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General Pharmacology of ^{13}C-Urea Powder Preparation in Helikit™

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Abstracts – The pre-mixed ^{13}C-urea powder preparation in Helikit™ for test of *Helicobacter pylori* was evaluated for pharmacological properties. The oral doses of the preparation used in mice were 30-fold as compared to human doses. The results obtained in the present study demonstrate that spontaneous movement, hexobarbital-induced hypnosis, rotarod performance, body temperature, acetic acid-induced writhing syndrome, chemical and electroshock convulsion, pupil size and intestinal propulsion had not been affected at the oral doses of 230, 700 and 2100 mg/kg in mice. The blood pressure was slightly elevated as given intravenously in rats at a dose of 5 mg/kg of the preparation, but respiration was not influenced at the dose. In isolated guinea pig ileum and rat fundus preparation, the preparation at a concentration of 1×10^{-4} g/ml neither caused any direct effect nor inhibited the contraction produced by acetylcholine, histamine or 5-hydroxytryptamine. These results reported here provide evidence that pre-mixed ^{13}C-urea powder preparation is free of general pharmacological properties performed in oral administration.

Keywords□ Helikit™, ^{13}C-urea powder, general pharmacology

Helikit™ is a breath test kit of *Helicobacter pylori* in human gastrointestine, which was developed by the company of Isodiagnostika Inc. in Alberta, Canada. The pre-mixed ^{13}C-urea powder preparation, the main component of Helikit™, is to be ingested 5 g in the clinical diagnosis. However, the general pharmacology of the powder preparation has not been reported. The present study was undertaken to survey the general pharmacological properties of pre-mixed ^{13}C-urea powder preparation.

MATERIALS AND METHODS

Materials and Animals

The pre-mixed ^{13}C-urea powder preparation contains 5 g in a cup of the Kit, of which components are 75 mg of ^{13}C-urea as a main component and diluents of citric acid (USP, 750 mg), sodium citrate (USP, 220 mg), mannitol (USP, 3925 mg), sodium cyclamate (NF, 5 mg) and orange essence FD-9825-B5 (USP, 25 mg). For administration to the animals, the sample powder dissolved in distilled water was given orally in a volume of 10 ml/kg of b.w. except a test on respiration and blood pressure which was given in intravenous route. The standard drugs and reagents used were of commercial sources. The animals used were male ICR mice weighing 20~25 g, male Sprague-Dawley rats weighing 250~320 g, Hartley guinea-pigs of either sex weighing 150~180 g, and male New Zealand White rabbits weighing 1.7~2.2 kg.

Effect on Spontaneous Activity

The test was conducted according to the method of Nahorski *et al.* (1975). Male ICR mice were used in groups of 12. Two animals were kept in an activity cage (Ugo Basile) for 30 min for adaptation. Next day 30 min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), the locomoter activity of the two animals was measured for 30 min. Caffeine was used as a reference drug.

Effect on Hexobarbital Hypnosis

Male ICR mice were used in groups of 8. Thirty min after *p.o.* administration of the sample(230, 700 or 2100 mg/kg), 50 mg/kg of hexobarbital · Na was injected *i.p.* and the time between righting reflex loss and arousal was measured. Chlorpromazine hydrochloride was used as a reference drug.

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Effect on Motor Coordination

The test was conducted according to the method of Dunham *et al.* (1957). Male ICR mice were used in groups of 8. Mice clining to the rotating rod for 1 min were selected for the test. Thirty min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), the number of animals falling off the rotarod within 1 min was counted. Chorpromazine hydrochloride was used as a reference drug.

Effect on Body Temperature

Male ICR mice were used in groups of 7. Before dosing the rectal temperature of each animal was measured using a digital rectal thermometer (Shibaura Electronica Co.). The temperature was measured before and at 30, 60 and 120 min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg). Aminopyrine was used as a reference drug.

Effect on Acetic Acid-induced Writhing Syndrome

The test was conducted according to the method of Whittle *et al.* (1957). Male ICR mice were used in groups of 8. Thirty min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), the animals were subjected to *i.p.* injection of 0.7% acetic acid solution. Ten min later, the number of writhing exhibited for 10 min was recorded. Aminopyrine was used as a reference drug.

Anticonvulsant Activity

Effect on Strychnine-induced Convulsion

The test was conducted according to the method of Araki *et al.* (1972). Male ICR mice were used in groups of 8. Sixty min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), the animals received intraperitoneally 1.5 mg/kg strychnine. The number of dead mice was counted. Phenobarbital-Na was used as a reference drug.

