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应用神经网络方法研究3-甲基芬太尼类似物的定量构-效关系

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目的：应用神经网络这种新型的信息处理系统研究定量构-效关系。 **方法**：应用自编的逆传播神经网络算法，结合偏最小二乘法，研究了25个3-甲基芬太尼类似物的量子化学指数和镇痛活性之间的定量关系。 **结果**：得到了良好的QSAR模型，3-甲基芬太尼类似物的量子化学指数即 N_1 和 O_{16} 上净电荷、C_{10}-C_9-N_5-C_4 二面角、C_7-PhA 中心的距离与镇痛活性之间具有很好的相关性，并根据计算结果，提出了芬太尼类似物与阿片受体的结合模式。 **结论**：神经网络方法研究结果优于单纯偏最小二乘法的结果，且能对化合物活性进行准确的预测。

关键词 神经网络(计算机)；3-甲基芬太尼；镇痛药；结构－活性关系

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jan; 16 (1): 32-35

Assay of metoprolol and α-hydroxymetoprolol in human urine by reversed-phase liquid chromatography with direct-injection[1]

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AIM: To develop an HPLC method with direct injection for the simultaneous determination of metoprolol (M) and α-hydroxymetoprolol (HM) in human urine. **METHODS**: Urine (200 μl) was diluted with eluate and injected into the chromatograph. Samples were seperated on an ODS column by isocratic binary elution and monitored by fluorescence detection. **RESULTS**: No potential interfering peaks were identified. M and HM gave rapid elution and baseline resolution. The linear curves of both analytes ranged between 0.2 and 100 $mg \cdot L^{-1}$. The response sensitivity was approximately 0.1 $mg \cdot L^{-1}$ and the coefficients of variation in the assay were within 8 % for both compounds. A typical application in oxidation phenotyping was presented for one healthy volunteer who received 100 mg of oral metoprolol. **CONCLUSION**: The method can be used for the investigation of genetic polymorphism of metoprolol oxidation in the large populations.

KEY WORDS metoprolol; α-hydroxymetoprolol; high pressure liquid chromatography; urine

[1] Supported by the National Natural Science Foundation of China, № 39200154 and 39270794, and by China Medical Board of New York, USA, Medical Research 92-568 and 82-410.

Received 1993-12-30 Accepted 1994-08-22

Metoprolol (M) is a cardioselective β_1-receptor blocker for treatment of hypertension and angina pectoris[1]. The oxidation of M to α-hydroxymetoprolol (HM) exhibits a debrisoquine-type genetic polymorphism in many ethnic populations[2-4]. M can be chosen instead of debrisoquine as a phenotyping probe by measurement of their metabolic ratio (MR = M/HM) in human urine[5-7]. Monitoring of the urine levels of M and HM is applied in oxidation phenotyping in various racial or ethnic populations. Several published HPLC methods have been available for analyzing the concentrations of M and HM in urine and/or plasma[8-11], but their utility is somewhat hampered by either time-consuming extraction procedures[8-11] or unduly long retention time (15 — 30 min)[7,9,11]. Accordingly, a rapid, simple, sensitive, and reproducible analytic method is required for this purpose. This paper described a direct-injection reversed-phase HPLC method for simultaneous quantitations of M and HM in human urine.

MATERIALS AND METHODS

Chemicals and drugs M tartrate was purchased from Sigma (St Louis, USA). HM *p*-hydroxybenzoate (H 119/60) was supplied by Astra Hassle AB (Mondal, Sweden). M tartrate (Betaloc 50 mg per tablet, code № 52 — 100B) was obtained from Sino-Swed Pharmaceutical Co. All other reagents were of AR grade unless otherwise indicated. Water was glass redistilled.

Standard solutions Stock solutions (250 mg · L^{-1}, each base equivalent) were prepared in HPLC-grade methanol and were kept at −4 C.

Sample preparation To 200 μl of unknown urine in an 1.5-ml conical polyethylene tube was added 200 μl of eluate. This mixture was vortexed for 10 s and then centrifuged at 1 000 × *g* for 10 min. The clear supernatant (50 μl) was injected into the chromatograph by autosampler.

