Research Article

Effect of Amitriptyline on the Pharmacokinetics of Divalproex Sodium in Normal Rabbits

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ABSTRACT

The present study was aimed at investigating the effect of Amitriptyline on the pharmacokinetic of Divalproex in rabbits. Divalproex and Amitriptyline are used for long duration and indicated for CNS disorder like depressive disorder, personality disorder and migraine, respectively. The pharmacokinetic parameters of Divalproex sodium (6 mg/kg, *p.o.*) was compared with concomitant administration of Divalproex sodium (6 mg/kg, *p.o.*) and Amitriptyline (10 mg/kg, *p.o.*). The blood samples were collected at different time interval of 30 min, 1st, 2nd, 4th, 8th, and 16th hour. Divalproex dissociates to release valproate and valproic acid in the gastrointestinal tract. The concentration of Valproic acid (VPA) in serum was then estimated by HPLC coupled with Mass Spectroscpoy (LC-MS/MS). The serum concentration of VPA was significantly increased after Amitriptyline treatment for 7 days. Pharmacokietic parameters like AUC, AUMC, $T_{1/2}$ and C_{max} of VPA showed significant change after Amitriptyline treatment in healthy albino rabbits. Divalproex and Amitriptyline both are metabolized by the same enzyme; CYP2C9. Amitriptyline inhibits UDP-glucuronosyltransferases (UTG), where Divalproex is substrate of UTG metabolism. Both reasons may lead to increase in serum VPA concentration. Change in the time and frequency of administration of Divalproex sodium is suggested when both drugs are administered concomitantly. Therapeutic drug monitoring should be performed as combination may lead to remarkable increase in valproic acid serum level.

Keywords: Amitriptyline, divalproex sodium, interaction, pharmacokinetic

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INTRODUCTION

Divalproex sodium has been indicated for the treatment of epilepsy, maniac disorder and known for its effective prophylactic treatment in migraine [1-5]. Amitriptyline (AMI) has been indicated for the treatment of migraine [6]. Divalproex (DVPX) may also be used concomitantly with amitriptyline in few migraine patients. The bioavailability of DVPX is nearly complete with all formulation [7]. Divalproex dissociates to release valproate and valproic acid in the gastrointestinal tract [8].Peak plasma level occurs from 15 to 60 minutes after ingestion of the syrup, 1 to 4 hours after a single oral tablet dose [9]. Valproate is highly protein bound, primarily to serum albumin [7]. Valproate is metabolized hepatically by 3 primary pathways to a large number of metabolites that have pharmacological activities; β-oxidation to 3-OH-valproate, 3-oxo-valproate and 2-en-valproate and glucuronidation to a number of inactive metabolites [10]. The 2-en-valproate metabolite is pharmacologically active and has a long half-life [11]. Plasma concentration-response data from one large, randomized, acute treatment trial [12] indicated a therapeutic range of 50 to 125 µg/ml, with increasing likelihood of response with higher plasma concentration within that range [13].

Amitriptyline is commonly prescribed tricyclic antidepressant, with therapeutic concentrations ranging from 160 to 200 ng/ml [14]. Amitriptyline is effective in the treatment of neuropathic cancer pain [15]

and effective in the treatment of migraine and chronic tension-type headache [16]. There is an overlap of psychiatric indications for both of the drugs, for example, for the treatment of recurrent depressive disorder, personality disorder and migraine [17-19]. Amitriptyline is metabolized by CYP2C9 and CYP2C19 enzyme system [20]. Divalproex sodium is substrate for CYP2C9 and inhibits drugs that are metabolized by this enzyme [21]. Because of the similar metabolism enzyme system and limited therapeutic window of valproate, concomitant use of medications could cause serious side effects at a previously well-tolerated dose of the Divalproex. It is observed that co-administration of high doses of valproic acid with drugs that are primarily metabolized by CYP2C9 may result in significant drug interactions [21].

The pharmacokinetic influence of Valpromide and Divalproex on Amitriptyline has been published. But the effect of Amitriptyline on Divalproex has not been studied yet. The objective of this study was to assess the effect of concomitant administration of Amitriptyline on the pharmacokinetic of Divalproex.

MATERIALS AND METHODS

Animals and experimental conditions

All experiments were performed on adult male albino rabbits weighing 1.8 to 2 kg. Four animals were housed in different cage with free access to food and *ad libitum*, and in standardized housing conditions (experimental temperature and natural light-dark cycle). The animals were kept for 7 days of adaptation to laboratory conditions with 12:12 h light/dark cycles and temperature of 20 to 25°C. All experiments were conducted between 10:00 to 16:00 to minimize confounding effect of circadian rhythms. The experiment was approved by Office of Institutional Animal Ethical Committee (Reg. no. 1432/PO/a/11/ CPCSEA dated 31.05.2012; Ref no. MCP/ AIEC/Clear/003/2012-13).

