

Pharmacokinetics and autoradiography of [³H] or [¹⁴C]stepholidine

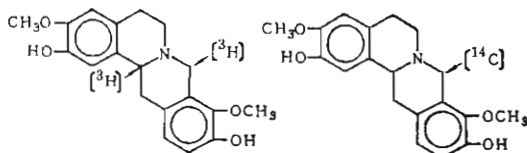
ZHANG Zhen-De, ZHOU Chen-Min, JIN Guo-Zhang, ZHANG Xin, YANG Liu
(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT After iv [³H]stepholidine (SPD) 12 MBq/kg, the concentration-time curve in rats was found to be a two-compartment open model. The distribution phase $T_{1/2\alpha} = 3.6$ min, the elimination phase $T_{1/2\beta} = 168$ min. The absorbed radioactivities of [³H] SPD in 15-30 min were 80-87%. The amounts of [³H]SPD bound to plasma protein, liver and kidney homogenates were estimated to be 37, 31, and 30%, respectively. During 3 d after ip [³H]SPD 50 MBq/kg, 56% of the radioactivity was excreted in urine and 5% in faeces, thus, it suggested that [³H]SPD was mainly excreted by kidneys.

After iv a single dose of [¹⁴C]SPD in mice, the whole-body autoradiography showed that [¹⁴C]SPD was rapidly distributed among various tissues. High radioactivities were found in kidneys, liver, brain, salivary glands, Harder's glands, heart blood and muscle at 2 min and intensively localized in kidneys and stomach mucosa at 30 min. The radioactivities in these tissues disappeared 4 and 8 h later, while that in intestine could not be detected 24 h later.

KEY WORDS stepholidine; berbines; pharmacokinetics; tissue distribution; autoradiography

l-Stepholidine (*l*-SPD), isolated from *Stephania intermedia* Lo in Yunnan province, is an analog of tetrahydroprotoberberine (THPB) and a new kind of dopamine receptor antagonist with some peculiar pharmacological characteristics^(1,2). *l*-SPD antagonized apomorphine-induced stereotypy in rats and vomiting in dogs and produced a transient catalepsy in mice⁽³⁾. Moreover, it declined the arterial blood pressure without any adverse effect on heart^(4,5). Recently, it has been found that *l*-SPD possesses the inhibitory effects on central nervous system, such as analgesia and sedation^(5,6). In order to introduce *l*-SPD to clinical trials, the pharmacokinetic and whole-body autoradiography of *l*-SPD in animals were studied in this work.



METHODS AND RESULTS

[¹⁴C]SPD and [³H]SPD were synthesized in our institute with the specific activity of 59.6 MBq/mmol and 6.7 GBq/mmol, respectively. The radiochemical purity was greater than 95% on thin layer chromatography⁽⁷⁾. [¹⁴C]SPD and [³H]SPD were dissolved in H₂SO₄ 5 mmol/L, then adjusted with NaOH 0.01 mol/L to pH 4.5.

Time curve of [³H]SPD concentration in plasma Six ♂ Wistar rats (305 ± 11 g) were used in this experiment. The blood samples were drawn from femoral artery after iv [³H]SPD 12 MBq/kg 1, 5, 10, 15, 30 min, 1, 2 and 3 h, and were put into heparinized tubes. 200 μl plasma from each sample was obtained after centrifugation and was extracted with 1,2-dichloride ethylene (3 × 10 ml). The organic layer was evaporated dry and the residue was dissolved again with small volume of 1,2-dichloride ethylene (50 μl). The solution was used for chromatography on the thin layer plate of fluorescent silica gel G (sizes: 7.5 × 2.5 cm). The plate was developed by an ascending system of *n*-butanol : acetic acid : water (3 : 0.5 : 3). A spot was obtained with *R_f* value (0.54) which was the same as the standard radiosample soaked in scintillation liquid. The recovery rate of *l*-SPD from the extraction was 60%. The pharmacokinetic analyses were computed according to westlake^(8,9). It has been shown that the concentration-time curve was simulated to a 2

compartment open model consisting of 2 phases (Fig 1). The pharmacokinetic parameters were: $T_{1/2\alpha} = 3.6$ min; $T_{1/2\beta} = 168$ min, $k_{12} = 8.43 \text{ h}^{-1}$, $k_{21} = 1.78 \text{ h}^{-1}$, $k_{10} = 1.63 \text{ h}^{-1}$, $V_c = 0.86 \text{ ml/g}$, $V_b = 5.60 \text{ ml/g}$, $AUC = 2.24 \times 10^5 \text{ dpm/(ml} \cdot \text{h)}$. It denotes that [^3H]SPD was rapidly distributed from central compartment to peripheral one.

