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Effects of Lithium on the Pharmacokinetics of Valproate in Rats

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Abstract

Combined treatment with lithium and valproate has been used for bipolar disorder. However, the studied interaction between these two drugs has not been fully investigated. We therefore examined the effects of lithium on the pharmacokinetics (plasma disappearance, metabolism and urinary excretion) of valproate in rats.

Lithium ($2\,mEq\,kg^{-1}$) was administered intraperitoneally twice a day for ten days. Plasma disappearance curves of valproate ($50\,mg\,kg^{-1}$, i.v.), valproate-metabolizing activities of UDP-glucuronosyltransferase (UGT) and cytochrome P450 (CYP) in liver microsomes and urinary excretion of free valproate and valproate-glucuronide were examined. The metabolizing activity of UGT and CYP were determined by enzyme assays and a fluorescence polarization immunoassay system. Urinary valproate-glucuronide was obtained using this system by subtracting the free level from total level, which was determined after deconjugating the sample with heat and NaOH.

The half-life of plasma disappearance of valproate was 25% reduced by lithium pretreatment ($0{\cdot}428 \pm 0{\cdot}031\,h$ with repeated lithium pretreatment vs $0{\cdot}578 \pm 0{\cdot}062\,h$ for controls). The valproate-metabolizing activity of UGT and CYP were not altered by lithium although lithium increased the urinary excretion of valproate-glucuronide.

In conclusion, lithium pretreatment causes a decrease in plasma valproate levels and an increase in urinary excretion of valproate-glucuronide in rats.

Lithium is used in the treatment of manic-depressive psychosis (Baldesarini 1996), and valproate in the treatment of both epilepsy and manic-depressive psychosis, especially acute mania (Brown 1989; McNamara 1996). It has been reported that combined treatment with lithium and valproate is effective in bipolar disorder (Emerich et al 1985; Post 1989). Accordingly, this combined therapy has been generally used for bipolar disorder (Solomon et al 1998).

Some investigators have reported that the combination of lithium and valproate induces a decrease in the plasma valproate level in rats (Vargas et al 1996), that lithium increases activities of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) of liver microsomes in rats (Feuerstein et al 1979), and that valproate is metabolized mainly by UGT and partially by CYP in rats (Granneman et al 1984). From these reports, we assumed that the decrease in the plasma valproate level by lithium is caused by lithium-induced activation of UGT or CYP (or both) in the liver microsomes, or possibly by increase in urinary excretion of valproate. However, such overall pharmacokinetic drug interactions between valproate and lithium have scarcely been investigated.

In this study we examined the effects of lithium, administered with repeated or single intraperitoneal injection, on plasma valproate levels in rats. Secondly, we measured the valproate-metabolizing activities of UGT and CYP in liver microsomes of the rats dosed repeatedly with lithium. We also studied the urinary excretion of valproate (free and glucuronate-conjugate (glucuronide)) in the rats.

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Materials and Methods

Reagents

Valproate (sodium salt) and lithium (chloride) for the use of administration to animals were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Other chemicals were of the highest purity available.

Animals

Male Wistar albino rats (200–250 g body weight), purchased from Japan SLC Inc. (Shizuoka, Japan), were housed in metal cages on wood-shaving bedding in a temperature- and humidity-controlled room, and were given free access to standard diet and drinking water. All experiments were conducted under the guidance of the Animal Use Committee in the University of Tokushima. The rats were divided at random into four groups: single lithium, single control, repeated-lithium and repeated-control group (four rats to a group).

Plasma disappearance curves of valproate

In the single-lithium and the control group (four rats to a group), valproate ($50\,mg\,kg^{-1}$) was administered through a femoral vein catheter one minute after intraperitoneal injection of lithium ($2\,mEq\,kg^{-1}$) or saline (control) under ether anaesthesia. In the repeated-lithium and the repeated-control group (four rats to a group), the same administration was performed 15 h after the end of the pretreatment: intraperitoneal injection of lithium ($2\,mEq\,kg^{-1}$) or saline (control) twice a day for ten days. Then, 0·5 mL of blood samples were collected in heparinized tubes through a femoral artery catheter at 1, 3, 5, 7, 10, 15, 20 and 30 min after the administration of valproate. Plasma samples were obtained by centrifuging the blood samples at $3000\,rev\,min^{-1}$ ($1500\,g$) for 15 min.

