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Antagonism of *l*-stepholidine on D_2 receptor-mediated inhibition of synaptosomal adenylate cyclase in rat corpus striatum[1]

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ABSTRACT In the presence of Sch 23390 0.1 $\mu mol \cdot L^{-1}$, dopamine (DA) inhibited the activity of synaptosomal adenylate cyclase (AC) isolated from rat striatum in a concentration-dependent manner with IC_{50} value of 2.2 $\mu mol \cdot L^{-1}$. The maximal inhibition corresponded to a 51% decrease of basal enzyme activity and was obtained at DA 100 $\mu mol \cdot L^{-1}$. The inhibitory effect of DA was reversed by selective D_2 receptor antagonist spiperone 10 $\mu mol \cdot L^{-1}$. N-0437, a selective D_2 DA receptor agonist also inhibited the activity of AC in the manner similar to that of DA. Both the inhibitions induced by DA and N-0437 were antagonized by *l*-stepholidine (*l*-SPD). However, in the presence of Sch 23390, *l*-SPD alone also inhibited the activity of AC by 29% and 33% at the concentrations of 10 and 100 $\mu mol \cdot L^{-1}$, respectively. The inhibition of *l*-SPD on AC activity was significantly antagonized by spiperone. In the presence of Sch 23390, both DA and N-0437 increased the activity of high affinity GTP phosphohydrolase in striatal synaptosomes. The increases of GTP phosphohydrolase activity stimulated by DA and N-0437 were completely reversed by *l*-SPD 100 $\mu mol \cdot L^{-1}$. These results suggest that *l*-SPD antagonizes or reverses the D_2 receptor-mediated inhibition of AC activity through affecting the regulation of G_i (inhibitory guanine nucleotide-dependent protein) on D_2 receptor-coupled AC and therefore affects the negative feedback of presynaptic DA receptors. Moreover, the inhibition of AC activity induced by *l*-SPD alone may provide a useful biochemical index for dual action of *l*-SPD.

KEY WORDS berbines; dopamine receptors; adenyl cyclase; GTP phosphohydrolase; corpus striatum; synaptosomes

l-Stepholidine (*l*-SPD), an alkaloid isolated from *Stephania intermedia* Lo, has been demonstrated to be a novel DA receptor antagonist by substantial evidence from biochemical, electrophysiological and pharmacological studies[1,2]. Previous results also showed that *l*-SPD augmented the activity of tyrosine hydroxylase (TH) and therefore increased the biosynthesis of DA through inhibiting the negative feedback regulation of presynaptic DA receptors[2]. TH is the rate-limiting enzyme in the biosynthesis of catecholamines. Abundant evidence has indicated that presynaptic DA receptors (autoreceptors) control the synthesis of DA in striatum and are involved in the feedback regulation of TH[3]. Pharmacological studies have shown that presynaptic DA receptors controlling DA synthesis belong to D_2 receptors[4] and that D_2 receptors are coupled with the inhibition of AC by the mediation of G_i, an inhibitory guanine nucleotide-dependent regulatory protein[5]. In the present study, we further investigated the effect of *l*-SPD on D_2 receptor-mediated inhibition of synaptosomal AC isolated from rat striatum in order to elucidate the mechanism by which *l*-SPD affects the feedback regulation of presynaptic DA receptors. The effect of *l*-SPD on D_2 receptors mediating stimulation of GTP phosphohydrolase activity was also investigated so as to search into the possible relationship between the effect of *l*-SPD on D_2 receptor-mediated inhibition of AC activity and the regulatory function of G_i.

MATERIALS AND METHODS

Chemicals and reagents *l*-SPD ($[\alpha]_D$ $-440°$ in pyridine): DA hydrochloride (Fluka Chemie AG, Switzerland): 2-(*N*-propyl-*N*-

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2-thienythylamino)-5-hydroxytetralin (N-0437), (+)-*R*-8-chloro-2,3,4,5,- tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-*ol* (Sch 23390) and spiperone (Research Biochemicals Inc, USA); cAMP, Na_2ATP and Na_2GTP (Sigma Chemical Co, USA); theophylline (Shanghai № 2 Reagent Factory, China); dithiothreitol (DTT, Serva Feinbiochemical, Heidelberg); cAMP assay kits were purchased from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. All other chemicals used in the present study were AR.

