

6. Pharmacokinetic Aspects of Sulfation

Chungsook Kim, Ph.D.

1. Abstract
2. Introduction

Department of Anesthesiology, Harvard Medical School, and Anesthesia Services of Massachusetts General Hospital and Shriners Burns Institute, Boston, MA 02114, U.S.A.

1. Abstract

In intact rats, 400 mg/kg acetaminophen depleted hepatic 3'-phospho-adenosine-5'-phosphosulfate (PAPS) and inorganic sulfate concentration in serum to approximately 40% and 20%, respectively, of the pre-drug value within 20 min, after which concentrations were relatively constant for 6hr and returned to the pre-drug value by 24hr. Brain PAPS, in contrast, remained at the pre-drug value by 2hr, declined to 60% of the pre-drug value at 6hr, and remained at approximately that value for 24hr. These results suggest that hepatic PAPS is rapidly formed from serum inorganic sulfate but brain PAPS is not. Studies in isolated rat hepatocytes show that intracellular PAPS concentration does not rise substantially above the value observed a physiologic inorganic sulfate concentration (ca. 1 mM) as inorganic sulfate concentration is raised to 5 mM. This result suggests that evaluation of serum inorganic sulfate concentration cannot accelerate the formation clearance of sulfoconjugates beyond the rate observed at 1 mM plasma inorganic sulfate. Acetaminophen in concentrations of 0.079 mM and 0.159 mM depleted PAPS concentration in isolated rat hepatocytes by 31% and 22%, respectively, when inorganic sulfate concentration in the medium was maintained at 5 mM. Thus, PAPS can be depleted through the formation of sulfoconjugates even when inorganic sulfate concentration is maintained. The role of PAP (3'-phospho-adenosine-5'-phosphate) as a product inhibitor was found not to be significant as a rate determining factor of drug sulfoconjugation in intact cells by accelerating PAP production with acetaminophen and evaluating the effect (none was observed) on the formation of dehydroepiandrosterone sulfate. The results of these studies suggest that the principal factor limiting the formation of acetaminophen sulfate is the availability of PAPS.

2. Introduction

Acetaminophen is eliminated by parallel pathways of oxidation and direct conjugation with sulfate and glucuronic acid.¹⁾ The clearance of the

drug through each of these processes influences the toxicity of a given dose.^{2,3)} The conjugation routes account for the elimination of 70% to 80% of a dose of acetaminophen and are therefore responsible for the pharmacokinetic characteristics of the drug,²⁾ while one of the two oxidative routes results in the formation of the toxic reactive intermediate, N-acetyl-p-benzoquinone imine.⁴⁾ Both of the direct conjugation routes are capacity limited.⁵⁾ The formation of the sulfate conjugate becomes compromised in the therapeutic concentration range, while that of the glucuronide pathway is apparently not diminished until large, potentially toxic doses are ingested.⁶⁾

The pharmacokinetic characteristics of acetaminophen are unusual in that the concentration of the drug in plasma declines log-linearly at all doses, but the apparent half-life increases as dose increases.⁷⁾ This phenomenon has been observed in humans and rats.⁷⁻⁹⁾ Since the phenomenon occurs in rats at non-toxic doses, it appears to be independent of the toxicity of the drug. The sulfation pathway is of particular interest with respect to the dose-dependent pharmacokinetics of the drug, because of its low capacity relative to the other principal route of elimination.⁷⁾

Depletion of PAPS and inorganic sulfate, an essential PAPS precursor, has been shown to limit the capacity of both rats and humans to form sulfate conjugates.¹⁰⁻¹²⁾ When large doses or constant rate infusion of acetaminophen are administered, inorganic sulfate concentration in plasma is reduced in dose- or infusion-time-dependent manner.⁷⁾ It has been demonstrated that depletion of plasma inorganic sulfate is accompanied by a decline in the formation clearance of sulfoconjugates and hepatic PAPS concentration.^{7,13-15)} The administration of inorganic sulfate or a precursor amino acid restores plasma sulfate and the formation clearance of sulfoconjugates to control values.^{10,11,16,17)}

Although the role of inorganic sulfate in limiting the formation of sulfoconjugates has been the subject of several investigations, other relevant kinetic issues have not been evaluated. In healthy rats and humans, it is very difficult to increase plasma sulfate concentration above the physiologic norm

(ca. 1 mM) because renal clearance of the ion increases with plasma concentration.^{17,18)} In rats with renal failure, plasma inorganic sulfate concentration is substantially elevated, but the formation clearance of the sulfate conjugate is accelerated relative to that observed at normal plasma sulfate only for large doses of drug.¹⁹⁾ The clearance at doses of acetaminophen which do not substantially deplete inorganic sulfate in healthy rats is not altered.^{7,10,17)} The cause of this discrepancy is not known.

