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NOTES

Plasma Protein Binding Interaction between Valproic and Salicylic Acids in Rhesus Monkeys

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Abstract □ The effects of three levels of salicylic acid on the steady-state plasma concentrations of free and total valproic acid were examined in catheterized rhesus monkeys. Valproate was infused intravenously for a total of 41 hr, and salicylate was added after the first 8 hr. The three salicylate infusions were randomly assigned to each monkey. Valproate free fraction was determined by equilibrium dialysis. Statistically significant increases in valproate free fraction and total body clearance were observed after addition of salicylic acid. The increase in valproate clearance was positively correlated with the molar ratio of salicylate to valproate steady-state plasma concentrations. There was no significant change in valproate free concentration after salicylate treatment. The proposed mechanism of this *in vivo* interaction includes plasma protein binding displacement with no change in valproate intrinsic clearance.

Keyphrases □ Valproic acid—plasma protein binding interaction with salicylic acid, rhesus monkey □ Salicylic acid—plasma protein binding with valproic acid, rhesus monkey □ Plasma protein binding—interaction between valproic and salicylic acids, rhesus monkey

Valproic and salicylic acids are known to bind extensively to plasma proteins in humans (1–8) and rhesus monkeys (9–11). Three *in vitro* studies showed that salicylic acid decreases valproate binding to plasma or human serum albumin (12–14). However, there is no indication that such an interaction occurs *in vivo*. In addition, these two acids may interact at a metabolic level.

Epileptic patients on valproate treatment occasionally receive salicylates and, depending on its mechanism, this interaction may be of therapeutic significance. The present study was designed to investigate this interaction *in vivo* at the systemic level. Both drugs were administered by constant-rate intravenous infusion to achieve one steady-state concentration of valproic acid and three steady-state salicylic acid levels.

EXPERIMENTAL

Four healthy male rhesus monkeys (*Macaca mulatta*) (mean weight 5.5 kg) were chair-adapted for 1 month prior to implantation of catheters. The jugular and femoral veins of each monkey were chronically catheterized to enable withdrawal of venous blood samples and drug infusion, respectively. The monkeys were maintained in three-level restraining chairs during the studies. Patency of catheters was assured by a slow, continuous saline infusion (1 ml/hr). Daily diet consisted of fresh fruit and monkey food.

Three studies were carried out in each monkey. In each study, valproic

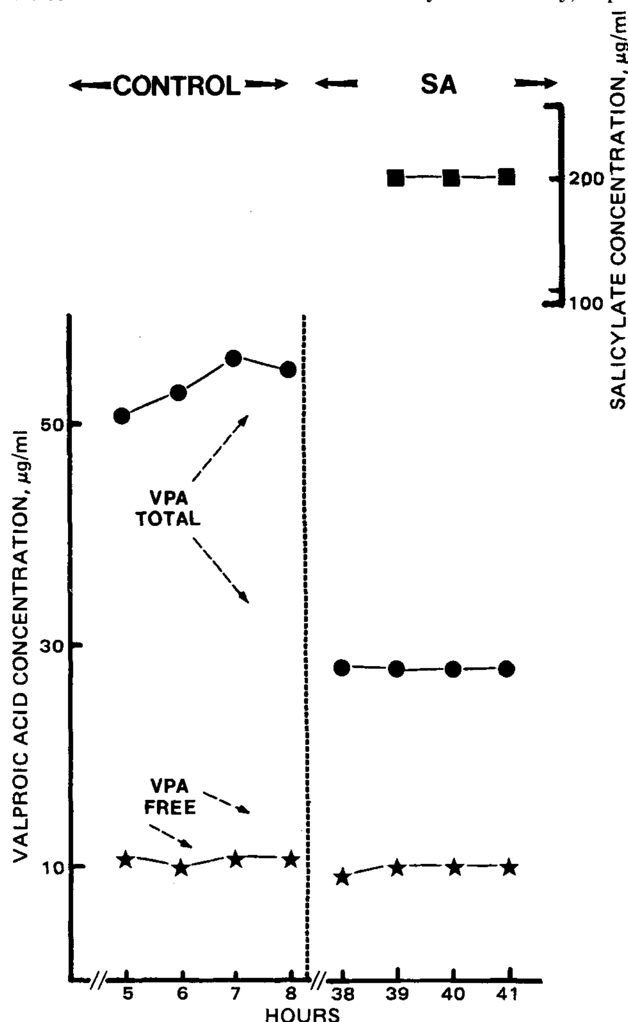

Figure 1—*Steady-state levels of total and free valproate (VPA) before and during salicylate (SA) infusion in Monkey 201.*