Drugs The following drugs were used in this study: Amitriptyline (Times pharmaceuticals, Nepal), Divalproex (Yarrow Chemicals, Mumbai). Acetonitrile. (S.D. Fine chemicals, Mumbai) Surgical spirit (Shiva Pharma Industries, Hyderabad). Amitriptyline and Divalproex were dissolved in distilled water. AMI (10 mg/kg) and DVPX (6mg/kg) were administered orally using oral feeding tube.

Study design In the first part of the study, the pharmacokinetic parameters of Divalproex sodium (6 mg/kg, *p.o.*) were established in healthy albino rabbits. The time of drug administration was noted for all the animals. Animals were left for a washout period of 15 days. In second part, the animals were administered with Amitriptyline (10 mg/kg, *p.o.*) once daily for a week. On the eighth day, the Divalproex sodium (6 mg/kg) was administered along with the Amitriptyline and the pharmacokinetic parameters of Divalproex were established.

Sample analysis: Separation and estimation were carried out at Biochemistry laboratory of Indian Institute of Horticulture Research (IIHR), Hesarghatta Main Road, Bangalore. The blood sample was collected at 0, 30 min, 1st, 2nd, 4th, 8th and 16th hour after the Divalproex administration. Two ml of blood was withdrawn at each time interval and centrifuged at 5000 r.p.m. for 20 minutes. The serum so collected was kept in refrigerator at -20°C until estimation process. One ml serum sample was precipitated by adding 3 ml of Acetonitrile followed by centrifugation at 10000 r.p.m. for 20 min. The aqueous/organic layer was transferred to a Silanized glass tube, evaporated under nitrogen, and recon-stituted with Acetonitrile. Reconstituted solution was filtered using membrane [Nylon-65] syringe filter. An Aquity UPLC instrument with a double diode PDA detector set at 254 nm [22] was coupled to Mass Spectroscopy (LC-MS/MS). Waters aquity software was used for data handling. The pre-column used was Vanguard 3/PK, 2.1× 5mm column. The column used was an Aquity UPLC@ BEH C_{18} 2.1x100 mm, 1.7µm. The 2 µl of the serum was injected into the column. The two solvent system used were, solvent A (0.01% Formic acid in water) and solvent B (75% Acetonitrile in water) in the ratio 90:10. Flow rate was maintained at 0.400 ml/min. The chromatogram was recorded and used for quantification. The

Divalproex sodium and amitriptyline was assumed to be absorberd 100% on oral absorption.

Pharmacokinetic analysis The blood concentration of Valproic acid before and after Amitriptyline treatment were applied to software Ramkin to calculate pharmacokinetic parameters like AUC_{0-t}, $AUMC_{0-t}$, C_{max}, T_{max}, $t_{1/2}$ and MRT.

Statistical analysis The data were expressed as mean ± S.E.M or percentage using GraphPad Prism Software (version 6.0). Differences between means were considered to be significant at $P<0.05$. The statistical evaluation of pharmacokinetic parameter was performed using Student's *t* test.

RESULTS AND DISCUSSION

Co-administration of two or more drugs may lead to beneficiary and sometime found to be a major interaction causing severe problem. We studied the influence of Amitriptyline on the pharmacokinetic of Divalproex in normal rabbits. Amitriptyline is tricyclic antidepressant drug mainly used for chronic depression as well as migraine. It is also prescribed for post traumatic pain management along with other medications. The dose of Amitriptyline was selected from human therapeutic dose extrapolated to rabbits, based on surface area. Single dose treatment of Divalproex (6 mg/kg, *p.o.*) alone and in combinations with Amitriptyline (10 mg/kg, *p.o.*) was studied. Plasma valproic acid concentrations were analyzed from serum withdrawn at different time interval of 30 min, 1st, 2nd, 4th, 8th, and 16th hour. The serum valproic acid concentration was estimated to study the effect on absorption, distribution, metabolism and excretion of valproic acid in the body. In the pharmacokinetic parameters, there was a significant change in absorption of valproic acid in presence of Amitriptyline.