Effect on Pentetrazol-induced Convulsion

The test was conducted according to the method of Swinyard *et al.* (1952). Male ICR mice were used in groups of 8. Thirty min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), 85 mg/kg of pentetrazol was injected *i.p.* Abolition of the hindleg tonic extensor phase of the seizure was used as a criterion for protection against convulsion. The number of protected mice was counted and the latency time until the tonic convulsion was recorded. Phenobarbital-Na was used as a reference drug.

Effect on Maximal Electroshock-induced Convulsion

The test was conducted according to the method of Woodbury *et al.* (1952). Male ICR mice were used in groups of 8. The animal received an electroshock in both corneas (60 Hz, 50 mA, 0.1 sec) 30 min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg). The number of death and duration of tonic extensor convulsion were recorded. Phenobarbital-Na was used as a reference drug.

Effect on the Respiration and Blood Pressure

The depth and rate of respiration of rats anesthetized by urethane-saline (2 g/kg, *s.c.*) were measured using the pneumatic pulse transducer (Narco Biosystem Inc.) and simultaneously the direct blood pressure recorded through the canula inserted into the left common carotid artery was measured using the blood pressure transducer (P-1000B, Narco Biosystem Inc.). Samples solublized in saline (0.5 ml/kg) were intravenously injected through the tail vein of rats placed on the temperature-controlled holder plate continuously kept at 37℃.

Effect on Isolated Smooth Muscles

Effect on the Isolated Ileum of Guinea Pig

The ileum of male guinea pig fasted 24 hr before the experiment was isolated. The ileum was kept in Locke-Ringer solution of Magnus organ bath at 32℃ into which carbogen (95% O_2+5% CO_2) was continuously supplied. After the ileum was stabilized at the resting tension of 0.5 g, the contraction and relaxation were recorded using the isometric transducer (Myograph F-60, Narco Biosystem Inc.). As reference drugs, acetylcholine and histamine each with the final concentration in the organ bath of 10^{-5} M were used. The sample was injected into the organ bath so as the final concentration was 10^{-5} g/ml or 10^{-4} g/ml in the organ bath.

Effect on the Rat Stomach Fundus Strip

Stomach fundus strip of male rat fasted 24 hr before the experiment, was isolated according to the method of Vane *et al.* (1957). The fundus strips were kept in Locke-Ringer solution of Magnus organ bath at 32℃ into which carbogen (95% O_2+5% CO_2) was continuously supplied. After the fundus was stabilized at the resting tension of 0.5 g, the contraction and relaxation were recorded using the isometric transducer (Myograph F-60, Narco Biosystem Inc.). As a reference drug, 5-hydroxytryptamine creatinine sulfate with the final concentration in the organ bath of 10^{-4} M was used. The sample was injected into the organ bath so as the final concentration could be 10^{-5} g/ml or 10^{-4} g/ml in the organ bath.

Effect on Pupil Size

The test was conducted according to the method of Takagi *et al.* (1972). Male ICR mice were used in groups of 8. Pupil diameters were measured with micrometer. Thirty min and 60 min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), the pupil diameters were measured.

Effect on Intestinal Propulsion

The test was conducted according to Takagi *et al.* (1972). Male ICR mice were used in groups of 8. Thirty min after *p.o.* administration of the sample (230, 700 or 2100 mg/kg), 0.2 ml of $BaSO_4$ suspension (1 g/ml of 0.1% CMC solution) was given orally and 30 min later, the animals were sacrificed. The intestine was isolated and the distance which $BaSO_4$ covered was measured. Its ratio to the full length of the small intestinal trace was calculated. Lidocaine was used as a reference drug.

Statistical Analysis

Statistical analysis of the data was evaluated using analysis of variance followed by Dunnett's t-test on the data of the sample, Student's t-test on the data of reference drugs, or Chi-square test specified in each Table. All tests were evaluated at the $p<0.05$ level of significance.

RESULTS

Effect on Spontaneous Activity

The sample given at 230, 700 or 2100 mg/kg, *p.o.* produced no effect on spontaneous movement. Caffeine at a dose of 10 mg/kg, *p.o.* increased significantly the movement (Table I).

Effect on Hexobarbital-induced Hypnosis

The sample given at 230, 700 or 2100 mg/kg, *p.o.* showed no effect on sleeping time induced by hexobarbital in mice. Chlorpromazine as a reference prolonged the sleeping time at a dose of 4 mg/kg, *p.o.* (Table II).

Effect on Motor Coordination

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice had no effect on motor coordination. Chlorpromazine as a reference interfered with motor coordination at a dose of 10 mg/kg, *p.o.* (Table III).

