Dynamic Studies on the Distribution and Excretion of ^{3}H-Kopsinine 1 in Rats

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ABSTRACT **OBJECTIVE**: To study the tissue distribution and excretion of ^{3}H-Kopsinine 1 in rats. **METHODS**: ^{3}H-Kopsinine 1 were administrated to 7 groups of 6 rats orally or intravenously, then blood, organ, urine, feces and bile were collected to prepare sample solutions. After 12 hours, the radioactivity was measured by Liquid Scintillation Counter. **RESUTS**: The radioactivity time curve can be described by a two compartment model. The T1/2α and the T1/2β is 12 minutes and 28.1 hours respectively. After oral administration the radioactivity in liver at 1 hour was 277.5± 17.8ng/g and that in Kidney was 182.4± 12.0ng/g. The majority (66%) of radioactivity was recovered from urine in 24 hours and 14% of radioactivity was from bile in 48 hours. **CONCLUSION**: Results suggest that liver is the main effective organ of Kopsinine 1, and also, Kopsinine 1 has a little possibility for enterohepatic circulation.

KEY WORDS Kopsinine 1, distribution,, excretion, pharmacokinetics

蕊木宁在大鼠体内分布与排泄的动态研究

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摘要 目的: 研究云南蕊木中有效成分蕊木宁在大鼠体内的组织分布与排泄。方法: 将蕊木宁用放射性元素^{3}H 标记, 分别以静注和口服方式给予受试大鼠, 收集血液、脏器、尿液、胆汁等, 用液体闪烁记数仪测量其放射活性。结果: 血中的放射活性呈二相衰减, t1/2α 为 12m in, t1/2β 28.1h。口服给药 1h 后, 肝脏中的放射物浓度为 277.5± 17.8ng/g, 肾脏为 182.4± 12.0ng/g; 并且, 48h 内, 大部分放射活性(66%)由尿液排泄, 小部分(14%)由胆汁排泄。结论: 肝脏可能是蕊木宁的主要靶器官, 且蕊木宁自身产生肝肠循环的可能性较小。

关键词 蕊木宁; 分布; 排泄; 药代动力学

One of the traditional Chinese medicines, Yunnan-Xin-Mu (YXM) is prepared from roots and leaves of Kopsia officinalis Tsiang et P. T. Li in China. Kopsinine 1 is known to be an active compound of YXM, usually used for the treatment of bone ache and hepatic disease. Although many pharmacological studies of Kopsinine 1 have been done, pharmacokinetic study has not been reported[1]. In this paper, pharmacokinetic studies of Kopsinine 1 were examined with radioactive compound. The relationship between pharmacological effects and pharmacokinetics of Kopsinine 1 was discussed.

MATERIAL AND METHOD

Drug: ^{3}H-Kopsinine 1 was prepared by China institute of Atom Energy (Beijing). Its specific radioactivity is 153.9 Ci/mol and the radio purity is more than 98%. The unlabeled Kopsinine I was isolated from YXM in our laboratory and identified. All other chemicals with the highest grade were from commercial source.

Animals: Male wistar/ST rats, 6 weeks old (150-200g body weight), were purchased from Bethune Medical University (China). The animals were bred in a breeding room with temperature of 24± 1℃, humidity of 50± 5% and 12 hours dark-light cycle for 3 days. They were given tap water and normal tbods ad libitun. The rats in oral administration group were fasted about 24 h before experiment.

Apparatus: Radioactivity Measurement was carried out by Parkart Model 4430 Liquid Scintillation Counter (Parkart Instrument Co. Inc. U.S.A)

Drug preparation: A) For i.v. injection: ^{3}H-Kopsinine 1 was dissolved in methanol to give the solution of 1 mg/mL. 3.5 mL of the solution was added to 96.5mL of distilled water under stirring. This solution (100ml) was named preparation A. Radioactivity of preparation A was adjusted to 26.08 μCi/mL, and then the dose (350 μg/kg) of preparation A was calculated to 260.8 μCi/kg. B) For oral

administration: 0.8 mL of ^{3}H-Kopsinine 1 methanol solution was added to 24.2 mL of distilled water under stirring. This solution (25 mL) was named preparation B. Radioactivity of preparation B was adjusted to 0.78 μCi/mL, and then the dose(640 μg/kg) of preparation A was calculated to 7.8 μCi/kg.

Blood sample preparation: After i.v. injection of ^{3}H-Kopsinine 1, blood was collected from orbital margin vein of rat in designed time period. 0.1 mL of blood was poured into the mixture of formic acid solution(88%)-hydrogen peroxide(30%)-noctanol(4∶3∶0.5), then the mixture was incubated in water bath at 80℃ for 45 min. After cooling to room temperature, the radioactivity of the mixture was measured.