Rats Sprague-Dawley ♀ rats ($223 \pm s$ 27 g, Shanghai Laboratorial Animal Center, China) were used in the present experiments, because ♀ rats have been reported to show a more consistent and robust sensitization than ♂ rats[6].

Preparation of synaptosomes The synaptosomes were prepared essentially according to Gray and Whittaker[7]. Rats were decapitated and the striatum were dissected out and placed on ice. The pooled tissues were homogenized in 10 vol of sucrose 0.32 $mol \cdot L^{-1}$ containing Tris-HCl 50 $mmol \cdot L^{-1}$ (pH 7.45), EGTA 1 $mmol \cdot L^{-1}$, DTT 1 $mmol \cdot L^{-1}$, $MgCl_2$ 2.5 $mmol \cdot L^{-1}$, and theophylline 10 $mmol \cdot L^{-1}$. After removal of nuclei and cell debris (P_1: $1000 \times g$ for 15 min at 4℃) the crude synaptosomal fractions were separated (P_2: $20\ 000 \times g$ for 20 min at 4℃) and the P_2 pellets were washed twice with sucrose 0.32 $mol \cdot L^{-1}$. After rehomogenization of the pellet, aliquots were distributed and stored at -25℃ for estimating the activity of AC and GTP phosphohydrolase as well as the protein content.

Adenylate cyclase assay AC activity was routinely assayed in a 150 μl reaction mixture containing Tris-HCl 50 $mmol \cdot L^{-1}$ (pH 7.45), Na_2ATP 1 $mmol \cdot L^{-1}$, $MgCl_2$ 2.5 $mmol \cdot L^{-1}$, theophylline 10 $mmol \cdot L^{-1}$, DTT 1 $mmol \cdot L^{-1}$, Na_2GTP 50 $\mu mol \cdot L^{-1}$, and NaCl 100 $mmol \cdot L^{-1}$. The reaction was initiated by adding the synaptosomal suspension (10-15 μg of protein) and was carried out at 25℃ for 20 min. The reaction was interrupted by boiling for 3 min and the reaction mixture was centrifuged at $800 \times g$ for 10 min. The resulting supernatants were used to determine the content of cAMP according to the method of competitive protein binding[8]. The unit of enzyme activity was expressed as nmol $cAMP \cdot mg^{-1} \cdot min^{-1}$.

Sch 23390, a selective D_1 receptor antagonist, was used at a concentration of 0.1 $\mu mol \cdot L^{-1}$. This concentration completely blocked the activation of AC by DA 100 $\mu mol \cdot L^{-1}$ and was 0.2% of the EC_{50} value of Sch 23390 in antagonizing the inhibition of AC by quinpirole hydrochloride 100 $\mu mol \cdot L^{-1}$, a selective D_2 receptor agonist[9]. Moreover, at this concentration, Sch 23390 did not affect basal AC activity[9].

GTP phosphohydrolase assay High affinity GTP phosphohydrolase activity was assayed in isolated synaptosomes according to the modification of the method of Cassel and Selinger[10]. The reaction mixture contained Tris-HCl 50 $mmol \cdot L^{-1}$ (pH 7.45), Na_2GTP 1 $mmol \cdot L^{-1}$, Na_2ATP 0.5 $mmol \cdot L^{-1}$, Na_2Cl 100 $mmol \cdot L^{-1}$, EDTA 0.1 $mmol \cdot L^{-1}$, EGTA 0.2 $mmol \cdot L^{-1}$, DTT 0.2 $mmol \cdot L^{-1}$, and Sch 23390 0.1 $\mu mol \cdot L^{-1}$. Theophylline 10 $mmol \cdot L^{-1}$ was also included to maintain the composition of the reaction mixture similar to that used for AC assay. The reaction was started by adding the synaptosomal suspension (5-10 μg of protein) and carried out at 30℃ for 5 min. The reaction was stopped by the addition of 0.1 ml of 50% (wt / vol) ice-cold trichloroacetic acid. The inorganic phosphorus (P_i) liberated was estimated in the supernatants resulted from the centrifugation at $800 \times g$ for 5 min. The unit of enzyme activity was expressed as pmol $P_i \cdot mg^{-1} \cdot min^{-1}$.

