去甲烏藥鹼於家兔體內之動態與代謝研究

Pharmacokinetic and Metabolic Studies of Higenamine in Rabbits

中文摘要

消旋體之去甲烏藥鹼((+/-)-Higenamine, (+/-)- HG)存在於中藥烏頭，及其他植物中 ，如釋迦葉、細辛及買麻藤具強心作用，而右旋體存在於蓮子芯具子宮平滑肌鬆弛作用； 爲非選擇性直接作用於交感神經乙型感受體。爲研究 HG 之藥物動力學，必需先建立高靈敏 度、精準且可靠之高效液相層析分析方法，利用其在鹼中與氧化鋁吸著、酸中釋出的固相 萃取原理純化生體液檢體，在逆相層析管柱(RP-C18)，使用 Acetonitrile-0.1 %磷酸 (9: 91)爲移動相，以氧化電位爲 0.75 伏特的電化學檢測器來偵測。偵測極限在血漿及尿液分 別爲 2.645 及 10.58 ng/mL。酸洗氧化鋁的固相萃取回收率在血漿及尿液分別爲 77.5 及 84.4 %。同日內及間日內之精密度及準確度試驗，其偏差係數均小於 7 %。

(+/-)-HG 以靜脈注射(10, 20, 30 mg/kg)、口服(50 mg/kg)及靜脈輸注(107 ug/min/kg)給於兔子作藥物動力學探討，結果靜脈注射後，HG 在體內快速消失，排除半衰期約 22 分 鐘，血中濃度經時變化呈二室模式，曲線下面積 (AUC)與劑量關係呈線性，而原型尿中排 泄量爲約 5.5 %；其平均藥物動力學參數分別清除率爲(CLtot) 127.7 mL/min/kg、腎清除 率爲 6.9 mL/min/kg、平均滯留時間爲(MRT) 9.28 min、穩定狀態分布體積(Vdss)爲 1.44 L/kg 及尿中排泄率(fe)爲 5.48 %。靜脈注射所得之藥動學參數供靜脈輸注電腦模擬，結果 靜脈輸注後 20 分鐘可達血中穩定狀態，並可維持 100 分鐘，與電腦模擬一致，輸注後第 100 分鐘測得 HG 與蛋白結合約爲 54.8 %，靜脈輸注與靜脈注射所得之藥物動力學參數間無統計 學差異，停止輸注後之血中濃度經時變化亦證實爲二室模式。

口服吸收快，約 10 分鐘達血中最高濃度。有趣的，血中濃度經時變化及尿液排泄似乎出 現二個不同的族群，其絕對生體可用率(F)以腎清除率校正分別約爲 20.5 及 5.5 %。尿液以 beta-glucuronidase 水解，無論是口服或靜脈注射均發現有很多結合態之代謝物約 20-35% 由尿中排泄。24 小時內(+/-)-HG 經靜脈注射後於膽汁排泄，主要爲結合態代謝物約 6%。另 以兩性之小鼠(MiceICR)以尾靜脈注射作急性毒性試驗，結果測得其 LD50 約爲 50mg/kg，無 性別差異。

口服投與消旋性之 HG，收集尿液以 MCI gel 作粗分後以製備式 HPLC 純化，分別得到八個 結晶型代謝物，以 LC/MS、2DNMR 及衍生甲基化反應判定其結構，至於確定其代謝物之非糖 體姿立體光學異構，則先用酸水解代謝物後，再經對掌鏡相異構管柱 (Chiral column)與 左旋及右旋 HG 之標準品比對確認。八個代謝物結構判定分別爲 S-(-)-HG-6,7-O-beta-D-

diglucuronide(M1)、S-(-)-HG-13-O-beta-D-glucuronide (M2)、R-(+)-HG-7-O-beta-D- glucuronide(M3)、 R-(+)-HG-13-O-beta-D-glucuronide (M4)、 S-(-)-HG-6-O-beta-D- glucuronide (M5)、R-(+)-HG-6-O-beta-D-glucuronide (M6)、 S-(-)-HG-7-O-beta-D-glucuronide (M7)及 S-(-)-HG-7-O-sulfate (M8)。從製備式 HPLC 純化所得八個代謝物的 量，及尿液收集 24 小時後於 HPLC 分析之波峰面積之相對比值，發現(+/-)-HG 於尿中主要代 謝途徑以結合態爲主，且以單取代之尿甘酸化物(glucuronide)爲多，約佔 85%；另取代之 位置及考量其立體光學異構情形，發現右旋體 R(+)-HG 主要代謝位置爲 C-6-OH，而左旋體 S(-)-HG 主要代謝位置爲 C-7-OH，其生成之代謝物 R(+)/S(-)比率在 C-6-及 C-7-OH，分別爲 3 :1 及 1:10。因此，HG 在兔子之代謝，可能有 PhaseII 結合位置及光學異構選擇性差異發生。

