Pharmacokinetics of a Novel N-Methyl-D-aspartate Receptor Antagonist (SM-18400): Identification of an N-Acetylated Metabolite and Pre-clinical Assessment of N-Acetylation Polymorphism

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SUMMARY

(S)-9-chloro-5-[p-aminomethyl-o-(carboxymethoxy)phenylcarbanoylmethyl]-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione hydrochloride trihydrate (SM-18400) was given intravenously to rats and dogs and its pharmacokinetics was investigated. By LC/MS/MS analysis, the major metabolite in the rat serum was identified as N-acetylated SM-18400 (SM-NAc). In rats, AUC ratio of SM-NAc to SM-18400 was approximately 50%. However, 71% of the dose was excreted as unchanged SM-18400 and only 9.8% as SM-NAc in the urine and bile, indicating that the contribution of N-acetylation clearance (CLNAc) to the total clearance (CLtot) is limited to 10-30% in rats. No SM-NAc or other metabolites were detected in the dog serum, urine or bile. The in vitro intrinsic clearance (CLint, ml/min/mg cytosolic protein) of N-acetyltransferase (NAT) activities of dog liver cytosol towards SM-18400 and hepatic N-acetylation clearance (CLNAc, ml/min/kg body weight) estimated by well-stirred model were both only 5% of the respective rat value, well reflecting the relative in vivo CLNAc/CLtot ratios. CLint values for human liver cytosol samples (n=4) and estimated CLNAc were all less than 18% and 7% of the rat, respectively. Based on these results, we concluded that the CLNAc/CLtot of human would be small enough to avoid major inter-individual variance in SM-18400 pharmacokinetics due to N-acetylation polymorphism. In addition, even a human liver cytosol sample lacking polymorphic NAT2 activity as determined by sulfamethazine (SMZ) N-acetylation analysis, proved capable of acetylatign SM-18400, suggesting that NAT2 is not the major enzyme responsible for N-acetylation of SM-18400 in human. This fact would also reduce the risk of N-acetylation polymorphism playing a role in clinical use of this drug.

Abbreviations: SM-18400=(S)-9-chloro-5-[p-aminomethyl-o-(carboxymethoxy)phenylcarbanoylmethyl]-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione hydrochloride trihydrate, NMDA=N-methyl-D-aspartate, CLNAc=N-acetylation clearance, SM-NAc=N-acetylated SM-18400, PABA=p-aminobenzoic acid, SMZ=sulfamethazine, DTT=dithiothreitol, AcCoA=acetyl coenzyme A, [14C]SM-18400 = [quinoxaline-2,3-14C]SM-18400
INTRODUCTION

(S)-9-chloro-5-[p-aminomethyl-o-(carboxymethoxy)phenylcarbamoylmethyl]-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione hydrochloride trihydrate (SM-18400) (Fig. 1) is a novel NMDA receptor antagonist. It has been shown to possess neuroprotective activity in several animal models [1,2] and developed as a drug to prevent neuronal degeneration induced by cerebral ischemia or seizure. In this paper, we describe a basic metabolism and pharmacokinetics study by using rats and dogs, which was performed as part of the pre-clinical evaluation program for this compound. At the commencement of the program, we attempted rapid metabolite identification, and by using LC/MS/MS technique, N-acetylated SM-18400 (SM-NAc, Fig. 1) could thereby be identified.

Human N-acetyltransferase (NAT) exhibits genetic polymorphism [3]. High plasma levels and unexpected adverse effects in slow acetylators have been reported in clinical use of NAT substrates such as isoniazid [4], hydralazine [5,6], procainamide [7] and others [3]. It has been recognized that among human NAT subtypes, NAT2 is a polymorphic enzyme, and pharmacokinetic variance is more frequently observed for the substrates of this isozyme [8,9]. Recently, NAT1 has also been reported to have polymorphic genes [10,11,12,13,14]. Therefore, when the metabolic pathway of a drug includes N-acetylation, we must take the possibility of clinical disadvantages due to NAT polymorphism into consideration. However, metabolic polymorphism could cause clinically significant problems only when the clearance of the concerned metabolic pathway is responsible for the greater part of the CLtot, and additionally when the drug has a narrow therapeutic range.

The investigation on the therapeutic range is underway by pharmaco- and toxicokinetics studies. Thus, the present study was performed with the focus on the following two aims: first, to clarify basic in vivo metabolic and pharmacokinetic profiles in laboratory animals and to determine the contribution of CLNAc to CLtot; second, to measure NAT activity in an in vitro system and assess the extent of metabolic polymorphism in human.

MATERIALS AND METHODS

Chemicals and reagents

SM-18400 and the authentic standard of SM-NAc (Fig. 1) were provided by Sumitomo Pharmaceuticals Co., Ltd. [quinoxaline-2,3-$^{14}$C]SM-18400([14C]SM-18400, Fig. 1) was synthesized at Sumitomo Chemical Co., Ltd. The radiochemical purity was greater than 97% on radio-TLC, and the specific activity was 3.68 GBq/mmol. PABA (sodium salt) and SM2 were purchased from Nacalai Tesque (Kyoto, Japan), OTT from Wako Pure Chemical (Tokyo, Japan) and AcCoA (sodium salt) from Sigma Chemical Company (St. Louis, MO). All other chemicals and solvents were reagent grade or HPLC grade.

Animals

Male Sprague-Dawley rats (seven weeks old, 249-301 g, Charles River Japan, Yokohama, Japan) and male beagle dogs (nine months old, 11.4-12.4 kg, White Eagle Laboratories Inc., Doylestown, PA) were used. Handling of the laboratory animals in this study has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. NIH.

Metabolite identification

SM-18400 was dissolved in the dosing vehicle [bicarbonate buffer (pH 11) containing 10% (w/v) mannitol] and administered to a rat at a dose of 25 mg/2 ml/kg via the tail vein. The blood sample (ca. 10 ml) was collected from the abdominal vein at 30 min after administration, and a serum sample obtained by centrifugation. The serum sample (1 ml) was mixed with 50 mM potassium biphthalate (3 ml) and applied to a Bond Elut SCX column (Varian, Harbor City, CA), which was pre-conditioned with methanol (3 ml), acetonitrile/triethylamine/0.2 M sodium chloride (80:0.1:20, v/v) (3 ml) and 50 mM potassium biphthalate (3 ml) in that order. After washing with 50 mM potassium biphthalate (3 ml), the adsorbed components were eluted with acetonitrile/triethylamine/0.2 M sodium chloride (80:0.1:20, v/v) (3 ml), and the eluate concentrated to dryness in vacuo. The residue was dissolved in an HPLC carrier and injected into an HPLC system equipped with a YMC-Pack MB-ODS column (5μm, 2.1 mm x 15 cm, YMC, Kyoto Japan), eluted with