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GLUCURONIDASE-RESISTANT GLUCURONIDES OF VALPROIC ACID: CONSEQUENCES TO ENTEROHEPATIC RECIRCULATION OF VALPROATE IN THE RAT

Ronald G. Dickinson, Mervyn J. Eadie and Wayne D. Hooper

Department of Medicine, University of Queensland,
Royal Brisbane Hospital, Herston, Qld 4029, Australia

Conjugates of carboxylic acids with D-glucuronic acid (acyl or ester glucuronides) have 1-*O*-acyl-β linkages which can be cleaved *in vivo* by β-glucuronidases. A consequence of this is exemplified in the pronounced enterohepatic recirculation of valproic acid (VPA) in the rat (1). Following i.v. administration of sodium valproate (NaVPA) at 15 or 150 mg/kg, *ca.* 60% of the dose is excreted in bile as the ester glucuronide (VPA-G). Subsequent hydrolysis by β-glucuronidases in the gut permits reabsorption of liberated parent VPA into the blood-stream, causing secondary increases in blood concentration of VPA at 4-5 hr after the i.v. bolus dose. Essentially no VPA or VPA-G is excreted in faeces.

In recent years, several reports (e.g. 2-4) have documented the intramolecular rearrangement of ester (but not ether) glucuronides, particularly at slightly alkaline pH, by migration of the aglycone moiety away from the C-1 position of the sugar ring. The resulting isomeric "glucuronides" are not substrates for β-glucuronidases. Previous studies of this *chemical* phenomenon have focussed on its occurence *ex vivo* where rearrangement during collection or storage of biological samples may compromise quantitative analysis when β-glucuronidase is used for hydrolysis. However, there is good evidence that rearrangement may occur *in vivo* e.g. with bilirubin (2), clofibric acid (3) and 3-(*p*-chlorophenyl)thiazolo-[3,2-*a*]benzimidazole-2-acetic acid (4) glucuronides.

We recently documented (5) pH-, temperature- and time-dependent rearrangement of VPA-G into at least six β-glucuronidase-resistant isomers (VPA-G-R). The present study assesses the effect *in vivo* on enterohepatic recirculation of VPA in the rat, when VPA-G entering the gut via the bile is replaced artificially by VPA-G-R.

METHODS: Donor rats (300-350 g male Sprague-Dawley) with jugular vein and bile duct catheters were given 150 mg NaVPA/kg i.v. and the bile collected for 2 hr (7-7.5 ml at pH 8-8.4 containing VPA-G at *ca.* 4 mg VPA/ml). To produce VPA-G-R, the pH was adjusted to 9.2 and the bile incubated at 25°C for 24 hr. VPA liberated by hydrolysis (*ca.* 10% of the VPA-G) was removed by adjustment of the pH to 4.5 followed by two extractions with 1-chlorobutane. The pH was then readjusted to 8.2 and the bile frozen until use. Acidification, extraction with 1-chlorobutane and readjustment to pH 8.2 as above was carried out also with bile containing non-rearranged VPA-G. Blank bile (2-2.5 ml) was collected for 2 hr from rats given saline i.v.; NaVPA in water was added to achieve a concentration of *ca.* 30 mg VPA/8 ml diluted bile, and the pH adjusted to 8.2. The VPA:VPA-G:VPA-G-R composition of these three dosing biles were: dose A 100:0:0, dose B 1.5:88:10.5, dose C 1:8:91; each contained *ca.* 30 mg VPA in non-conjugated or conjugated form indicated in the above ratio in a volume of *ca.* 8 ml.

Recipient rats were prepared with a catheter in the jugular vein and two in the bile duct, one draining bile from the liver and the other permitting infusion into the gut. Rats were maintained conscious and unrestrained in metabolic cages throughout the experiment. No

food was given, and dehydration was prevented by frequent i.v. infusions of saline. The prepared doses A, B and C were infused into the gut via the second biliary catheter over 2 hr. Blood, urine and bile samples were collected for analysis. At termination of the experiments at 6, 22 and 36 hr after doses A, B and C respectively, the small intestine including contents and large intestine including contents and faeces were homogenised.

RESULTS AND DISCUSSION: VPA appeared rapidly in blood following infusion of dose A, reached a peak concentration of ca. 33 µg/ml at 2 hr after which it was rapidly eliminated (<0.2 µg/ml at 4 hr). After infusion of β-glucuronidase-susceptible conjugate, dose B, VPA appeared slowly and steadily in blood, reaching a peak plateau concentration of ca. 5 µg/ml at 6-9 hr, thereafter being steadily eliminated to <0.2 µg/ml at 22 hr. Infusion of the rearranged, β-glucuronidase-resistant conjugates, dose C, caused VPA to appear even more slowly in blood, reaching peak plateau concentrations of ca. 2 µg/ml at 12-24 hr and becoming non-measureable at <0.2 µg/ml at 36 hr. The profile of blood concentrations of VPA was paralleled by the profile of excretion in bile of VPA-G arising from hepatic glucuronidation of VPA from the blood.

At termination of the experiments at 6, 22 and 36 hr after doses A, B and C respectively, none of the dose was recovered from the small intestine and contents, whereas 0, 2 and 12% was recovered from the large intestine, contents and faeces. Of the 12% of dose C recovered in the large intestine etc., most (80%) was still in conjugated form (presumably VPA-G-R). In bile, 61, 53 and 39% of doses A, B and C respectively was recovered; the composition of the recovered drug (i.e. ratio of VPA:VPA-G:VPA-G-R) was 1.5:93:5.5, 1.5:96:2.5 and 2.5:91:6.5 respectively. In urine, 18, 16 and 14% of doses A, B and C respectively was recovered over the 6, 22 and 36 hr respectively of study, and the composition (VPA:VPA-G: VPA-G-R ratio) was 1.5:96.5:2, 1:96:3 and 1.5:74:24.5 respectively. The ratio in urine after dose C shows that about one quarter of the 14% recovered, or about 3.5% of dose C, is still in the form VPA-G-R. This indicates that this portion of VPA-G-R administered via the bile duct has been absorbed intact from gut to blood and eliminated by the kidneys.

The results above were confirmed in rats without surgery, where doses B and C were administered orally. Hydrolysis of β-glucuronidase-resistant VPA-G-R in the gut of the rat was shown to be enzymic rather than chemical from *in vitro* experiments using homogenised duodenum, jejunum, ileum, caecum and colon, each including contents. The presumed non-specific esterases were located predominantly in the caecum and contents (and were probably, therefore, of microbial origin) and were inactivated by heat treatment.

These experiments show that β-glucuronidase-resistant "glucuronides" of valproic acid are disposed along three pathways after biliary administration to rats: (a) part hydrolysis by non-specific esterases in the gut leading to a reduced enterohepatic recirculation (b) part excretion in faeces (c) part absorption intact from gut to blood and excretion in urine.

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