Preparation of organ tissue sample: After i.v. injection of ^{3}H-Kopsinine 1, the organs of rat were collected in designed time period. The organs were washed with isolonic solution, and was cleaned with Kimtowel(Kimbery-Clark Co. Inc, Japan) before homogenization. 50 mg of each homogenate was put into the mixture of formic acid solution (88%)-hydrogen peroxide(30%)-noctanol(4∶3∶0.5). Then it was treated in the same way as mentioned above.

Urine sample preparation: After oral administration of preparation B, urine was collected about 0.5mL in designed time period. Then the collected urine was diluted to 20 mL with water.

Bile sample preparation: A polyethylene tube (fr, No3, Hibiki) was inserted into the rat bile duct under amobarbital anesthesia. After oral administration of preparation B to the operated rat, bile was collected about 0.5mL at designed interval. Then it was treated with the same method mentioned in blood sample[2].

Radioactivity detection: ^{3}H-Kopsinine 1 were administrated to 7 groups of 6 rats by orally or intravenously. Blood, urine, feces and bile were collected prior to organ in designed time period(before the organs were collected, the animals must be put to death). Then they were treated to get sample solutions by the method mentioned above. 0.1 mL of sample solution was added to the mixture of 2-ethoxyethanol-PPO xylene solution(4∶6). After 12h, the radioactivity was measured by Liquid Scintillation Counter (Parkart Model 4430).

Data analysis: The radioactivity-time curve was plotted semilogarithmically. The half-life (T1/2) was calculated from the linear region by linear regression analysis. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal method from a graph for up to 48 h[3].

RESULT

Rodioactivity-time course in blood: Blood was collected at 3, 5, 20 min and 1, 8, 24 and 4g h after i.v. injection of ^{3}H-Kopsinine 1 and their radioactivities were detected (Table 1). The radioactivity in blood is shown as 320.3±18.5ng/mL after administration and 90.0±4.4ng/mL 60 min later. The radioactivity time curve can be described by a two compartment model. The T1/2 α and the T1/2 β is 0.20h, 28.1h respectively(Fig.1, Table2).

Distribution of radioactivity in organ tissue: After i.v. injection, organ were collected from 3 min to 48 h. The highest level of radioactivity was found in kidney and liver. The level of radioactivities were moderated in blood, lung and heart, and those in testis and fat were the lowest(Table 1). The elimination rate of radioactivity in liver was much slower than that of kidney. As the radioactivity in liver at 1 hour was 277.5±17.8 ng/g and that in kidney is 182.4±12.0ng/g, it was considered that the drug still concentrated in liver.

Table 1 Distribution of Radioactivity after Orai Administration of ^{3}H-Kopsinine 1

Tissue	Concentration(ng/g or ml of ^{3}H-Kopsinie 1)[a]						
	3min	5min	20min	1h	8h	24h	48h
Blood	320.3±18.5	260.1±34.1	175.2±3.6	90.0±4.4	75.1±3.8	50.4±8.0	26.8±2.9
Liver	389.4±147.6	944.5±54.8	438.5±30.5	277.5±17.8	136.0±9.1	104.1±21.4	71.5±7.6
Spleen	107.7±14.1	142.5±7.6	111.0±8.2	89.2±15.2	68.9±6.5	49.7±5.8	38.1±5.4
Lung	308.7±61.3	397.5±45.7	165.8±9.8	127.7±10.5	92.5±6.9	56.9±12.0	28.7±2.2
Kidney	1391.4±214.0	2154.9±94.3	640.2±60.2	182.4±12.0	131.7±16.7	56.9±2.5	35.2±2.5
Testis	41.7±6.5	79.1±5.1	68.2±4.4	58.0±7.6	47.2±4.7	40.6±2.2	31.6±5.4
Far	31.9±8.7	72.2±2.2	66.0±2.5	46.1±4.7	28.3±7.3	19.6±3.6	14.1±2.5
Brain	51.1±7.6	80.5±6.5	58.0±8.7	49.7±8.3	37.0±2.9	29.0±2.9	22.5±2.2
Sub. Gland	119.3±24.7	164.3±18.9	108.8±8.7	76.9±5.8	44.6±2.2	30.5±2.5	20.7±4.7
Adr. gtand	98.3±16.7	146.9±32.3	124.8±10.9	87.4±18.9	64.6±10.9	45.0±5.1	25.4±5.8
Thymus	81.2±21.0	156.7±21.8	96.8±7.6	62.4±16.7	41.0±2.5	30.1±3.3	18.5±2.9

a) The Data are expressed as the mean ± SD of six rats.

Table 2 Pharmacokinetic Parameters of ^{3}H-Kopsinine 1 in Blood

Parameter	Value
T1/2α(h)	0.20
T1/2β(h)	28.09
AUC(mg.h/L)	2.67
Vd(L/kg)	3.86

a) The mean value from 7 groups of 6 rats.

Excretion in urine and bile: ^{3}H-Kopsinine 1 was excreted from urine and bile when administrated orally. The majority of radioactivity(66%) was excreted in urine in 24 hours and small amount of it(14%) was excreted in bile in 48h. The general result was shown in Fig. 2 and Fig. 3.