英文摘要

Pharmacokinetic and Metabolic Studies of Higenamine in Rabbits

((+/-)-Higenamine, (+/-)- HG), a potent cardiotonic principle, is isolated as a racemate from Aconitum japonicium, and other natural resource such as Annona squamosa, Asiasarum heterotropodes, and Gnetum parvifolium. It acts directly on the adrenergic beta-1 and beta-2 receptors. (+)-HG is isolated from Nelumbo nucifera as smooth muscle relaxant. A method for the pharmacokinetic studies of HG in plasma and urine based on high - performance liquid chromatography (HPLC) with electrochemical detection was developed. The plasma and urine was treated with acidic alumina and then HG was released by acid treatment. HPLC was performed on an ODS column with a mobile phase of acetonitrile-0.1 % phosphoric acid (9:91) and electrochemical detector at an oxidation potential of0.75 V. The lower limits of quantitation for HG in plasma and urine was 2.645 and 10.58 ng/mL, respectively. The recoveries of HG after alumina treatment in plasma and urine were ca. 77.5 and 84.4 %, respectively. Intra- and Inter-day precision and accuracy reported as coefficients of variation in plasma and urine were less than 7 %.

The pharmacokinetics of HG were investigated in rabbits by iv bolus (10, 20,30 mg/kg), peroral route (50 mg/kg) and iv infusion (107 ug/min/kg). Plasma HG concentration declined rapidly in a biexponential pattern, with a terminal half -life

of 22 min.The AUC increased proportionally with increasing doses, whereas the percentage of unchanged HG excreted from urine remained constant although dose was increased. The mean of total plasma clearance, renal clearance, mean residence time, volume of distribution at steady-state, and fraction of urinary excretion were 127.7 mL/min/kg, 6.9 mL/min/kg, 9.28 min, 1.44 L/kg and 5.48 %, respectively. The mean percentage of protein binding of HG in plasma was 54.8 % at steady-state after iv infusion. The results from post-infusion were also confirmed that HG displayed a two-compartment open model in animals. After oral administration,HG was rapidly absorbed to reach peak concentration within 10 min. Interestingly, the plasma concentration-time profiles revealed two distinguishable groups with different Cmax,extent of absorption and urinary excretion. The average absolute bioavailabilities of HG calculated by AUCs and corrected with renal clearance were about 20.5 % and 5.5 % for the two groups, respectively.

Upon hydrolysis of urine samples with beta - glucuronidase, urinary concentrations of HG were greatly enhanced in both groups in spite of the administration routes. Parent drug and its conjugated excreted from urine was about 20 - 40 % of the doses. After iv bolus of HG.HCl at 20 mg/kg to a rabbit , HG was mainly excreted as conjugated metabolites from bile about 6 %. The acute toxicity test was intravenously performed on mice, the LD50 was about 50 mg/kg with no sex difference. Using the column chromatography (MCI gel) and preparatived HPLC (RP-C18) with photodiode array detector, at least 8 distinct urinary metabolites from the 24 h pooled urine sample after oral administration were isolated as crystalline solids. Metabolites and its derivatizations were characterized using a combination of negative-ion LC/MS, and 2DNMR spectroscopy to determine the conjugation position. After acid hydrolysis of metabolites, the configuration of each optical aglycone of HG metabolites was determined by using a beta-cyclodextran chiral column and compared with the optically active HG enantiomers. One metabolites was identified as S-(-)-HG-6,7-O-beta-D- diglucuronide (M1). Six metabolites were HG mono glucuronides, S-(-)-HG-13-O- beta-D-glucuronide (M2),

R-(+)-HG-7-O-beta-D-glucuronide (M3), R-(+)-HG-13-beta -D-glucuronide (M4), S-(-)-HG-6-O-beta-D-glucuronide (M5), R-(+)-HG-6-O-beta-D- glucuronide (M6) and S-(-)-HG-7-O-beta-D-glucuronide (M7). Metabolite 8 was determined as S-(-)-HG-7-O-sulfate (M8). Based on the HPLC profiles, the major formation of conjugation were glucuronidation occurred at the C-6 and C-7 -OH about 40 % and 45 %, respectively, but minor glucuronidation at the C-13-OH (ca 3 %), sulfation and methylation via COMT. Interestingly, there are marked differences in stereoselective glucuronidation between the enantiomers of HG at the catechol moiety. The conjugated ratios of R-(+)/S-(-) isomer of HG at the C-6 and C-7-OH were about 3 and 0.1, respectively. The peak area ratios of HG metabolites indicated that (+/-)-HG was present of regio- and enantioselective metabolism in rabbits.