13 Siess W. Molecular mechanisms of platelet activation. *Physiol Rev* 1989; 69: 58—178.
14 Kopec M, Latallo ZS. Fibrinogen and fibrin degradation products. In: Markwardt F, editor. *Fibrinolytics and antifibrinolytics*. NY: Springer-Verlag, 1978: 81—105.
15 Dikshit M, Srimal RC. Evidence for the presence of a new plasma factor which acts synergistically to ADP induced platelet aggregation. *Life Sci* 1990; 47: 117—26.

214-218

富含血小板的血浆凝块溶解液通过影响 Ca^{2+} 的自稳态介导的血小板高反应性

廖昌龙，邹其俊 （深圳市老年医学研究所血栓研究室，深圳 518001，中国）

摘要 大鼠富含血小板的血浆凝块溶解液增加血小板的聚集和丙二醛的形成，促进体内动脉血栓形成，但对凝血酶诱导的血小板钙内流及释放无影响. 然而，使诱导剂导致的血小板游离钙持续维持在高水平. 尿激酶或链激酶并不增加血小板的反应性. 因此，我们认为溶栓治疗后的血小板功能亢进是血凝块溶解产物介导的，而与溶栓药物无关.

关键词 尿激酶；链激酶；溶栓治疗；血小板聚集；钙

R965.2

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 May; **14** (3) : 218—221

Second peak of plasma diazepam concentration and enterogastric circulation

MA Yue—Ming, SUN Rui—Yuan
(*Department of Pharmacology, Wannan Medical College, Wuhu 241001, China*)

ABSTRACT Intragastric food administration caused a pronounced second peak of plasma diazepam concentration in rabbits after iv diazepam 5 mg · kg^{-1}. The second peak disappeared after gastrostomy and choledochostomy. A large amount of diazepam was found in the gastric juice while its content in bile remained at a much lower level during the whole experiment. These results suggested that diazepam may undergo an enterogastric circulation in addition to its enterobepatic circulation, with the former mainly contributing to the appearance of the second peak.

KEY WORDS diazepam; pharmacokinetics; gastric juice; bile; gastrostomy; choledochostomy

The plasma concentration-time curve of diazepam exhibits 2 distinct peaks after a single dose[1]. The second peak appeared postprandially and seemed not to have much to do with the enterohepatic circulation[2], nor with changes in its serum protein binding[3]. In a preliminary experiment, we found that a large amount of diazepam appeared in gastric juice after iv the drug in rabbits. The present study was designed to investigate the possibility that diazepam in gastric juice should be related to the rebound of its plasma levels in rabbits.

Received 1992-03-24 Accepted 1992-12-24

MATERIALS AND METHODS

Rabbits New Zealand rabbits of either sex, weighing 2.5 ± *s* 0.3 kg were used, and were fasted for 12 h before experiment.

Drugs Diazepam powder was purchased from Yiming Pharmaceutical Factory (Beijing), and diazepam injection from Shanghai № 13 Pharmaceutical Factory. Standard *N*-desmethyldiazepam was a product of Sigma Chemical Co, USA.

Drug analysis[4,5] Pipette 1 ml sample of

diazepam and 3 ml benzene into a 10 ml test tube. Vortex-mix each tube for 3 min. After centrifugation (500 × *g*) for 5 min, the organic phase was transferred to a tapered tube and evaporated to dryness on boiling water bath. The residue was redissolved in mobile phase (100 μl) and a portion (10—80 μl) was injected into the chromatograph. A Hitachi 635A HPLC system was equipped with a variable-wavelength uv detector and a C—E IB data processor. The column, 500 mm × 4 mm ID, was packed with Hitachi gel 3050 (10 μm). Chromatography was performed in a reverse-phase mode using a mobile phase of methanol 16 mmol · L^{-1} : water (65 : 35, vol : vol) at pH 7.0. The column effluent was monitored at 242 nm.

Pharmacokinetic study The rabbits were given a bolus injection of diazepam 5 mg · kg^{-1} into a marginal vein of the ear. Subsequent gastrogavage of food (5 g ordinary feed and 50 ml normal saline containing 5% glucose) was given after an interval varying from 0.25 h to 4.25 h. Blood samples (2.0 ml) were drawn from a femoral artery at 2, 1, 0.17 h before and 0.25, 0.5, 1, 2, 3, 4, 6 h after intragastric food administration. The rabbits were left undisturbed for 10 d. Then gastrostomy and choledochostomy were performed, and the time course of plasma diazepam concentration was measured again following the same procedure as described above, except that the food was given through an incision intubation into the deuodenum. The gastric juice and bile from separate drainage tubes were sampled regularly.

Paired comparisons of *t* test was used for the statistical analysis.

RESULTS

Diazepam concentration in plasma The intragastric food administration evoked a pronounced second peak of plasma diazepam concentration-time curve in normal rabbits (Fig 1). It lasted about 3 h before descending to the original level. There was a close correlation between the time of food administration and the starting time of the second peak (Fig 2) with an average delay of 0.25 h. In contrast, the intraduodenal food administration failed to produce the second peak in gastrostomized and choledochostomized rabbits. Moreover, the diazepam concentration in plasma also declined monotonically in normal rabbits when intragastric food administration was omitted.