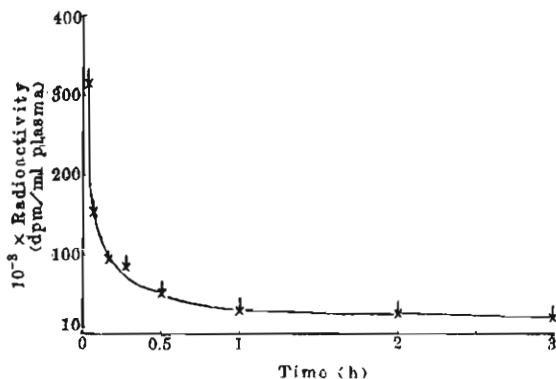


Fig 1. Plasma level of [^3H]stepholidine in rats after iv 12 MBq/kg. $n = 6$, $\bar{x} \pm \text{SD}$.

Distribution of [^3H]SPD in tissues Twelve $\text{♀} \delta$ Kunming species mice (22.6 ± 1.4 g) were used. After iv [^3H]SPD 202 MBq/kg at 5, 30, 60 and 180 min, 10 μl of blood samples from the eyes of mice were drawn. Mice were killed and the various tissues were dis-

sected and weighed (10–20 mg). It was found that [^3H]SPD was widely distributed in body tissues at 5 min after iv and the highest radioactivity of [^3H]SPD was in kidneys. The concentration order of radioactivity in tissues: kidneys > liver > brain > lung > heart > stomach > intestine, whereas a very low level was present in the spleen. The radioactivity tended to decrease in the tissues 3 h later (Fig 2).

Absorption of [^3H]SPD from gastrointestinal tract Fifteen $\text{♀} \delta$ Kunming mice (21.5 ± 1.4 g) were starved for 24 h before administration of drug. At 15, 30 and 60 min after ig [^3H]SPD 202 MBq/kg, the absorbed radioactivities of [^3H]SPD were 80, 87 and 90%, respectively. This suggested that [^3H]SPD was rapidly penetrated through gastrointestinal membrane.

Protein-binding determination of [^3H]SPD The blood samples were drawn from the femoral vein of three δ Wistar rats (180 ± 2 g) and were separately put into the heparinized tubes. Plasma was obtained after centrifugation and diluted to 1:2 with phosphate buffer (0.2 mol/L, pH 7.4). The kidneys and livers were dissected, and homogenized separately in 5 ml/g tissues of

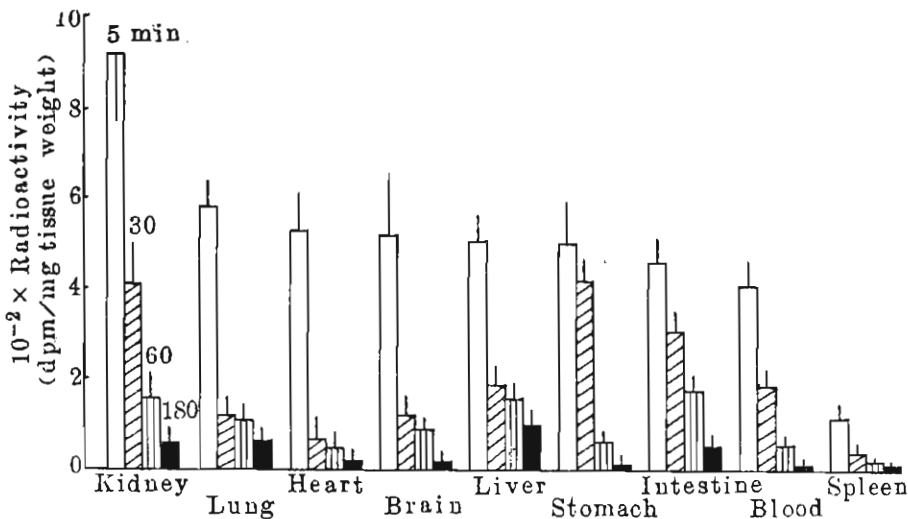


Fig 2. Tissue distribution of [^3H]stepholidine in mice at 5, 30, 60 and 180 min after iv 202 MBq/kg. $\bar{x} \pm \text{SD}$.