Plasma samples (0·2 mL) were applied to the TDX analyzer system (Abbott Laboratories, USA) using a fluorescence polarization immunoassay, detecting only free valproate, and the plasma valproate levels were measured. Distribution volume (Vd), half-life ($t_{\frac{1}{2}}$) and total clearance (CL) were calculated with a one-compartment model program, using the obtained plasma valproate levels.

Valproate-metabolizing activities of UGT and CYP

In the repeated-lithium group and its control (four rats to a group), enzyme assays were performed to measure valproate-metabolizing activities of UGT and CYP.

Rats were killed by cervical cutting, the liver was removed and the microsomes were prepared as previously reported (Kamataki & Kitagawa 1974). The amount of CYP was determined spectrophotometrically as the carbon monoxide complex (Omura & Sato 1964).

The valproate-metabolizing activity of UGT in microsomes was measured by modifying the method reported previously (Yu & Shen 1996). The obtained microsomes (0·1 mL; with 2·5 mg (mg protein)$^{-1}$ Brij 35, a surfactant) were mixed with 0·2 mL of 0·25 M Tris–HCl (pH 7·4), 0·02 mL of 125 mM $MgCl_2$ and 0·1 mL of 15 mM UDP-glucuronic acid (or, as a reference, 0·1 mL of distilled water). The mixture was pre-incubated at 37°C for 3 min, then 0·1 mL of valproate (0·05, 0·1, 0·2, 0·4 or $0{\cdot}8\,mg\,mL^{-1}$) was added and the mixture was incubated at 37°C for 30 min. The reaction was stopped with 0·5 mL of 0·4 M trichloroacetic acid (TCA) plus 0·6 M glycine. The mixture was iced and centrifuged at $3000\,rev\,min^{-1}$ ($1500\,g$) for 20 min. The supernatants were applied to the TDX analyzer, and the reaction rates were calculated from the free valproate levels in the supernatants. The values of Km and V_{max} were obtained with the Lineweaver–Burk plot.

The valproate-metabolizing activity of CYP in the microsomes was measured by modifying the method reported previously (Fabre et al 1992). The mixture (1·0 mL), which consisted of 0·1 mL of the microsomes, 0·5 mL of valproate ($1\,mg\,mL^{-1}$), 0·3 mL of 0·167 nM EDTA/0·33 M Na-K phosphate buffer (pH 7·4) and 0·1 mL of 10 mM NADPH (or, as a reference, 0·1 mL of distilled water), was incubated at 37°C for 15 min. The reaction was stopped with 1·0 mL of 10% TCA and the mixture was centrifuged at $3000\,rev\,min^{-1}$ ($1500\,g$) for 10 min. The supernatants were applied to the TDX analyzer, and the reaction rates were calculated from the free valproate levels in the supernatants.

Urinary excretion of valproate

In the repeated-lithium and the repeated-control group (four rats to a group), the urinary excretion of valproate (free valproate and its glucuronide) was examined. Urine was collected every 6 h for 24 h after the administration of valproate. Each sample was divided into two: one half for the measurement of the free valproate and the other for total valproate (free and glucuronide). Free valproate in urine was directly measured with the TDX analyzer; total valproate was measured similarly after the glucuronide was deconjugated by treatment of the urinary sample with 0·5 N NaOH at 100°C for 2 h (Tatsuhara & Muro 1990). Each value for urinary valproate-glucuronide concentration was obtained by subtracting the free level from the total level. The cumulative amounts of valproate excreted into urine were expressed as percentages of the administered dose.

Statistics

The results were reported as mean values ± s.e.m. for four rats, and significance was evaluated by Student's *t*-test. Differences were considered significant at $P < 0{\cdot}05$.

Results

Plasma disappearance curves of valproate

Figure 1 shows plasma disappearance curves after the intravenous administration of a 50 mg kg^{-1} dose of valproate to rats. Figure 1a shows the curves obtained in the single lithium and the control group (no significant difference was observed). Figure 1b shows the curves obtained in the repeated-lithium and repeated control group (repeated pretreatment with lithium or saline, respectively). The curve of the repeated-lithium group was lower than that of the control. As shown in Table 1, the half-life of the repeated-lithium group was significantly shorter than that of the control. That is, the plasma disappearance rate of valproate was increased by repeated pretreatment with lithium.