Protein assay Protein of synaptosomes

was determined by the method of Lowry, *et al.* Bovine serum albumin used as standard.

Statistical analysis of data The means $(\bar{x}) \pm s$ of the determinations (n) were analyzed with t test.

RESULTS

Effect of DA on D_1 receptor–stimulated activity of synaptosomal AC from rat striatum In the absence of Sch 23390, DA increased the activity of synaptosomal AC in a concentration–dependent manner (Fig 1A). The activity of AC was increased by 15% by DA at the concentration of 1.0 μmol · L^{-1} ($P<0.05$). However, the maximal activation elicited by DA 100 μmol · L^{-1} corresponded to a 54% increase of basal enzyme activity.

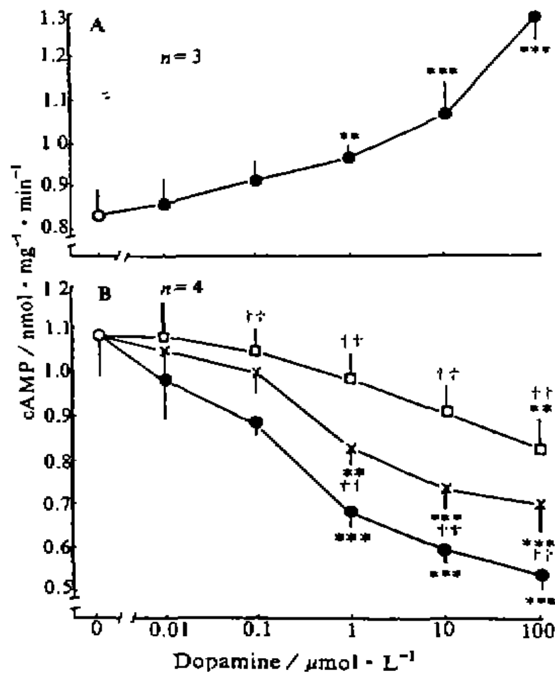

Fig 1. (A) Activation of DA on activity of synaptosomal adenylate cyclase isolated from rat striatum. (B) Antagonism of *l*–SPD and spiperone on DA–induced inhibition of the activity of synaptosomal adenylate cyclase from rat striatum. (○) control activity; (●) DA; (×) DA + *l*–SPD 100 μmol · L^{-1}; (□) DA + spiperone 100 μmol · L^{-1}. $\bar{x} \pm s$. **$P<0.05$, ***$P<0.01$ *vs* control. ††$P<0.05$ *vs* corresponding DA.

Antagonism of *l*–SPD on D_2 receptor–mediated inhibition of synaptosomal AC from rat striatum When synaptosomal AC activity was assayed in the presence of Sch 23390 0.1 μmol · L^{-1} to block the D_1 receptor activation, DA inhibited the activity of synaptosomal AC in a concentration–dependent manner (Fig 1B). DA 1 μmol · L^{-1} significantly decreased the basal enzyme activity by 24% ($P<0.05$). The maximal inhibition elicited by DA corresponded to a 51% decrease of basal enzyme activity. The apparent IC_{50} of DA is 2.2 μmol · L^{-1}. The inhibitory effect of DA on AC activity was reversed by selective D_2 receptor antagonist spiperone 10 μmol · L^{-1} (Fig 1B). Under the same experimental conditions, *l*–SPD 100 μmol · L^{-1} exhibited an antagonism on the inhibition of DA on AC activity, enabling the concentration–response curve to uplift. The inhibitions of the activity of synaptosomal AC elicited by DA 1, 10, and 100 μmol · L^{-1} were decreased by 36, 29, and 26% by *l*–SPD 100 μmol · L^{-1}, respectively.

Antagonism of *l*–SPD on N–0437–induced inhibition of synaptosomal AC from rat striatum N–0437, a potent selective D_2 receptor agonist, also inhibited the activity of synaptosomal AC in the manner similar to that of DA (Fig 2). The inhibitory effect of N–0437 was also antagonized by *l*–SPD (Fig 2). *l*–SPD 100 μmol · L^{-1} decreased the inhibition of N–0437 0.1, 1, and 10 μmol · L^{-1} on AC activity by 48, 34, and 20%, respectively.