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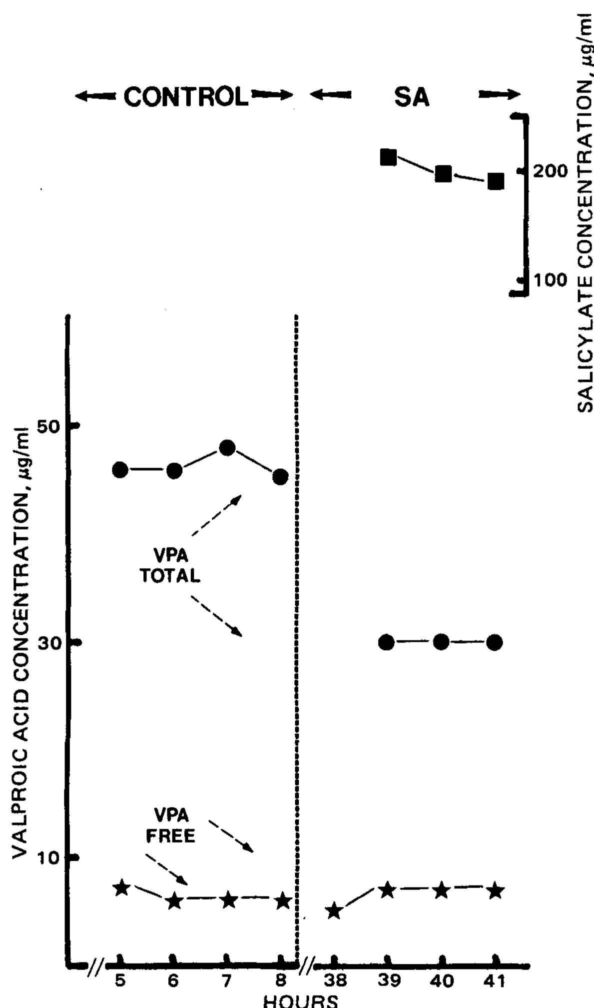

Figure 2—*Steady-state levels of total and free valproate (VPA) before and during salicylate (SA) infusion in Monkey 304.*

acid was infused for 41 hr (mean rate 74.4 mg/hr) and salicylic acid was added after the first 8 hr [valproic acid has a half-life of 1 hr in the monkey (15)]. The infusion rate of valproic acid was selected to yield levels in a clinically realistic range (30–80 µg/ml). The infusion rates of salicylic acid in the three studies were 55, 70, and 90 mg/hr. Study order was randomly assigned to each monkey, and a washout period of at least 2 weeks was allowed between studies.

Valproic and salicylic acids were administered as equivalents of sodium salts in sterile saline. All infusion equipment was covered with aluminum foil to protect salicylic acid from light.

Plasma samples were obtained at 5, 6, 7, and 8 hr following the start of infusion for control valproate steady-state levels and at 38, 39, 40, and 41 hr for steady-state levels of both drugs.

Valproate free fraction was determined by equilibrium dialysis. Each dialysis unit consisted of two 1-ml chambers separated by a dialysis membrane[1]. Plasma containing the drug (0.5 ml) was introduced into one chamber, and 0.5 ml of phosphate buffer (pH. 7.4) was added to the opposite chamber. The cells were kept immersed in a water bath at 37° for 24 hr. Valproate concentrations were measured from the buffer side (free drug) and plasma side (total drug) using a previously described GLC procedure (16). Plasma salicylate concentrations were determined as total salicylate by the spectrophotometric procedure of Wiseman *et al.* (17).