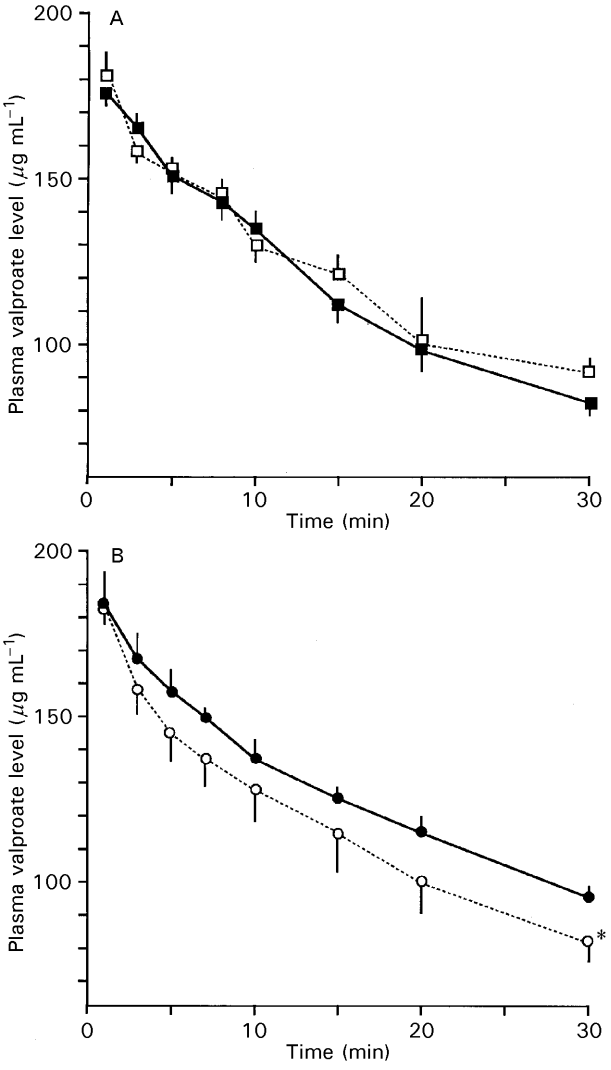

Figure 1. Plasma disappearance curves of valproate (50 mg kg^{-1}) administered intravenously to rats (A) 1 min after intraperitoneal pretreatment with lithium (2 mEq kg^{-1}) (single lithium group: □) or saline (single control group: ■), or (B) 15 h after the end of repeated pretreatment with lithium (2 mEq kg^{-1}) twice a day for ten days (repeated-lithium group: ○), or with saline (repeated-control group: ●). Each point represents mean ± s.e.m. of four rats. * $P < 0.05$, compared with the control.

Table 1. Pharmacokinetic parameters of valproate in plasma, calculated with a one-compartment model, after its intravenous administration to rats in combination with pretreatment with lithium or saline.

	$t_{\frac{1}{2}}$ (h)	CL (L kg^{-1} h^{-1})	Vd (L kg^{-1})
Single pretreatment			
Control	0·461 ± 0·033	0·438 ± 0·035	0·287 ± 0·007
Lithium	0·512 ± 0·044	0·404 ± 0·034	0·290 ± 0·005
Repeated pretreatment			
Control	0·578 ± 0·062	0·352 ± 0·028	0·292 ± 0·017
Lithium	0·428 ± 0·031*	0·467 ± 0·099	0·286 ± 0·041

Valproate-metabolizing activities of UGT and CYP

The glucuronidation rate constants of valproate are shown in Table 2. No significant difference in Vmax or Km was observed between the repeated-lithium group and the control.

The valproate-metabolizing activities of CYP (mean ± s.e.m.) were 0·45 ± 0·41 nmol min^{-1} mL^{-1} in the repeated-lithium group and 0·62 ± 0·62 nmol min^{-1} mL^{-1} in the control. These values were low level and were not significantly different. The amount of CYP was also not changed by lithium (data not shown). The valproate-metabolizing activities of UGT and CYP were not changed by lithium, although Feuerstein et al (1979) had reported a 44–81% increase in UGT and a 15–17% increase in CYP by lithium.

Urinary excretion of valproate

Figure 2 shows cumulative urinary excretion curves for valproate after its intravenous administration (50 mg kg^{-1}) in the repeated-lithium and the repea-

Table 2. Glucuronidation rate constants of valproate in liver microsomes obtained from rats dosed repeatedly with lithium or saline (control).

	V_{max} (nmol min^{-1} mL^{-1})	Km (μmol mL^{-1})
Repeated pretreatment		
Control	4·83 ± 0·06	0·467 ± 0·047
Lithium	5·38 ± 0·23	0·408 ± 0·064

Values are expressed as mean ± s.e.m. for four rats.