In addition, it has not been determined whether PAPS concentration can be depleted through the formation of sulfoconjugates when inorganic sulfate is not limiting. In theory, this should be possible if the formation rate of PAPS does not increase in response to the increased elimination rate caused by administration of an exogenous sulfotransferase substrate. Thus, sulfoconjugate formation may be limited by PAPS availability even when PAPS precursors are in abundant supply, as may be the case with UDP-glucuronic acid depletion during the formation of glucuronide conjugates.

It has been shown in studies with isolated phenolsulfotransferases that PAP is a very potent product inhibitor of this class of enzymes.²⁰⁻²²⁾ The potential role of PAP in limiting the rate of sulfoconjugation in intact cells is not known.

Lastly, PAPS and phenolsulfotransferases are present in several tissues and are of particular importance in the metabolism of neurotransmitters in the central nervous system.^{22,23)} It is not known if a large dose of a substrate of the enzymes will reduce the concentration of PAPS in tissue other than liver, potentially limiting the formation of sulfoconjugates in those tissues.

The purpose of the work discussed in this report was to address these issues by determining; (a) the effect of acetaminophen on the time course of PAPS concentration in brain relative to PAPS concentration in liver and inorganic sulfate concentration in plasma; (b) the ability of acetaminophen to depress intracellular PAPS in the presence of an excess of inorganic sulfate; (c) the response of intracellular PAPS to high extracellular

sulfate concentration; and (d) the influence of PAP produced at high rates of sulfoconjugation as an inhibitor of the reaction.

References

- 1) M. Davis, C.J. Simmons, N.G. Harrison, and R. Williams: Paracetamol overdose in man: relationship between pattern of urinary metabolites and severity of liver damage. *Q.J. Med.*, **45**, 181-191 (1976)
- 2) D.J. Jollow, S.S. Thorgeirsson, W.Z. Potter, M. Hashimoto, and J.R. Mitchell: Acetaminophen-induced hepatic necrosis VI. Metabolic disposition of toxic and non-toxic doses of acetaminophen. *Pharmacol.*, **12**, 251-272 (1974)
- 3) V.F. Price and D.J. Jollow: Induced resistance of diabetic rats to acetaminophen-induced hepatotoxicity. *J. Pharmacol. Exp. Ther.*, **220**(3), 504-513 (1982)
- 4) D.C. Dahlin and S.D. Nelson: Synthesis, decomposition kinetics, and preliminary toxicological studies of pure N-acetyl-p-benzoquinone imine, a proposed toxic metabolite of acetaminophen. *J. Med. Chem.*, **25**(8), 885-886 (1982)
- 5) J.T. Slattery and G. Levy: Acetaminophen kinetics in acutely poisoned patients. *Clin. Pharmacol. Ther.*, **25**(2), 184-195 (1979)
- 6) K.S. Pang, H. Koster, I.C.M. Holsema, E. Scholten, and G.J. Mulder: Aberrant pharmacokinetics of harmol in the perfused rat liver preparation: Sulfate and glucuronide conjugations. *J. Pharmacol. Exp. Ther.*, **219**(1), 134-140 (1981)
- 7) R.E. Galensky and G. Levy: Dose- and time-dependent elimination of acetaminophen in rats: Pharmacokinetic implications of cosubstrate depletion. *J. Pharmacol. Exp. Ther.*, **219**(1), 14-20 (1981)
- 8) J.A.H. Forrest, J.A. Clements, and L.F. Prescott: Clinical pharmacokinetics of paracetamol. *Clin. Pharmacok.*, **7**, 93-107 (1982)
- 9) L.F. Prescott: Paracetamol overdosage: pharmacological considerations and clinical management. *Drugs*, **25**, 290-314 (1982)
- 10) J.H. Lin and G. Levy: Sulfate depletion after acetaminophen administration and replenishment by infusion of sodium sulfate or N-acetylserine in rats. *Biochem. Pharmacol.*, **30**(19), 2723-2725 (1981)
- 11) R.E. Galinsky, J.T. Slattery, and G. Levy: Effect