phosphate buffer. One ml of the homogenate suspensions and plasma were put separately into a small bag of cellulose dialysis membrane. The bag was placed into a glass-stoppered centrifuge tube containing 10 ml phosphate buffer and [^3H]SPD 14 035-17 544 dpm. The tube was incubated at 37°C for 24 h in water bath. Protein-binding of [^3H]SPD was determined by equilibrium dialysis method⁽¹⁰⁾. It was showed that the amounts of [^3H]SPD bound to plasma, liver and kidney homogenates in tissues were 37, 31 and 30%, respectively.

Excretion of [^3H]SPD Three ♂ Wistar rats (303 ± 6g) were anesthetized with ether. The polyethylene catheter was inserted into the rat total bile duct. After iv [^3H]SPD 3.4 MBq/kg, the bile was collected at 5, 15, 30 min, 1, 2, 4 and 8 h. The maximum peak value of excretion of [^3H]SPD in bile appeared at 60 min and its amount was only 12% in total.

Three ♀ ♂ Kunming species mice (20.3 ± 0.6) were separately placed in the cages constructed to allow collection of urine and faeces. After ip [^3H]SPD 50 MBq/kg, urine and faeces were collected at various intervals (0-24, 24-48 and 48-72 h). Samples of 10 μl urine and 5-10 mg faeces were used for determination. It was showed that the radioactivity in urine excreted within 0-24 h was 49 ± 6. The cumulative radioactivity excreted in 72 h were 56%, whereas only 5% in faeces was found in the same period. Therefore, [^3H]SPD was mainly excreted by kidneys.

Whole-body Autoradiography of [^{14}C]SPD Each dose of [^{14}C]SPD 15.6 MBq/kg was iv to five Kunming species mice (29.0 ± 0.8 g) at 2, 30 min, 4, 8 and 24 h. The ether anesthetized mice were immersed immediately into a mixture of hexane and dry ice (-70°C). Then, the mice were embedded in an aqueous gel of carboxymethyl cellulose (3% CMC) and immersed into a mixture of hexane and dry ice. The whole-body auto-

radiography was performed according to Slanina^(11,12). Sagittal whole-body sections of 20 μm was performed with a microtome of LKB-2250 type at -20°C and the sections of different levels of the body were taken out on tape (NO. 800, USA). Then, they were freeze-dried and exposed against X-ray films at -20°C in the cryostat lasted 5 wk. Thereafter, the films were developed and fixed. The results pointed out that radioactivity rapidly disappeared from blood and was unevenly distributed in tissues. There was also to be a time-dependent shift in the distribution profile. At 2 min after iv [^{14}C]SPD, the highest labelling was observed in brain, fat, liver, kidneys, heart, blood, salivary glands and Harder's glands and moderate in lung, stomach and intestine (Fig 3A, plate 1). At 30 min after iv, the radioactivities of [^{14}C]SPD disappeared in tissues of brain, fat, vertebra, spleen and muscle, and reduced in the lung, salivary gland, heart blood, stomach and intestine. On the contrary, the more radioactivities in the kidneys and stomach mucosa were further observed (Fig 3B, plate 1).

At 4 and 8 h after iv the activities of [^{14}C]SPD in the liver, kidney and stomach mucosa could not be visualized, while some activities markedly revealed in the intestinal contents (Fig 3C, plate 1). At 24 h after iv the radioactivity could not be seen.

DISCUSSION

It was showed that the activity of [^3H]SPD in the blood decreased rapidly, and the disposition of *l*-SPD consisted of a fast distribution phase α and slow elimination phase β . It indicates that *l*-SPD disposition has a 2 compartment of pharmacokinetic open model, rapidly departing from central compartment to peripheral one. Furthermore, *l*-SPD was absorbed very well from digestive tract and easily penetrated into the brain tissue through the blood brain-barrier. Thus, *l*-

SPD exhibited the analgesic and sedative effects quite rapidly in animal experiment. The whole body autoradiography is a useful method to get a general idea of distribution and excretion of a compound or its metabolites in animal tissues. The selective accumulation of them in tissue or tissues may also give a clue for the most possible mechanism of the drug action. It has showed that [¹⁴C]SPD in all tissues would not last for a long time and the results of autoradiography indicate that the central pharmacological effects of *l*-SPD may be related to its well absorption and easy penetration into blood brain barrier.