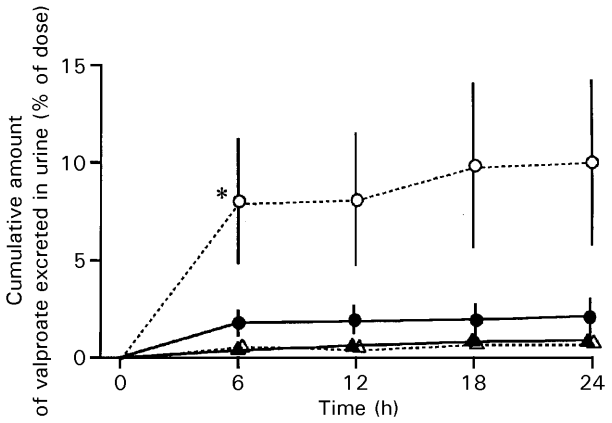

Figure 2. Cumulative urinary excretion curves of valproate ($50\,mg\,kg^{-1}$) administered intravenously to rats 15 h after the end of repeated pretreatment with lithium ($2\,mEq\,kg^{-1}$) twice a day for ten days (repeated-lithium group), or with saline (repeated-control group). ○, ●: the valproate-glucuronide in the repeated-lithium and the repeated-control group, respectively. △, ▲: the free valproate in the repeated-lithium and repeated-control group, respectively. Each point represents mean ± s.e.m. of four rats. * $P < 0{\cdot}05$, compared with the control.

ted-control group. The urinary excretion rate of the valproate-glucuronide in the repeated-lithium group was higher than that in the control, and a significant difference in the urinary glucuronide excretion was observed at 6 h after administration. Excretion rates of free valproate were not different between the two groups and were much lower than that of the glucuronide. Therefore, the valproate-glucuronide excretion was increased by lithium.

Discussion

In this study, the plasma disappearance rate of valproate was significantly increased with repeated pretreatment by lithium, suggesting that there is pharmacokinetic interaction between valproate and lithium. The half-life of valproate in the repeated-lithium group was 25% lower than that in the control group, indicating 25% reduction of mean plasma valproate levels in a steady state after repeated treatment with lithium. However, it is uncertain how such reduced levels of valproate can affect the overall efficacy or toxicity of valproate plus lithium, although the usual minimum effective–maximum safe concentration range of valproate is quite narrow ($50–100\,mg\,L^{-1}$) (Holford & Tett 1997).

It was assumed that the decrease in plasma valproate level caused by lithium is due to the UGT- or CYP-inducing effect of lithium (Feuerstein et al 1979; Rogiers et al 1995). We therefore examined the valproate-metabolizing activities of UGT and CYP in the liver microsomes. As a result, it was seen that the activity of UGT was not changed by lithium; CYP was also not changed and showed low levels. These results may be due to the different substrates used in our study and in the previous reports. Therefore, the lithium-induced increase in plasma disappearance of valproate is suggested to be independent of the microsomal valproate-metabolizing activities. Otherwise, it might be induced by an increase in the urinary excretion of valproate.

The urinary excretion of valproate after its intravenous administration was examined. Pretreatment with repeated doses of lithium increased the urinary excretion of valproate-glucuronide. This may contribute, in part to the decrease in plasma valproate, which probably indicates increase in the renal excretion rate. However, the plasma levels of valproate-glucuronide and other metabolites in plasma and urine, possibly affected by lithium, may also need to be checked, since urinary excretion of free valproate and its glucuronide was not high. This phenomenon may be caused by lithium-induced nephropathy, which has been reported by some investigators (Christensen et al 1992; Nyengaard et al 1994; Pospishil 1998). The increase in renal excretion of valproate-glucuronide may be caused by a decrease in renal tubular reabsorption of the glucuronide, possibly due to tubular injury or urinary pH change by lithium. Further study is required to confirm the mechanism of the increase in urinary excretion of valproate-glucuronide by lithium.

Increase in biliary excretion of valproate may also relate to the decrease in its plasma level, although the total biliary excretion of valproate was less than 10% of the administered dose (data not shown). However, the biliary excretion may not have much to do with the plasma valproate level, because the liver extraction rate of valproate is regarded as being metabolism-limited.

In conclusion, it is suggested that repeated administration of lithium decreases valproate plasma levels in part by reducing the urinary excretion of valproate-glucuronide in rats. It should be confirmed whether this phenomenon occurs in patients with bipolar disorder (and bipolar disorder combined with renal insufficiency) treated with both lithium and valproate.

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