Inhibition of *l*–SPD on activity of synaptosomal AC from rat striatum In the presence of Sch 23390, *l*–SPD inhibited sinificantly the activity of synaptosomal AC at higher concentrations of 10 and 100 μmol · L^{-1} ($P<0.05$, Fig 4). The basal enzyme

activity was decreased by 29 and 33% by *l*–SPD 10 and 100 μmol · L^{-1}, repectively. The inhibition of *l*–SPD on AC activity was reversed by spiperone 10 μmol · L^{-1} (Fig 3).

Effect of *l*–SPD on D_2 receptor–mediated stimulation of high affinity GTP phosphohydrolase activity In the presence of Sch 23390, DA 10 and 100 μmol · L^{-1} significantly stimulated the GTP phosphohydrolase activity by the activation of D_2 receptors and increased the GTPase activity by 24 and 34%, respectively (Tab 1). Similarly, GTPase activity was increased by 14 and 25% by N–0437 at the concentrations of 10 and 100 μmol · L^{-1}, respectively. *l*–SPD did not affect the enzyme activity at the concentrations of 10 and 100 μmol · L^{-1}, while *l*–SPD 100 μmol · L^{-1} completely reversed the stimulations of GTP phosphohydrolase activity by both DA and N–0437 (Tab 1).

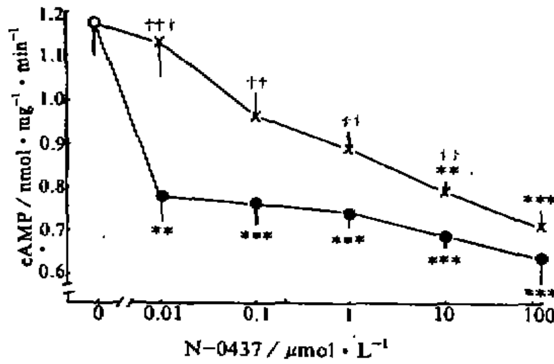

Fig 2. Antagonism of *l*–SPD on N–0437–induced inhibition of activity of synaptosomal adenylate cyclase from rat striatum. (○) control activity. The enzyme activity was assayed in the absence (●) and presence (×) of *l*–SPD 100 μmol · L^{-1}. $n=3$, $\bar{x} \pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. $^{+}P<0.05$, $^{++}P<0.01$ *vs* corresponding N–0437.

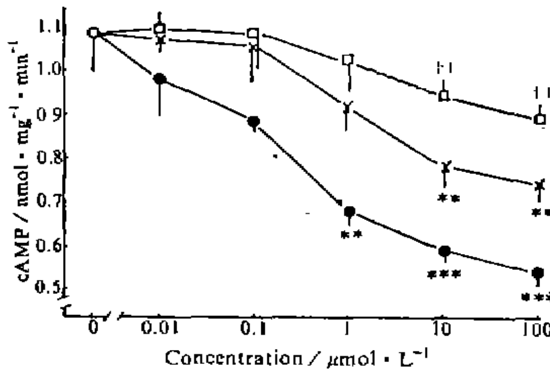

Fig 3. Inhibition of *l*–SPD and DA on activity of synaptosomal adenylate cyclase from rat striatum. (○) control activity; (●) DA; (×) *l*–SPD; (□) *l*–SPD + spiperone 10 μmol · L^{-1}. $n=4$, $\bar{x} \pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. $^{++}P<0.05$ *vs* corresponding *l*–SPD.

Tab 1. Effects of DA, N–0437 and *l*–SPD on activity of synaptosomal GTP phosphohydrolase from rat striatum. The enzyme activity was assayed in the presence of Sch 23390 0.1 μmol · L^{-1}. $n=4$, $\bar{x} \pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug / μmol · L^{-1}	Activity / pmol P_i · mg^{-1} · min^{-1}
Control	465 ± 8
DA 10	576 ± 13***
DA 100	625 ± 24***
N–0437 10	528 ± 20**
N–0437 100	579 ± 27***
l–SPD 10	476 ± 6
l–SPD 100	468 ± 20
DA 10 + *l*–SPD 100	474 ± 16
DA 100 + *l*–SPD 100	482 ± 22
N–0437 10 + *l*–SPD 100	463 ± 23
N–0437 100 + *l*–SPD 100	471 ± 18

DISCUSSION

DA receptors have been classified into two subtypes, based on functional and pharmacological profiles[11]. D_2 receptor is characterized by its ability to inhibit AC activity whereas D_1 receptor stimulates it[5,11]. In the present study, we investigated the stimulatory effect of DA on AC activity in the absence of Sch 23390 so as to assess our experimental conditions. The results indicate that

DA activates D_1 receptors and therefore stimulates the activity of synaptosomal AC isolated from rat striatum in a concentration-dependent manner.