Effect on Body Temperature

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice showed no effect on the normal rectal temperature. Aminopyrine as a reference caused a significant decrease in body temperature at a dose of 200 mg/kg, *p.o.* (Table IV).

Table I. Effect of the sample on spontaneous activity in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	No. of data	No. of movement before treatment (for 30 min)	No. of movement after treatment		
					30~40 min	40~50 min	50~60 min
Saline	–	12	6	2095.8±226.5	469.2±60.7	302.5±62.4	228.8±49.7
Sample	230	12	6	2100.7±193.4	540.3±33.2 (15.2)	350.8±59.5 (16.0)	283.0±71.4 (23.7)
	700	12	6	2105.5±204.4	468.2±54.3 (-0.2)	316.3±88.7 (4.6)	273.8±62.4 (19.7)
	2100	12	6	2241.7±188.8	519.7±56.5 (10.8)	309.3±14.9 (2.3)	213.8±18.8 (-6.6)
Caffeine	10	12	6	2114.7±90.6	1054.2±97.6* (124.7)	900.7±65.5* (197.7)	960.7±101.3* (319.8)

Significantly different from the saline group (*; $p<0.01$). The figures in parentheses indicate increase percents.

Table II. Effect of the sample on hexobarbital-induced sleeping time in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Initiation time to sleep (sec)	Sleeping time (sec)	Increment (%)
Saline	–	8	185.6±15.2	413.4±83.9	–
Sample	230	8	185.9±18.4	421.5±94.2	2.0
	700	8	190.3±18.7	490.0±96.6	18.5
	2100	8	227.4±14.8	418.9±50.7	1.3
Chlorpromazine	4	8	131.1±11.9*	1011.1±91.7**	144.6

Significantly different from the saline group (*; $p<0.05$, **; $p<0.01$).

Table III. Effect of the sample on rota-rod test in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of tested mice	No. of fallen mice
Saline	–	8	0
Sample	230	8	0
	700	8	0
	2100	8	0
Chlorpromazine	10	8	8*

Significantly different from the saline group (*; $p<0.01$ in χ^2-test).

Effect on Acetic Acid Writhing Syndrome

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice showed only a tendency to inhibit writhing syndrome, but it was not significant as compared with control group. Aminopyrine 200 mg/kg, *p.o.* in mice as a reference caused significant inhibition of writhing by approximately 91% (Table V).

Anticonvulsant Activity

Effect on Strychnine-induced Convulsion

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice did not inhibit strychnine-induced death. Phenobarbital · Na 100 mg/kg, *p.o.* as a reference caused complete inhibition of death due to convulsion (Table VI).

Effect on Pentetrazol-induced Convulsion

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice did not inhibit pentetrazol-induced convulsion. Phenobarbital · Na as a reference caused complete inhibition of the convulsion at 100 mg/kg, *p.o.* (Table VII).

Effect on Maximal Electroshock-induced Convulsion

The sample given at 230, 700 or 2100 mg/kg, *p.o.* in mice did not inhibit significantly the electroshock-induced convulsion. Phenobarbital · Na as a reference caused complete inhibition of the convulsion at 50 mg/kg, *p.o.* (Table VIII).

Effect on Respiratory and Blood Pressure

A typical pattern of 4 experiments was shown in Fig.

Table V. Effect of the sample on acetic acid-induced writhing syndrome in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	No. of writhing	Inhibition (%)
Saline	–	8	13.6±3.0	–
Sample	230	8	14.1±4.4	-3.7
	700	8	10.8±4.5	21.1
	2100	8	7.3±1.5	46.8
Aminopyrine	200	8	1.3±0.7*	90.8

Significantly different from the saline group (*; $p<0.01$).

Table VI. Effect of the sample on strychnine-induced death in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of tested mice	No. of died mice	Death time (sec)
Saline	–	8	8	204.4±19.3
Sample	230	8	8	212.8±14.8
	700	8	8	213.6±14.5
	2100	8	8	216.4±12.9
Phenobarbital · Na	100	8	0*	—

Significantly different from the saline group (*; $p<0.01$ in χ^2-test).

Table VII. Effect of the sample on pentetrazol-induced convulsion in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of tested mice	No. of convulsed mice	Convulsion time (sec)
Saline	–	8	8	208.0±18.3
Sample	230	8	8	209.5±16.5
	700	8	8	214.3±17.9
	2100	8	8	203.0±45.7
Phenobarbital · Na	100	8	0*	—

Significantly different from the saline group (*; $p<0.01$ in χ^2-test).