RESULTS AND DISCUSSION

The effects of salicylate infusion on valproate steady-state levels are illustrated in Figs. 1 and 2 for Monkeys 201 and 304, respectively. Valproate free fraction and total body clearance before and after salicylate treatment are summarized in Table I; both increased significantly after salicylate treatment ($p < 0.05$). However, there was no significant change in the valproate free concentration ($p > 0.05$). As a result, total valproate levels decreased during valproate treatment. A significant correlation was obtained between the increase in valproate clearance and the molar ratio of salicylate to valproate ($y = 10.9x + 0.7$, $r = 0.93$, Fig. 3). Over 80% of the variability in the increase in valproate clearance can be explained by the salicylic acid concentration.

These findings indicate that salicylic acid is able to displace valproate acid from plasma protein binding sites *in vivo*. The results are consistent with the fact that valproic acid has a low extraction ratio in the rhesus monkey (15, 18). Pharmacokinetic theory predicts that the valproate free concentration should not be changed if salicylate affects valproate plasma binding without changing its intrinsic metabolic clearance (19). Several studies (12, 13) indicated that an *in vitro* plasma binding interaction exists between salicylic and valproic acids. If the interaction mechanism found in the rhesus monkey is also applicable in humans, this interaction should have little or no pharmacological consequence. However, since changes in total valproate levels would not reflect the changes in free levels, monitoring of the valproate free concentration would be indicated in patients receiving salicylates (20).

Table I—Effect of Salicylic Acid on Valproic Acid Free Fraction and Systemic Clearance

Monkey	Free Fraction		Systemic Clearance, liters/kg hr	
	Control	During Salicylate	Control	During Salicylate
201	0.22	0.23[a]	0.14	0.19
	0.33	0.75[b]	0.15	0.21
	0.20	0.35[c]	0.19	0.30
200	0.33	0.46	0.22	0.28
	0.15	0.25	0.30	0.57
	0.11	0.22	0.28	0.64
301	0.24	0.23	0.45	0.43
	0.29	0.74	0.30	0.45
	0.12	0.40	0.38	0.77
304	0.36	0.58	0.46	0.29
	0.19	0.23	0.24	0.30
	0.15	0.34	0.28	0.46
	$p < 0.002$[d]		$p < 0.0008$[d]	

[a] Salicylate infusion rate of 55 mg/hr. [b] Salicylate infusion rate of 70 mg/hr. [c] Salicylate infusion rate of 90 mg/hr. [d] Comparisons were made by analysis of variance.

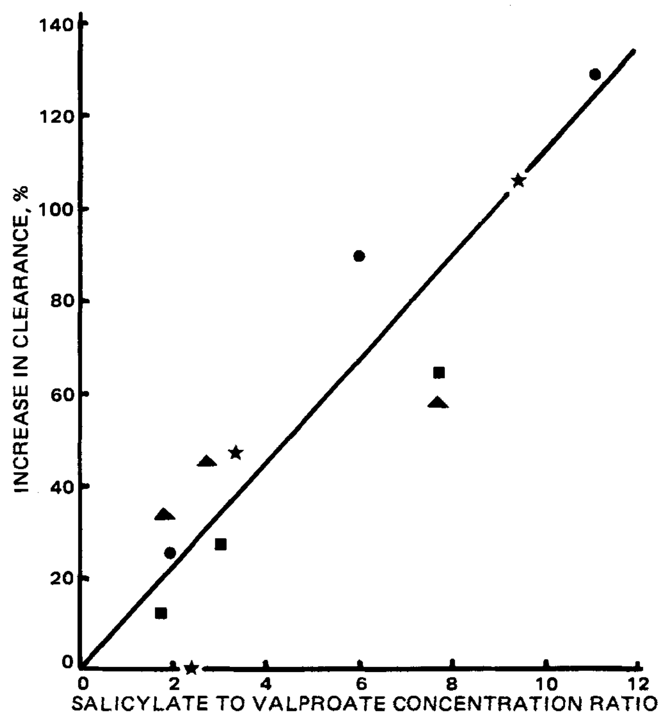

Figure 3—*Percent increase in valproate clearance correlated with the molar ratio of salicylate to valproate.*

[1] Dialyzer tubing, VWR Scientific, Seattle, WA 98124.

