Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 813-818, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

EVALUATION OF THE INTESTINAL PERMEABILITY AND HEPATIC HANDLING OF PEPTIDOMIMETIC ANALOGS

H. Hamilton,*† B. Steinbaugh,† J. Blankley,† M. Taylor,† O.H. Chan,§ B. Stewart,§ R. Schroeder,‡ M. Ryan,‡ S. Rapundalo,‡ J. Cook,§ A. Bernabei,† C. Stewart†

Departments of Medicinal Chemistry, Pharmacology, and Pharmacokinetics and Drug Metabolism, Parke-Davis Pharmaceutical research Division of Warner-Lambert Co., 2800 Plymouth Road, Ann Arbor, Michigan 48105, USA.

(*Received* 5 *June* 1992; *accepted* 2 *October* 1992)

Abstract

Three structurally similar peptidomimetic analogs with varying logP's and molecular weights were evaluated in the in situ rat gut perfusion assay and isolated perfused rat liver to analyze their absorption and clearance characteristics.

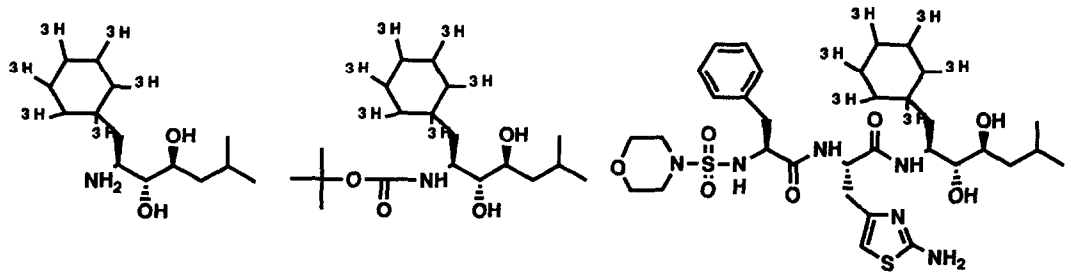
Introduction

In an effort to understand factors important to the bioavailability of peptidomimetic drugs,[1,2] a study was initiated of renin inhibitor analogs and structurally related compounds in isolated in vitro and in situ assays to evaluate intestinal absorption and hepatic clearance. Herein results are reported with the orally active renin inhibitor and CI-992,[3] the transition-state analog portion of this molecule (termed ACDMH, aminocyclohexyl-diolmethylhexane), and the butyloxycarbonyl-protected form of ACDMH (BOC-ACDMH) in the isolated perfused rat liver and in situ perfused rat gut preparations. While structurally similar, these compounds span a significant range of molecular weight and lipophilicity, two parameters believed to be important for absorption and clearance.

Methods and Results

Compounds were synthesized with a common tritiated structure (the cyclohexyl ring of ACDMH) to allow detection in biological matrices. Radiolabel was essential since the intermediates had renin inhibition IC_{50}'s which were not meaningful for bioassay, and had no chromophore for UV/HPLC detection. Physical properties of these analogs are given in Table 1.

Table 1. Structure and Properties of Compounds Evaluated for Absorption and Clearance



	ACDMH	BOC-ACDMH	CI-992
Molecular Weight	243	343	708
Log P (pH 7.5)[4]	2.0	4.0	3.1
pKa[5]	9.3	--	6.3
Aq. Solubility[6]	5.87 mg/ml	< 0.02 mg/ml	0.01 mg/ml
Renin Inhibition[3]	0% @ 10-6 M	0% @ 10-6 M	IC_{50}=0.32 nM

Intestinal Absorption

An in situ single-pass rat intestinal perfusion technique was used to study the intestinal absorption of these compounds.[7] Compounds were studied at concentrations below their solubility limits, and flux across the intestinal membrane was measured from the disappearance of drug from the perfusate effluent. Effective permeability was calculated by mass balance analysis.[8] The results are summarized in Table 2.

Table 2. Effective Permeability of Reference Compounds and Analogs.