More recent evidence shows that the negative coupling of D_2 receptors with AC activity is the most possible mechanism by which D_2 receptors regulate TH activity by preventing or reversing cAMP-dependent activation and phosphorylation of the enzyme[5,12]. The synaptosome isolated from striatum has been shown to be an effective model system for study of presynaptic DA receptor feedback[13]. In the present study using this model system we found that in the presence of Sch 23390 to block D_1 receptor activation, both DA and selective D_2 receptor agonist N-0437 inhibited the activity of synaptosomal AC in a concentration-dependent manner and the inhibitory effect of DA on AC activity was significantly antagonized by selective D_2 receptor antagonist spiperone. The results indicate that the inhibition of DA on synaptosomal AC activity from rat striatum results from the activation of D_2 receptors.

We also found that *l*-SPD antagonized the D_2 receptor-mediated inhibition of synaptosomal AC activity resulting from the activation of D_2 receptor by both DA and N-0437. The results are consistent with the characteristics of *l*-SPD blocking DA receptors[1]. According to these results, we suppose that the effect of *l*-SPD on presynaptic negative feedback regulation observed in previous study may be related to its antagonistic effect on D_2 receptor-mediated inhibition of synaptosomal AC activity. In fact, this supposal has been confirmed by our recent experimental results that *l*-SPD reverses the inhibition of N-0437 on synaptosomal TH activity from rat striatum (data to be published). It is thus possible that *l*-SPD prevents the inhibition of AC activity and the decrease of cAMP formation produced by the activation of D_2 receptor and therefore augments the phosphorylation and activation of TH so that the formation of *l*-dopa is increased. Interestingly, in the presence of Sch 23390, *l*-SPD alone also exhibited the inhibitory effect on the activity of synaptosomal AC at higher concentrations (10 and 100 $\mu mol \cdot L^{-1}$). And this inhibitory effect was effectively antagonized by selective D_2 receptor antagonist spiperone. It seems to support the idea that *l*-SPD possesses dual actions on DA receptors[1,2]. However, the inhibitory mechanism of *l*-SPD on AC remains to be further studied.

Abundant evidence indicates that GTP phosphohydrolase activity is activated via stimulation of D_2 receptor[14,15] and that the activation of D_2 receptor on GTP phosphohydrolase constitutes a useful index of the efficiency of the transduction mechanism that couples the D_2 receptor to AC[14,15]. The results presented in this study indicate that in the presence of Sch 23390, both DA and N-0437 increased the activity of high affinity GTP phosphohydrolase significantly. Although *l*-SPD did not affect GTP phosphohydrolase activity directly, it could completely reverse the increases of GTP phosphohydrolase activity elicited by both DA and N-0437. These results reveal that *l*-SPD antagonizes the inhibition of D_2 receptor-mediated synaptosomal AC activity and reverses the negative feedback regulation of presynaptic DA receptor on TH activity by decreasing the G_i activity. A schematic model for action of *l*-SPD on modulation of DA synthesis by presynaptic DA receptors (i.e., D_2 receptors or autoreceptors) is shown in Fig 4.