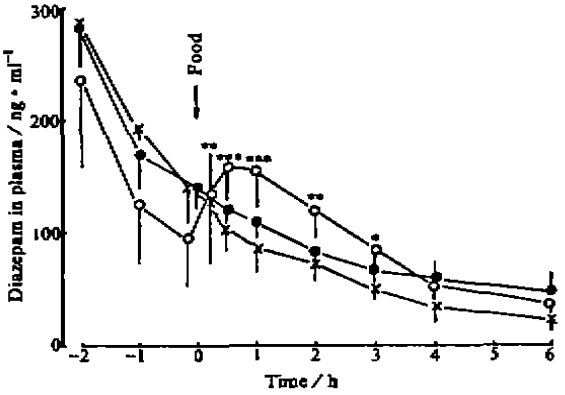

Fig 1. Diazepam concentration in plasma after iv 5 mg · kg^{-1} in 8 normal rabbits (○), 4 normal rabbits without food administration (●), and 6 gastrostomized and choledochostomized rabbits (×). $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* the last values before intragastric food administration.

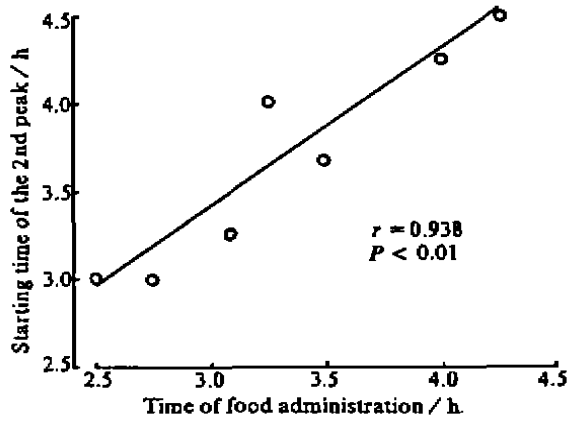

Fig 2. Relationship between starting time of the 2nd peak and time of intragastric food administration in rabbits after iv diazepam 5 mg·kg^{-1}.

Diazepam content in gastric juice and bile Much higher concentration of diazepam was found in gastric juice in comparison with that in bile and in plasma (both $P < 0.01$, Fig 3).

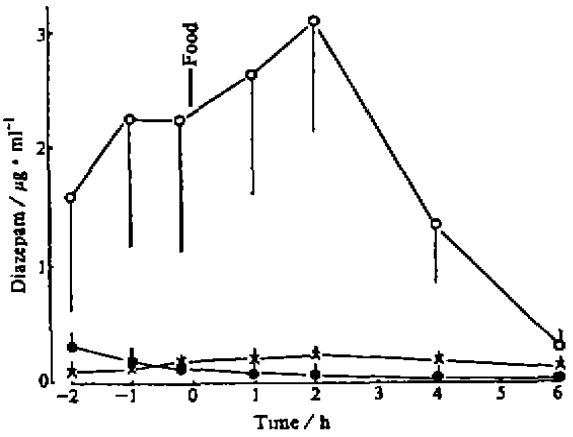

Fig 3. Diazepam concentrations in gastric juice (○), plasma (●), and bile (×) after iv 5 mg·kg^{-1} in 6 gastrostomized and choledochostomized rabbits. $\bar{x}\pm s$.

Intraduodenal food administration brought about significant increases in gastric secretion and in its diazepam concentration, whereas the bile flow and its diazepam concentration remained at low levels and varied less during 8 h of observation in gastrostomized and choledochostomized rabbits (Tab 1). When plasma diazepam concentration attained the second peak, the drug content in gastric juice accumulated to a level of 125±83 μg, while in bile the value was only 0.5±0.6 μg ($n=6$, $P<0.01$).

Tab 1. Secretions of gastric juice and bile in 6 gastrostomized and choledochostomized rabbits before and after intraduodenal food administration (at 0 h) with iv (3 h previously) diazepam 5 mg·kg^{-1}. $\bar{x}\pm s$, $^{*}P>0.05$, $^{**}P<0.05$ *vs* before food administration. $^{++}P<0.05$, $^{+++}P<0.01$ *vs* bile.

Time/h	Gastric juice/ml	Bile/ml
−1～0	$9\pm4^{+++}$	1.3±0.7
0～1	$16\pm4^{**+++}$	1.2±0.7
1～2	$9\pm3^{*+++}$	1.0±0.5
2～4	$7\pm3^{+++}$	1.0±0.5
4～6	$4\pm2^{++}$	0.9±0.2

DISCUSSION

The present study revealed that intragastric food administration induced a pronounced second peak of the plasma diazepam concentration in rabbits, and the second peak did not occur when the food administration was omitted. These results were quite in agreement with the observation in human experiments[1].

The enterohepatic circulation was believed responsible for the formation of the second peak of some drugs, such as ranitidine[6] and piroxicam[7]. But it was not the case for diazepam[2]. Our results provided further evidence for this conclusion in that only a very small amount of diazepam could be detected from bile and it showed no variation in response to the intraduodenal food administration.