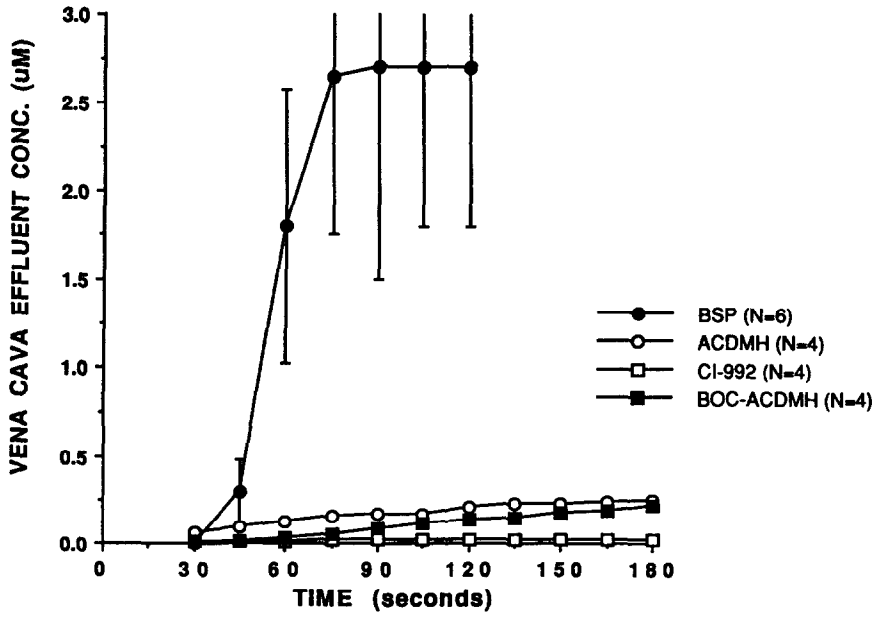
Compound	Effective Permeability	Compound	Effective Permeability
L-Phenylalanine[a]	3.57 ± 0.21	ACDMH	1.28 ± 0.12
D-Mannitol	0.05 ± 0.03	BOC-ACDMH	5.76 ± 0.72
Gabapentin[a]	0.46 ± 0.05	CI-992	1.68 ± 0.25

[a] 0.01mM

Liver Clearance.

Hepatic handling of the analogs was examined in the isolated perfused rat liver model, using both the single pass[9-11] and recirculating models.[11] With single pass perfusion (Figure 1), a rapid initial uptake was observed for all compounds, and they were not well differentiated. BSP (sulfobromophthalein), a highly extracted compound, was tested as a reference in this model, and unlike the test compounds rapidly achieved a period of steady-state extraction.

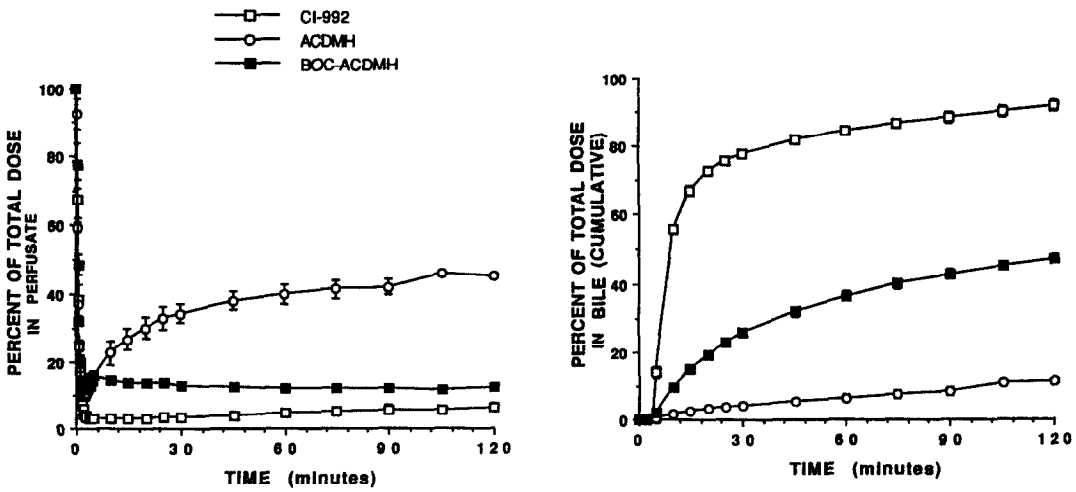
Figure 1. Single Pass Isolated Perfused Rat Liver



Concentration of drug appearing 'downstream' after a single pass through an isolated rat liver. Test compounds were given at an initial (portal vein) concentration of 1 mM, and BSP at 30 mM. BSP data was qualitatively the same at the lower dose and is shown at 30 mM for clarity.

In order to enable better differentiation among compounds, they were then tested in recirculating preparations (Figure 2). This model was run for a much longer period of time, allowing more kinetic information to be derived.[11] All compounds were extracted rapidly from perfusate, however a distinction in hepatic handling of the compounds was seen in this model. The radioactivity from CI-992 appeared primarily in the bile over the two hour time period of the experiment, while ACDMH radioactivity was found to efflux back into the perfusate. BOC-ACDMH was intermediate in these two routes of handling in this model.

Figure 2. Recirculating Isolated Perfused Rat Liver



Percent of total radioactivity of test compound in the perfusate and bile over time. 1 mM initial concentration, n=2.