1. The sample at 1 mg/kg *i.v.* in rats did not affect the respiration and the blood pressure, and at 5 mg/kg *i.v.* in rats the blood pressure was slightly elevated. However,

Table IV. Effect of the sample on rectal temperature in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Rectal temperature (℃)			
			0 min	30 min	60 min	120 min
Saline	–	7	37.66±0.19	37.44±0.14	37.59±0.16	37.43±0.10
Sample	230	7	37.69±0.16	37.54±0.22	37.56±0.20	37.47±0.20
	700	7	37.70±0.16	37.43±0.26	37.69±0.21	37.50±0.21
	2100	7	37.66±0.17	37.51±0.14	37.51±0.24	37.37±0.15
Aminopyrine	200	7	37.64±0.12	35.57±0.47*	35.37±0.36*	35.87±0.34*

Significantly different from the saline group (*; $p<0.01$).

Table VIII. Effect of the sample on maximal electroshock seizure in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of tested mice	No. of survived mice	Death rate (%)	Convulsion time (sec)	Death time (sec)
Saline	–	8	3	62.5	53.0±5.9	26.4±2.7
Sample	230	8	3	62.5	51.3±8.0	27.4±2.5
	700	8	2	75.0	57.5±19.5	29.5±1.8
	2100	8	4	50.0	54.5±5.1	27.5±4.9
Phenobarbital · Na	50	8	8*	0.0	14.9±1.4#	–

Significantly different from the saline group (*; $p<0.01$ in χ^2-test, #; $p<0.01$ in t-test).

the effect was slight as compared with those of acetylcholine (5 μg/kg, *i.v.*) and epinephrine (5 μg/kg, *i.v.*). The sample at 5 mg/kg, *i.v.* did not influence respiratory and heart rates in the rats (not shown).

Effects on Isolated Smooth Muscles

Effect on Isolated Guinea Pig Ileum

Results are shown in Figs. 2 and 3. The sample at a concentration of 1×10^{-4} or 1×10^{-3} g/ml did not effect the resting tone of the guinea pig ileum. The presence of the sample at a concentration of 1×10^{-4} or 1×10^{-3} g/ml did not inhibit the contraction produced by acetylcholine (1×10^{-3} M) or histamine (1×10^{-3} M).

Effect on Isolated Rat Fundus Strip

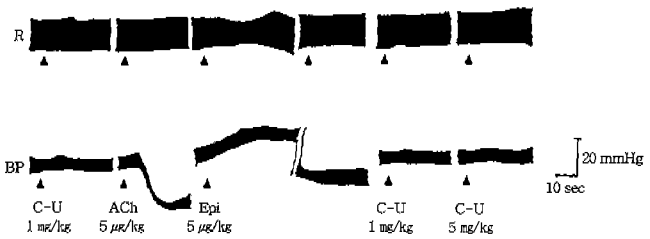

Fig. 1. Effects of the ^{13}C-urea powder (C-U) on respiration and blood pressure of an anesthetized rat. Each arrowhead represents the administration point of each sample. R: Respiration, BP: Blood pressure, ACh: Acetylcholine, Epi: Epinephrine.

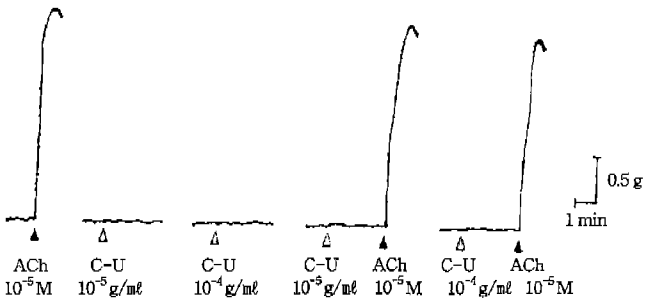

Fig. 2. Effects of the ^{13}C-urea powder (C-U) on contration of guinea pig ileum by acetylcholine (ACh). Each arrowhead symbol represents the administration point of each sample.

As shown in Fig 4, the sample at a concentaration of 1×10^{-5} or 1×10^{-4} did not affect the resting tension of rat fundus strip. The presence of the sample at a concentration of 1×10^{-5} or 1×10^{-4} g/ml did not influence the contraction produced by 5-hydroxytryptamine (1×10^{-4} M).

Effect on Pulpil Size

The sample at doses of 230, 700 or 2100 mg/kg, *p.o.* did not affect the pupil size of mice until 2 hrs. Atropine as a reference caused mydriatic effect at 2 mg/kg, *p.o.* (Table IX).