REFERENCES

- 1 Jin GZ, Wang XL, Shi WX. Tetrahydroprotoberberine—A new chemical type of antagonist of dopamine receptors. *Sci Sin [B]* 1986; 29: 527
- 2 Shi WX, Chen Y, Jin GZ. Effect of *l*-stepholidine on rotational behavior in rats. *Acta Pharmacol Sin* 1984; 5 : 222
- 3 Xu J, Jin GZ, Yu LP, Liu XJ. Differentiation between catalepsies induced by *l*-tetrahydropalmatine and by haloperidol and morphine. *Ibid* 1981; 2 : 152
- 4 Zhang ZD, Jin GZ, Xu SX, Yu LP, Chen Y, Jiang FY, Zhang YR. Effects of *l*-stepholidine on central nervous and cardiovascular system. *Ibid* 1986; 7 : 522
- 5 Xiong ZL, Sun Z, Jin GZ, Chen Y. Influence of *l*-stepholidine on blood pressure and its relation to α -adrenoceptors. *Ibid* 1987; 8 : 497
- 6 Bian CF, Duan SM, Xing SH, Yu YM, Qin W. Interaction of analgesics and *l*-stepholidine. *Ibid* 1986; 7: 410
- 7 Yang L, Zhang X. Synthesis of [¹⁴C]labeled stepholidine. *J Label compounds Radiopharm* 1988; 25 : 569
- 8 Westlake WJ, Problems associated with analysis of

- pharmacokinetic models. *J Pharm Sci* 1971; 60 : 882
- 9 Jusko WJ, Gibaldi M. Effects of change in elimination on various parameters of the two-compartment open model. *Ibid* 1972; 61 : 1270
- 10 Yu YW, Sung CY. Studies on oral diuretics: 1. A comparative study on the physiological dispositions of chlorothiazide and hydrochlorothiazide in rats. *Acta Physiol Sin* 1962; 25 : 215
- 11 Slanina P, Ullberg S, Hammarström L. Distribution and placental transfer of [¹⁴C]thiourea and [¹⁴C]thiouracil in mice studied by whole-body autoradiography. *Acta Pharmacol Toxicol* 1973; 32 : 358
- 12 Cassano GB, Hansson E. Uptake of [¹⁴C]glutamine in the tissues of the mouse studied by whole-body autoradiography. *J Neurochem* 1965; 12 : 851

[³H]与[¹⁴C]千金藤立定的药物动力学和放射自显影

张振德、周臣民、金国章、章辛、杨柳
(中国科学院上海药物研究所, 上海 200031, 中国)

提要 大鼠 iv [³H] SPD 12 MBq / kg 后, 血药-时间曲线符合二室开放性模型, 分布相 $T_{1/2\alpha} = 3.6 \text{ min}$, 消除相 $T_{1/2\beta} = 168 \text{ min}$. ig[³H] SPD 后, 在 15-30 min 吸收为 80-87%, 与血浆、肝和肾蛋白结合分别为 37, 31 和 30%. [³H]SPD 50 MBq / kg ip 后, 3 d 内从尿中排出量为 56 %, 粪中 5 %, 排泄主要途径是尿液。

整体放射自显影中, 小鼠[¹⁴C] SPD 2 min 后, 在肾、肝、脑、唾液腺、副泪腺、心、肺和肌肉内有较强的放射性; 30 min 后, 在肾、胃和肠内溶物中仍较高; 4 和 8 h 后, 除肠的内溶物外, 其它组织中的放射性减低; 24 h 后趋向消失。

关键词 千金藤立定; 小檗因类; 药物动力学; 组织分布; 放射自显影

《中国药理学报》启事

本刊已采用计算机编辑排版. 为加快稿件处理, 本刊欢迎作者将稿件输入磁盘后, 用磁盘投稿. 磁盘规格为 13.3cm (5.25 inch), 360K 或 1.2M 容量都可. 同时, 必需附有磁盘内容的打印稿, 一式两份, 一并寄给编辑部, 以便送审.