naturally occurring endometrial adenocarcinoma has increased in carcinogenicity studies using F344 rats since 2000 as follows. Ando *et al*. (2008) reported that the mean incidence was 3.3% (range, 0-8%) in 1990-1999, whereas it was 12%

(range, 9-16%) in 2000-2004. Nyska *et al.* (1994) also reported that the incidence rates of endometrial adenocarcinoma were 13.3% and 24% in 2 lifetime feeding studies using F344 rats, and the NTP background data showed a rate of 11%. The incidence of endometrial adenocarcinoma observed in this study fell within the above range.

In view of the above results, any correlation between acotiamide-HH administration and increased incidence of endometrial adenocarcinoma in rats can be considered low. However, caution should be exercised and the profile of acotiamide-HH for human use should be carefully considered. Finally, it is necessary to conduct further studies to examine the relationship between endometrial adenocarcinoma and acotiamide-HH as part of the safety assessment of this drug for human use.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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