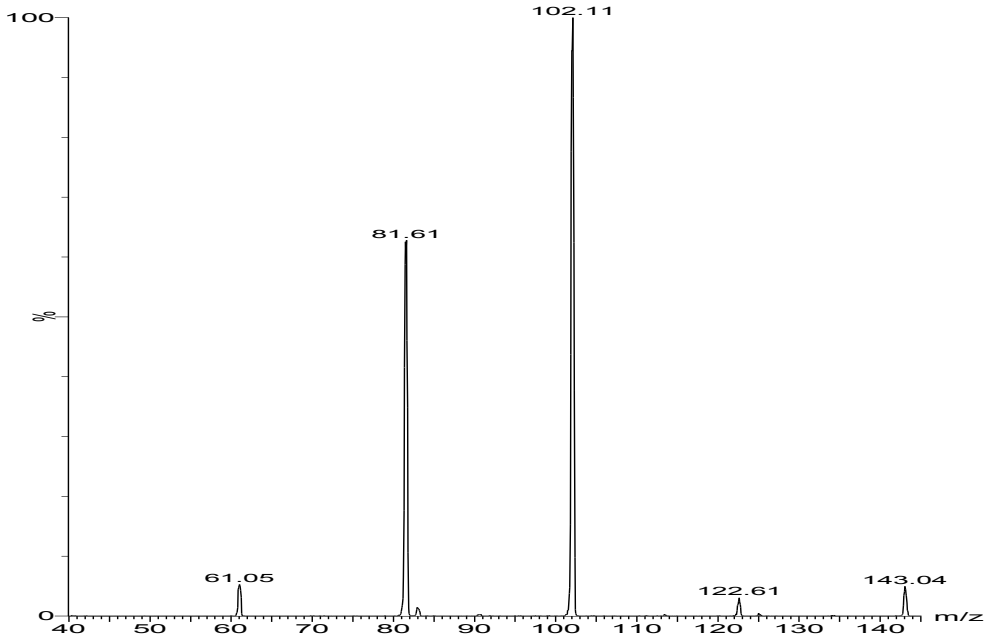

Figure 1: Mass spectroscopy of Valproic acid (LC-MS/MS)

Divalproex sodium is substrate for CYP2C9 and inhibits drugs that is metabolized by this enzyme [21]whereas Amitriptyline is metabolized by CYP2C9 and CYP2C19 enzyme system [20]. It is observed that co-administration of high doses of valproic acid with drugs that are primarily metabolized by CYP2C9 may result in significant drug interactions [21]. The AUC also increased from 1500.738 to 1851.9, half-life also increased from 10.37 to 12.57 and mean residence time increase more than 3 hours, exhibiting higher chances of side effects.

UDP-glucuronosyltransferase (UGT) enzymes catalyze the conjugation of various endogenous substances and exogenous compounds [23]. Drugs that affect the level

of expression of hepatic enzymes, particularly those that elevate levels of glucuronosyltransferases, may increase the clearance of valproate [24]. VPA is primarily hepatically metabolized by UGT enzymes and oxidation to a much lesser extent by CYP 2C9 and CYP 2C19 [25]. Amitriptyline acts as an inhibitor of UGTs [26]. There is evidence to suggest that Amitriptyline may inhibit Valproic acid glucuronidation *in vitro* and/or *in vivo* [27] resulting in free plasma valproic acid concentration. As free drug is a major determinant of pharmacologic effects, these drug interactions could result in toxicity and/or enhanced efficacy [28].

Table 1: Serum concentration of Divalproex sodium before and after Amitriptyline treatment in healthy albino rabbits

	Serum concentration of Valproic acid in mcg/ml	
Time (hrs)	**Divalproex sodium (6 mg/kg) #**	**Divalproex sodium (6 mg/kg, *p.o.)* + Amitriptyline (10 mg/kg, *p.o.*) #**
0	0	0
0.5	54.15 ± 4.32	76.18 ± 6.33*
1	171.095 ± 12.95	166.41 ± 14.25
2	182.25 ± 16.29	218.33 ± 17.63***
4	121.65 ± 9.48	162.67 ± 13.58***
8	82.33 ± 7.47	93.25 ± 7.21**
16	53.26 ± 4.39	78.50 ± 5.32***

Number of rabbit per group = 4
Values are in Mean ± SEM
*p<0.05,**p<0.01 and ***0<0.001

A significant change in parameters like $AUC_{0\text{-}t}$. $AUMC_{0\text{-}t}$, $T_{1/2}$, C_{max} and MRT were seen. The time of maximum concentration was same in both the case.

Table 2: Effect of Amitriptyline treatment on pharmacokinetic parameters of Divalproex sodium in healthy albino rabbits

Parameters	**Divalproex Sodium (6 mg/kg, p.o.) #**	**Divalproex (6 mg/kg, p.o.) + Amitriptyline (10 mg/kg, p.o.) #**
$AUC_{0\text{-}t}$ (µg/ml/h)	1500.738±144.56	1851.903±158.7**
$AUMC_{0\text{-}t}$ (µg/ml/h)	12568.26±1085.33	16604.56±1276.5***
$Tt_{1/2}$ (hrs)	10.37±1.37	12.57±1.76*
C_{max} (µg/ml)	182.25±13.33	218.33±18.6**
T_{max} (hrs)	2±0	2±0
MRT (hrs)	16.21±2.14	19.90±2.36**

Values are in Mean ± SEM
*p<0.05, **p<0.01 and ***p<0.001

CONCLUSION

Amitriptyline treatment showed significant change in pharmacokinetic parameters of Divalproex sodium like AUC, AUMC, $t_{1/2}$, C_{max} and MRT in healthy albino rabbits**.** Hence, change in the time and frequency of administration of Divalproex sodium is suggested when both drugs are administered concomitantly. The experimental results in this study were performed on healthy rabbits. These findings have to be confirmed on studies on humans before practicing in therapeutics.

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