- of sodium sulfate on acetaminophen elimination by rats. *J. Pharm. Sci.*, **68**(6), 803-805 (1979)
- 12) J.B. Houston and G. Levy: Drug biotransformation interactions in man VI: acetaminophen and ascorbic acid. *J. Pharm. Sci.*, **65**(8), 1218-1221 (1976)
 - 13) G.A. Hazelton, J.J. Hjelle, R.L. Dills, and C.D. Klassen: A radiometric method for the measurement of adenosine 3'-phosphate-5'-phosphosulfate in rat and mouse liver. *Drug Metab. Disp.*, **13**(1), 30-34 (1985)
 - 14) J.J. Hjelle, G.A. Hazelton, and C.D. Klassen: Acetaminophen decreases adenosine 3'-phosphate-5'-phosphosulfate and uridine diphosphoglucuronic acid in rat liver. *Drug Metab. Disp.*, **13**, 35-41 (1985)
 - 15) E.J. Glazenburg, I.M.C. Jedel-Halsema, E. Scholtens, A.J. Baars, and G.J. Mulder: Effects of variation in the dietary supply of cysteine and methionine on liver concentration of glutathione and "active-sulfate" (PAPS) and serum levels of sulfate, cysteine, methionine, and taurine; relation to the metabolism of acetaminophen. *J. Nutr.*, **113**, 1361-1379 (1983)
 - 16) E.J. Glazenburg, I.M.C. Jekel-Halsema, A. Baranczyk-Kuzma, K.R. Krijgheld, and G.J. Mulder: D-cysteine as a selective precursor for inorganic sulfate in the rat *in vivo*. *Biochem. Pharmacol.*, **31**(24), 3997-4000 (1982)
 - 17) J.H. Lin and G. Levy: Renal clearance of inorganic sulfate in rats: effect of acetaminophen-induced depletion of endogenous sulfate. *J. Pharm. Sci.*, **72**(3), 213-217 (1983)
 - 18) K.R. Krijgheld, E. Scholtens, and G.J. Mulder: The dependence of the rate of sulfate conjugation on the plasma concentration of inorganic sulfate in the rat *in vivo*. *Biochem. Pharmacol.*, **31**(24), 3997-4000 (1982)
 - 19) J.H. Lin and G. Levy: Effect of experimental renal failure on sulfate retention and acetaminophen pharmacokinetics in rats. *J. Pharmacol. Exp. Ther.*, **221**(1), 80-84 (1982)
 - 20) R.A. Ryen and J. Carroll: Studies on a 3'-hydroxysteroid sulfotransferase from rat liver. *Biochem. Biophys. Acta*, **429**, 391-401 (1976)
 - 21) R.D. Sekura and W.B. Jakoby: Phenol sulfotransferases. *J. Biol. Chem.*, **254**(13), 5658-5663 (1979)
 - 22) W.B. Jakoby, R.D. Sekura, E.S. Lyon, C.J. Marcus, and J. Wang: Sulfotransferases, in *Enzymatic Basis of Detoxication* (W.B. Jakoby, ed.), vol II. New York: Academic Press, 1980, pp. 199-128
 - 23) E.J.M. Pennings, R. Vrieling, and G.M.J. Van Kempen: Kinetics and mechanism of the rat brain phenol sulfotransferase reaction. *Biochem. J.*, **173**, 299-307 (1978)
 - 24) M.W. Duffel and W.B. Jakoby: On the mechanism of aryl sulfotransferase. *J. Biol. Chem.*, **256**(21), 11123-11127 (1981)
 - 25) C.J. Marcus, R.D. Sekura, and W.B. Jakoby: A hydroxysteroid sulfotransferase from rat liver. *Anal. Biochem.*, **107**, 296-305 (1980)
 - 26) E.S. Lyon and W.B. Jakoby: The identity of alcohol sulfotransferases with hydroxysteroid sulfotransferases. *Arch. Biochem. Biophys.*, **202**(2), 474-481 (1980)
 - 27) D. Acost and E.M.B. Sorenson: Role of calcium in cytotoxic injury of cultured hepatocytes. *Ann. N.Y. Acad. Sci.*, **497**, 78-92 (1983)
 - 28) I. Bartosek, A. Guaitani, and L.L. Miller: *Isolated Liver Perfusion and its Applications*. New York: Raven Press, 1973
 - 29) L.E. Gerschenson, J. Berliner, and M.B. Davidson: The isolation and culture of liver cells. *Meth. Enzymol.*, **32**, 733-740 (1974)
 - 30) K.F. Wong and T. Yeo: Assay of adenosine 3'-phosphate-5'-sulpho-phosphate in hepatic tissues. *Biochem. J.*, **181**, 107-110 (1979)
 - 31) W.H.B. Denner, A.M. Strokes, F.A. Rose, and K.S. Dodgson: Separation and properties of the soluble 3'-phosphoadenosine 5'-phosphosulfate degrading enzymes of bovine liver. *Biochem. Biophys. Acta*, **315**, 394-401 (1973)
 - 32) R. Bailey-Wood, K.S. Dodgson, and F.A. Rose: Purification and properties of two adenosine-5'-phosphosulfate sulphohydrolases from rat liver and their possible role in the degradation of 3'-phosphoadenosine 5'-phosphosulphate. *Biochem. Biophys. Acta*, **315**, 402-411 (1973)
 - 33) A.M. Strokes, W.H.B. Denner, and K.S. Dodgson: Kinetic properties of the soluble adenosine 5'-phosphosulphate sulphohydrolase from bovine liver. *Biochem. Biophys. Acta*, **315**, 402-411 (1973)
 - 34) K.P. Wong: Measurement and kinetic study of the formation of adrenaline sulfate *in vitro*. *Biochem. J.*, **174**, 777-782 (1978)
 - 35) G.J. Mulder and A.H. Hagedoorn: UDP-glucuronyltransferase and phenol sulphotransferase *in vivo* and *in vitro*: conjugation of harmol and har-