On the contrary, the intraduodenal food administration elevated the diazepam content in gastric juice so much as to approach a level capable of increasing the plasma concentration to produce a second peak. Because the gastric secretion of intestinal phase (induced by intraduodenal food infusion) accounts for only a small portion of the total gastric secretion in response to a meal, it is rational to expect a higher diazepam content in the gastric juice of intact rabbits that received intragastric food administration. These findings favored an idea that diazepam, as a weak base, may diffuse easily from blood into gastric juice and accumulated there to a great extent before it became available for reabsorption from the intestine, i.e. the drug may undergo an enterogastric circulation. Therefore, interrupting the circulation, the gastrostomy eliminated the occurrence of the second peak. Food administration, by stimulating the secretion of gastric juice and increasing the blood flow in the digestive tract[8], may accelerate the accumulation of diazepam in stomach and then its transport into blood, thus acting as a trigger of the appearance of the second peak. In the case without food administration, the enterogastric circulation operated at a slow rate, the reab-

sorbed amount of diazepam was not sufficient to counteract the elimination of the drug from blood, thus the plasma concentration declined steadily without the second peak.

The results of this study suggested a general implication for the drugs with biochemical characteristics similar to those of diazepam, such as temazepam and veralipride, which also exhibited double peaks of their blood concentration-time curves following a single dose[9,10].

Enterogastric circulation, like enterohepatic circulation, may exert some influence on the pharmacokinetic behavior of certain drugs. Its theoretical and practical significance calls for further investigation.

REFERENCES

1 Linnoila M, Korttila K, Mattila MJ. Effect of food and repeated injection on serum diazepam levels. *Acta Pharmcol Toxicol* 1975; **36**: 181—6.

2 Eustace PW, Hailey DW, Cox AG, Baird ES. Biliary excretion of diazepam in man. *Br J Anaesth* 1975; **47**: 983—5.

3 Korttila K, Kangas L. Unchanged protein binding and the increase of serum diazepam levels after food intake. *Acta Pharmacol Toxicol* 1977; **40**: 241—6.

4 Arnold E. A simple method for determining diazepam and its major metabolites in biological fluids; application in bioavailability studies. *Acta Pharmacol Toxicol* 1975; **36**: 335—52.

5 Brodie RR, Chasseaud LF, Taylor T. High-performance liquid chromatographic determination of benzodiazepines in human plasma. *J Chromatogr* 1978; **150**: 361—6.

6 Miller R. Pharmacokinetics and bioavailability of ranitidine in humans. *J Pharm Sci* 1984; **73**: 1376—9.

7 Zhou HW, Shen JQ, Lu M, Liang WQ, Lin W, Zhao WH. Pharmacokinetic analysis of enterohepatic circulation of piroxicam in rabbits. *Acta Pharmacol Sin* 1992; **13**: 180—2.

8 Reitberg DP, Love SJ, Quercia GT, Zinny MA. Effect of food on nifedipine pharmacokinetics. *Clin Pharmacol Ther* 1987; **42**: 72—5.

9 Kroboth PD, Smith RB, Rault R, Silver MR, Sorkin MI, Pushchett JB, *et al*. Effects of end-stage renal disease and aluminum hydroxide on temazepam kinetics. *Clin Pharmacol Ther* 1985; **37**: 453—9.

10 Staveris S, Houin G, Tillement JP, Jamet G, Schneider M, Jung L, *et al*. Primary dose-dependent pharmacokinetic study of veralipride. *J Pharm Sci* 1985; **74**: 94—6.

(8)

218-221

地西泮血浆浓度的第二峰与肠胃循环

马越鸣，孙瑞元

R 965.2

（皖南医学院药理教研室，芜湖 241001，中国）

摘要 兔 iv 地西泮 5 mg·kg^{-1}，随即经胃管灌食物可使血药浓度出现明显的第二峰。对兔行胃造瘘术和胆总管造瘘术后，第二峰消失，从胃液中检测到大量地西泮，同期只有很少量的药物出现在胆汁中。结果提示，地西泮在体内除肠肝循环外，还可进行肠胃循环，而第二峰的出现主要与后者有关。

关键词 地西泮；药物动力学；胃液；胆汁；胃造瘘术；胆总管造瘘术

药代动力学

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 May; **14** (3) : 221—224

Effects of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin on immune function in mice

JIA Zheng—Ping, XIE Jing—Wen (*Department of Pharmacy, General Hospital of Lanzhou Command of PLA, Lanzhou 730050, China*)

FENG Pu, NIU Ji—Guo

(*Department of Immunology, Gansu Institute of New Medicine, Lanzhou 730050, China*)

ABSTRACT 4-[4″-(2″,2″,6″,6″-Tetramethyl-1″-piperidinyloxy)amino]-4′- demethylepipodophyllotoxin (GP-7) 10—40 mg·kg^{-1} ip daily for 7 d reduced the specific antibody formation of splenocytes, serum

Received 1992-06-03 Accepted 1992-12-23