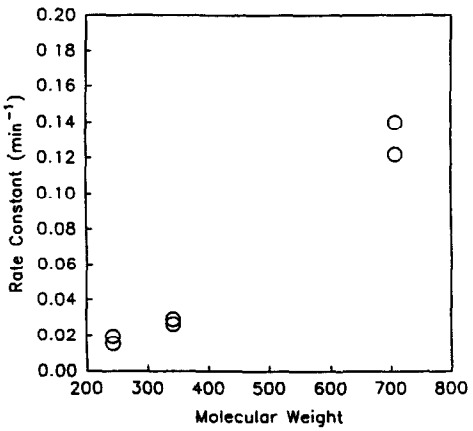
Compartmental analysis of the data was consistent with the radioactivity detected not being parent compound in both the CI-992 and ACDMH experiments. This was confirmed by HPLC analysis in the bile-duct cannulated rat.[12]

Discussion

In the intestinal absorption studies, L-phenylalanine, gabapentin and D-mannitol were used as reference compounds with known values for the fraction of dose absorbed in humans (1.0, 0.74, and 0.05, respectively). Although zwitterionic at physiologic pH, permeabilities were high for L-phenylalanine and gabapentin; at 0.01 mM, both compounds are in the linear region of a carrier-mediated transport process, an unlikely process for the renin inhibitor analogs. The effective permeabilities of the tritiated analogs increased with increases in partition coefficient. All three compounds demonstrated an effective permeability above unity, suggesting that the compounds would be well absorbed from solution in the intestine. The fraction absorbed for these compounds would depend on the rate and extent of dissolution. A molecular weight cut-off for absorption may be suggested by the lower permeability of CI-992. ACDMH is 99.8% ionized in the perfusion medium (pH 6.5), which may explain the relatively low effective permeability of this compound.

The rate of initial uptake into hepatic cells was independent of log P or molecular weight, as all compounds disappeared from the perfusate within minutes. Once within the hepatic cell, the compounds were metabolized prior to efflux back into the perfusate or bile, so conclusions as to the rate of transport into bile or back into perfusate would depend on the molecular weight and log P of these metabolites, not the parent compounds. The liver has sufficient holding capacity for these compounds to allow time for preferential routes of administration (note the total radioactivity for the compounds in perfusate and bile does not equal unity, and the remainder must therefore be in the liver itself). The type of metabolite produced, and the resulting hepatic fate of that compound, may very well depend on the parent compound's lipophilicity or size. With this caveat, there appears to be a relationship between the first order rate constant associated with rate of appearance of radioactivity in bile and molecular weight of the three compounds (Figure 3), although the exact structure of the compounds in the bile is not known.

Figure 3. Rate of Appearance in Bile of Radioactivity vs. Molecular Weight



In this study, compounds with a higher log P are better absorbed in the intestine, while uptake into hepatic cells is independent of log P or molecular weight. Excretion into bile occurs to a greater extent with higher molecular weight compounds. These results are consistent with the conventional wisdom that compounds with a higher log P and lower molecular weight offer the best chance of achieving good oral bioavailability. With renin inhibitors, the problem of maintaining sufficient receptor potency with a lower molecular weight compound and incorporating desirable physiochemical parameters remains a challenge.

†Department of Medicinal Chemistry §Department of Pharmacokinetics and Drug Metabolism
‡Department of Pharmacology

Acknowledgements The authors wish to acknowledge the helpful discussions of Drs. M. Plummer and J. Wright

References and Notes

1. Humphrey, M.J.; Ringrose, P.S. *Drug Metab. Rev.* **1986,***17*,283-310.

2. Greenlee, W.J. *Med. Res. Rev.* **1990,***10*,173-236.

3. Patt, W.C.; Hamilton, H.W.; Ryan, M.J.; Painchaud, C.A.; Taylor, M.D.; Rapundalo, S.T.; Batley, B.L.; Connolly, C.J.C.; Taylor, D.G. *Med. Chem. Res.* **1992,***2*,10-15.

4. Haky, J.E.;Young, A.M. *J. Liq. Chromatog.* **1984,***7*,675-689.

5. pKa's were determined by potentiometric titration with standardized hydrochloric acid.

6. Solubility was determined in pH 7 phosphate buffer; samples were shaken for 1 hour and the supernatant filtered and assayed by HPLC.

7. Johnson, D.A.; Amidon, G.L. *J. Theor. Biol.* **1988,***131*,93-106.

8. Permeability was calculated from ratio of drug concentration exiting the intestine to drug concentration entering the intestine, corrected for water flux using the nonabsorbable marker, PEG 4000, or gravimetric analysis, and normalized for intestinal length, flow rate and drug diffusivity : Amidon, G.L.; Sinko, P.J.; Fleisher, D. *Pharm. Res.* **1988,***5*,651-654. $P_{eff} = \ln (^{Cout}/_{Cin})/ -4GZ$; GZ = (;Dl/2Q)

9. Tokumo, H.; Aoyama, N.; Busch, N.; Mancuso, D.J.; Holzbach, R.T. *Hepatology* **1991,***13*,62-67.

10. Sorrentino, D.; Licko, V.; Weisiger, R.A. *Biochem. Pharmacol.* **1988**, *37*,3119-3126.

11. Gores, G.; Kost, L.J.; LaRusso, N.F. *Hepatology* **1986,***6*,511-517.

12. Stewart, B.H.; Lu, R.H.H.; Chan, O.H. Unpublished results