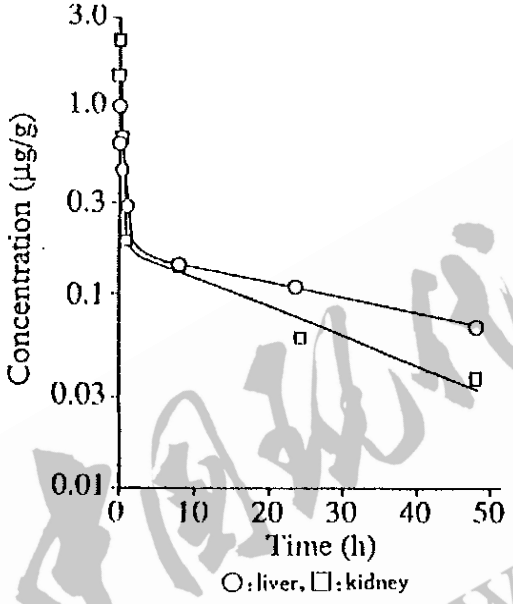

Fig. 1 Time Course of Radioactivity in Liver and Kidney after Intravenous Administration of ^{3}H-Kopsinine 1

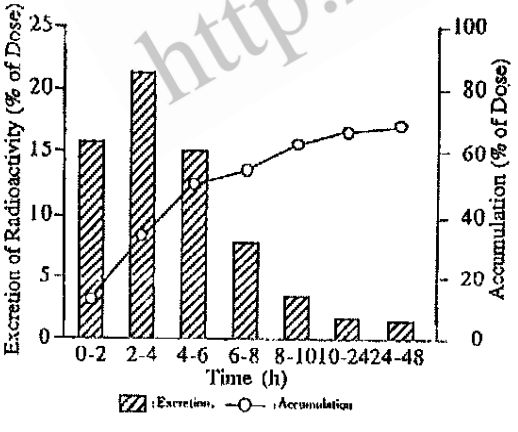

Fig. 2 Time Course of Radioactivity in Urine after Oral Administration of 0.35mg/kg of ^{3}H Kospinine 1

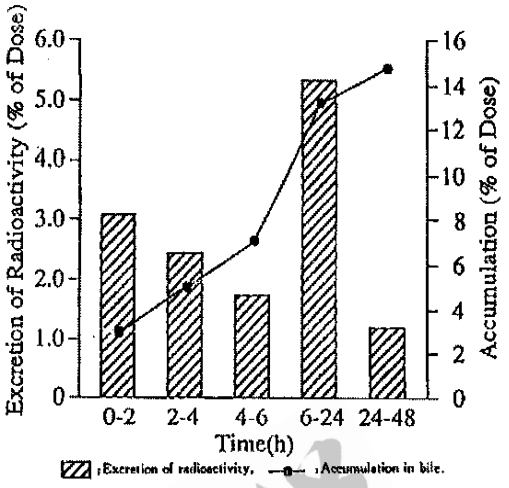

Fig. 3 Time Course of Radioactivity in Bile after Oral Administration of ^{3}H-Kopsinine 1(n= 6)

DISCUSSION

The radioactivity in blood after intravenous injection of ^{3}H-Kopsinine 1 distributed to organ tissue very soon. The blood level of radioactivity decreased in two phases, namely the distribution phase and the elimination phase, with the T1/2 α of 0.20h and T1/2 β of 28.1 h. The pharmacokinctic properties are different from the parameters obtained by HPLC method. By using radioactive Kopsinie 1, the original form as well as its metabolites can be detected. But chemical method determined only the original form of Kopsinine 1. Since both Kopsinine 1 and its metabolites have the pharmacological activity[4]. So the sensitivity of HPLC-UV method is much lower than that of the radioactivity. Therefore, results from the HPLC method missed the elimination phase. The T1/2 (11 min) of Kopsinine 1 obtained by HPLC was almost the same as T1/2 α by radioactivity. The pharmacokinetic parameters by radioactivity can explain the maintenance of choleretic effect much better than that by HPLC.

After intravenous injection of ^{3}H-Kopsinine 1 the level of radioactivity was the highest in kidney and liver. Kidney and liver are the main organ for excreting and metabolism. The radioactivity in liver was the highest at 1 hour after administration, and the decreasing rate was the slowest. This phenomenon suggested that liver is the main affinitive organ of the crude drug, which is conformed to traditional Chinese meaicine theory. Finnally it was excreted in urine and bile. The majority of radioactivity was excreted in urine. Comparing to the excretion in urine, the radioactivity excreted in bile was at a very low level. The reason might be own to the molecule weight (338) of Kopsinine 1, because the compound below 400 molecule weight is difficult to

excrete from bile[5]. Our result confirms to the above theory. And the results also suggest that Kopinine 1 has a little possibility for enterohepatic circulation.

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