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Synthesis and Evaluation of Guanazole Prodrugs as Antineoplastic Agents

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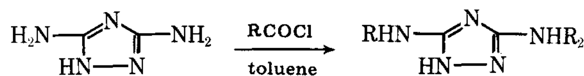
Received January 22, 1981, from the **Section of Biomedicinal Chemistry, School of Pharmacy, and the ‡ Department of Pathology, School of Medicine, University of Southern California, Los Angeles, CA 90033.* Accepted for publication April 16, 1981.

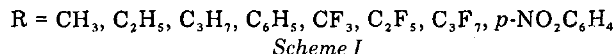
Abstract ☐ Nine amide derivatives of guanazole were synthesized, and eight of them were tested for antineoplastic activity *versus* L1210 cells *in vitro*. Several of these compounds exhibited activity comparable to or greater than that of guanazole.

Keyphrases ☐ Guanazole—synthesis and evaluation of prodrugs as antineoplastic agents ☐ Prodrugs—of guanazole, synthesis and evaluation as antineoplastic agents ☐ Antineoplastic agents—synthesis and evaluation of guanazole prodrugs

Guanazole (3,5-diamino-1,2,4-triazole, NSC 1895) was synthesized by Pellizzari (1) and discovered in a general screen by the National Cancer Institute. Subsequently, Brockman *et al.* (2) reported that guanazole inhibited DNA synthesis by inhibiting ribonucleotide reductase. Antitumor activity against various animal leukemias and tumors (3) and remissions in some patients with actute myelocytic leukemia (4) were reported. However, extensive clinical utilization of this drug has been limited by its high polarity and water solubility, low molecular weight, and relatively low potency, which warrants continuous intravenous infusion at frequent intervals (5).

Structure–activity relationship analysis of past and current work on guanazole derivatives established the importance of both amino groups for antineoplastic activity (2, 3). Thus, to circumvent the problem of the high elimination rate and to increase the efficacy of guanazole as an antieoplastic agent, amide derivatives with decreased polarity, water solubility, and increased molecular weight were synthesized (Scheme I). These compounds were then tested for anticancer activity *versus* L1210 cells *in vitro*.





Scheme I

The compounds and their physical properties are outlined in Table I.

EXPERIMENTAL

Chemistry—All solvents were analytical grade. All melting points were taken in open capillary tubes[1] and are corrected. Elemental analysis[2] was within ±0.4% of the theoretical values. All proton NMR spectra were recorded on a 60 MHz spectrometer[3] with tetramethylsilane as the internal standard and solvents such as deuterochloroform, dimethyl sulfoxide-d_6, and trifluoroacetic acid. IR spectra were recorded on a spectrophotometer[4] as potassium bromide pellets or Fluorolube mulls. UV spectra were recorded on a double-beam spectrophotometer[5]. TLC was performed using Eastman-type 6060 chromatogram sheets (silica gel), and the sheets were developed in an iodine chamber to check the purity of the amides.

Most of these derivatives were synthesized by a general procedure for the acylation of amines. The appropriate acid chloride or anhydride was condensed with guanazole, usually in the presence of a nonpolar solvent. Two typical reactions for preparing the amides are described using III and VI as examples.

One gram (0.01 *M*) of guanazole and 4.2 g (0.02 *M*) of trifluoroacetic anhydride were refluxed in dry toluene for 8 hr. The white precipitate

[1] Thomas Hoover melting-point apparatus.
[2] Performed by the Analytical Laboratory, California Institute of Technology, Pasadena, Calif.
[3] Hitachi Perkin-Elmer R-24.
[4] Beckman IR-4240.
[5] Perkin-Elmer Coleman 124.

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