Effect on Intestinal Propulsion

The sample at doses of 230, 700 or 2100 mg/kg, *p.o.* did not influence intestinal propulsion of barium sulfate meal

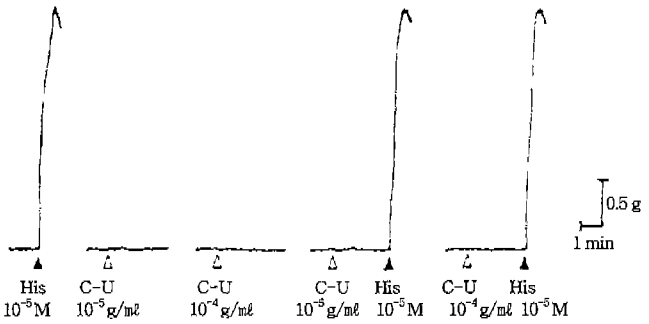

Fig. 3. Effects of the ^{13}C-urea powder (C-U) on contration of guinea pig ileum by histamine (His). Each arrowhead symbol represents the administration point of each sample.

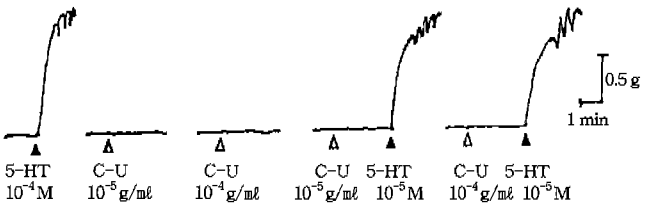

Fig. 4. Effects of the ^{13}C-urea powder (C-U) on contration of rat fundus strip by 5-hydroxytryptamine (5-HT). Each arrowhead symbol represents the administration point of each sample.

Table IX. Effect of the sample on pupil size in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Pupil size (mm)		
			0 hr	1 hr	2 hr
Saline	–	8	0.659±0.012	0.685±0.014	0.671±0.014
Sample	230	8	0.661±0.012	0.684±0.012	0.686±0.010
	700	8	0.674±0.018	0.680±0.013	0.663±0.007
	2100	8	0.685±0.012	0.691±0.018	0.674±0.013
Atropine	2	8	0.670±0.028	1.325±0.054*	1.694±0.059*

Significantly different from the saline group (*; $p<0.01$).

Table X. Effect of the sample on intestinal propulsion in mice

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Propulsion (%)	Inhibition (%)
Saline	–	8	53.7±2.5	–
Sample	230	8	53.3±4.5	0.7
	700	8	57.2±4.8	-6.6
	2100	8	49.3±4.3	8.1
Lidocaine	100	8	35.1±3.5*	34.6

Significantly different from the saline group (*; $p<0.01$).

in mice. Lidocaine at a dose of 100 mg/kg, *p.o.* caused considerable inhibition of intestinal propulsion (Table X).

DISCUSSION AND CONCLUSION

In clinical use, 5 g of pre-mixed ^{13}C-urea preparation is to be ingested for a diagnostic purpose. In this pharmacological work, three dose levels of 230, 700 and 2100 mg/kg were administered orally to the animals. Three doses of the sample in animals correspond to the amount of 3-, 10- and 30-fold as compared to human doses. The results obtained in the present study demonstrate that spontaneous movement, hexobarbital-induced hypnosis, rotarod test, body temperature, acetic acid-induced writhing syndrome and chemical and electroshock convulsion had not been affected at the doses given orally in mice. The respiration and blood pressure were not influenced as given intravenously in rats at a dose of 1 mg/kg. At a dose of 5 mg/kg *i.v.*, the blood pressure of rats was slightly increased, but respiration was not influnced at the dose. It is described here that the contents of ^{13}C-urea are as little as 75 mg in 5 g of this preparation. Therefore, it is unclear that the effect on blood pressure might be due to ^{13}C-urea or the diluents in the preparation. However, the intravenous administration route of the sample is regardless of clinical oral uses. In isolated guinea pig ileum and rat fundus preparation the concentrations of 1×10^{-5} and 1×10^{-4} g/ml of the sample did neither cause any contraction nor relaxation and did not inhibit the contraction produced by acetylcholine, histamine or 5-hydroxytryptamine, of which facts indicate that the sample is neither agonistic nor antagonistic to acetylcholine, histamine or 5-hydroxytryptamine. The oral administration of the sample at 2100 mg/kg in mice did not affect pupil size and intestinal propulsion of barium sulfate meal. These results reported here provide evidence that pre-mixed ^{13}C-urea powder preparation is devoid of serious general pharmacological properties.

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