- malol. *Biochem. Pharmacol.*, **23**, 2101-2019 (1974)
- 36) T. Slotkin and V. DiStefano: Urinary metabolites of harmine in the rat and their inhibition of monoamine oxidase. *Biochem. Pharmacol.*, **19**, 125-131 (1970)
- 37) J. Jorritsam, J.H.N. Meerman, R.J. Vonk, and G.K. Mulder: Biliary and urinary excretion of drug conjugates: Effect of diuresis and choleresis on excretion of harmol sulfate and harmol glucuronide in the rat. *Xenobiotica*, **9**(4), 247-252 (1979)
- 38) T.J. Mende and P.L. Whitneu: Microdetermination of inorganic sulfate using thin-layer plates. *Anal. Biochem.*, **84**, 570-573 (1978)
- 39) P. Lundquist, J. Martensson, B. Sorbo, and S. Ohman: Turbidity of inorganic sulfate, ester sulfate, and total sulfur in urine. *Clin. Chem.*, **26**(8), 1178-1182 (1980)
- 40) P.I. Adriaenssen and L.F. Prescott: High performance liquid chromatographic estimation of paracetamol metabolites in plasma. *Br. J. Clin. Pharmacol.*, **6**, 87-88 (1978)
- 41) G. Levy and H. Yamada: Drug biotransformation interactions in man III: acetaminophen and salicylamide. *J. Pharm. Sci.*, **60**(2), 215-221 (1971)
- 42) G.J. Mulder and E. Scholtens: The availability of inorganic sulphate in blood for sulphate conjugation of drugs in rat liver *in vivo*: [35S] sulphate incorporation into harmol sulphate. *Biochem. J.*, **172**, 247-251 (1978)
- 43) M.E. Morris and G. Levy: Serum concentration and renal excretion by normal adults of inorganic sulfate after acetaminophen, ascorbic acid, or sodium sulfate. *Clin. Pharmacol. Ther.*, **33**(4), 529-536 (1983)
- 44) L.R. Drewes and D.D. Gilboe: Nutrient transport system in dog brain. *Fed. Proc.*, **36**(2), 166-170 (1977)
- 45) D.D. Dziewiatkoski: Metabolism of sulfate esters, in Symposium: *Sulfur in Nutrition*, O.H. Muth and J.E. Oldfield, eds. Connecticut: AVI Publishing, 1970, pp.97-125