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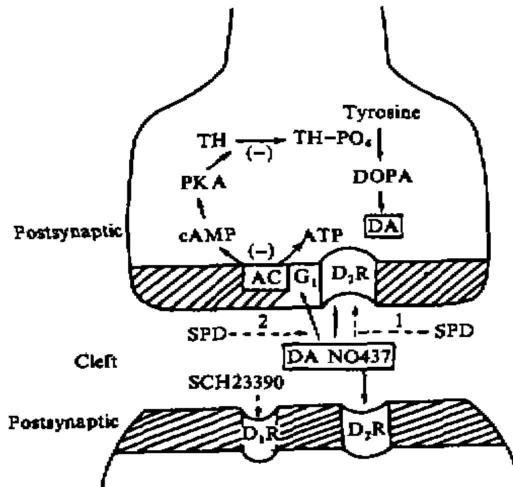

Fig 4. Schematic model for action of *l*–SPD on modulation of striatal DA synthesis by presynaptic DA receptors. PKA, cAMP–dependent protein kinase; D_1R, D_1 DA receptor; D_2R, D_2 DA receptor; TH–PO_4, phosphorylated TH. DA and N–0437 activate D_2 receptors and augment G_i activity by which cAMP formation is reduced resulting from the inhibition of AC. Phosphorylation and activation of TH are attenuated and synthesis of DA is inhibited. *l*–SPD reverses the inhibition of DA and N–0437 on synthesis of DA through 1) blocking D_2 receptors and 2) reversing the augmentation of G_i activity.

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104–110

左旋千金藤立定对 D_2 多巴胺受体介导的大鼠纹状体突触体腺苷酸环化酶活性抑制的拮抗

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提要 在 Sch 23390 存在时，DA 和 N–0437 以浓度依赖的方式抑制大鼠纹状体突触体 AC 的活性，D_2 受体拮抗剂 spiperone 和 *l*–SPD 拮抗 DA 和 N–0437 的抑制. 在相同条件下，DA 和 N–0437 也显著增加突触体高亲和力 GTP 酶的活性，*l*–SPD 能完全逆转二者的激活效应. 这些结果表明，*l*–SPD 通过逆转 D_2 受体对 GTP 酶的兴奋和 G_i 对 AC 的抑制性调控，从而影响突触前 DA 受体的负反馈调节.

关键词 小檗因类；多巴胺受体；腺苷酸环化酶；鸟苷三磷酸水解酶；纹状体；突触体

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Pharmacokinetics and relative bioavailability of ofloxacin tablets in 12 healthy volunteers

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ABSTRACT Single oral dose of tablet A (Daiichi Pharmaceutical Co Ltd, Japan) and B (Jining Pharmaceutical Factory, Shandong, China) of 300 mg ofloxacin (Ofl) were given to 12 Chinese healthy male volunteers in an open, randomized crossover study. Drug concentrations in serum and urine were assayed by HPLC and partial least squares spectrophotometric method, respectively. The serum concentration–time course after medication conformed to a 2–compartment open model with a first order absorption. Pharmacokinetic parameters after tablet B did not differ significantly from the corresponding values after tablet A. The bioavailability of tablet B was comparable to that of tablet A.

KEY WORDS ofloxacin; tablets; pharmacokinetics; biological availability

Ofloxacin (Ofl), a fluorinated quinolone, is a new broad–spectrum antibiotic for oral use[1]. Its primary effect is the inhibition of bacterial DNA–gyrase (topoisomerase II). The spectrum of Ofl includes Gram–positive bacteria, highly potent against *Staphylococcus aureus*, as well as Gram–negative bacilli with an efficacy comparable to those of modern parenteral antibiotics. After oral administration, the drug is rapidly absorbed and widely distributed to the body tissues and fluids[2]. Over 90% of Ofl is excreted in the urine unchanged. These properties make Ofl a potential remedy in many types of infections.

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The aim of this study was to determine the pharmacokinetics of Ofl in 12 Chinese volunteers upon oral administration, and to investigate the relative bioavailability of tablet B as compared to tablet A.

MATERIALS AND METHODS

Drugs and instrument Ofl tablet A (lot No AN 549, Daiichi Pharmaceutical Co Ltd, Japan) and B (lot 900315, Jining Pharmaceutical Factory, Jining 272131, China) were compared. Both forms of tablet contained 100 mg Ofl each. The HPLC instrument consisted of Waters 510 HPLC system equipped with a 490 E wavelength adjustable uv detector and a Baseline 810 data processor. The UV–visible recording spectrophotometer was UV–240.

Subjects Twelve healthy male Chinese volunteers aged $24 \pm s$ 4 a and weighing $64 \pm s$ 3 kg entered the study. All volunteers gave their written consents and underwent thorough physical examination. There were no