Chromatography The chromatograph consisted of LC-6A liquid chromatograph, SCL-6A system controller, CTO-6A column oven, SIL-6A auto injector, RF-530 fluorescence HPLC monitor, FDD-1A floppy disc driver, C-R3A 6-slot expansion case and chromatopac from Shimadzu, Japan. The analytical column (250 mm · 4.6 mm ID) was filled with Spherisorb C_{18} material, at a 5-μm particle size (Dalian Institute of Chemical Physics, Chinese Academy of Sciences). The mobile phase was a 50:50 isocratic binary eluate (final pH 3.4). Solution A was methanol. Solution B was a mixture of 1000 ml of water, 1.8 ml of glacial acetic acid, and 150 μl of triethylamine. The flow-rate was 1.2 ml · min^{-1} and the column temperature was 40 C. The fluorescence was monitored at 299 nm with excitation at 277 nm.

Validation study Urine standards were prepared by spiking an aliquot of each standard solution into blank urine from a healthy drug-free volunteer as described in sample preparation above. Seven calibration samples containing mixtures of M and HM were made at levels between 0.2 and 100.0 mg · L^{-1} for standard curves. Three spiked urine samples at 2.5, 10.0, and 25.0 mg · L^{-1} of both compounds were prepared to test the accuracy and precision of the assay. The minimum detectable amount was defined as a signal-to-noise ratio of 4:1. The potential interference from endogenous sources was investigated with blank human urine.

Amounts of M and HM in the unknown samples were quantitated by response factors (mV per mg · L^{-1}) of the calibration samples at 2 different levels within the linear range.

Applications of the method Urine was voided immediately before *po* M 100 mg and was collected through the next 8 h. Aliquots (10—20 ml) of the collection were stored at −30 C.

RESULTS AND DISCUSSION

Chromatography The retention times of M and HM were 4.1 and 7.1 min, respectively (Fig 1). M and HM gave rapidly eluting, fully resolved and sharp symmetrical peaks, the total elution time per run for both analytes was within 10 min. The use of triethylamine as a modifier of eluent[8] gave excellent resolution and rapid elution, and also enhanced the resolution performance of weak bases like M

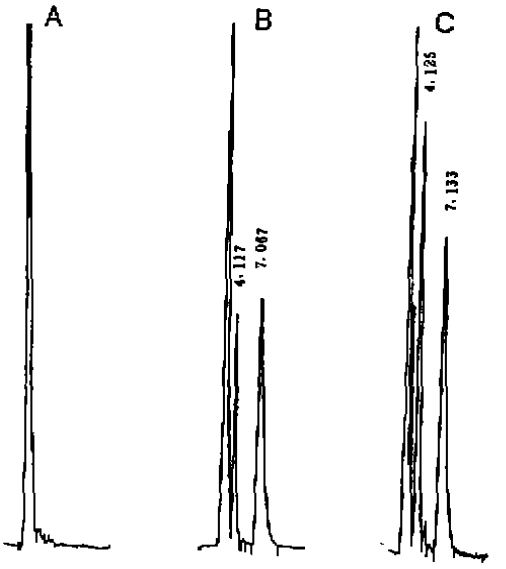

Fig 1. Chromatograms of predosing blank urine (A), spiked urine (B), and postdosing 0—8 h urine from a subject *po* metoprolol 100 mg (C). Retention time: HM 4.1 min; M 7.1 min.

(pK_a 9.70) on reversed-phase chromatography.

Calibration curve Standard curves were made by plotting the peak height (mV) *vs* the spiked concentration ($mg \cdot L^{-1}$). The M curve was linear over the range of 0.25 — 100 $mg \cdot L^{-1}$ with correlation coefficient (r) of 0.998, and the HM curve was also linear over the range of 0.20—100 $mg \cdot L^{-1}$ with an r of 0.997. No internal standard was employed due to a nearly complete extraction recovery of direct-injection samples and relative difficulty of obtaining a required adequate internal standard.

Validation test The reproducibility of the procedures was evaluated by analyzing replicate samples. Coefficients of variation (CV) within 8 % were observed in both intra- and inter-days (Tab 1). Extraction recovery of direct-inject samples was theoretically almost complete (Tab 2). This is due to the fact that there is no loss of compounds if the samples is chromatographed by direct injection. The lower limit of detection for both analytes was roughly 0.1 $mg \cdot L^{-1}$. No endogenous interfering peaks were found in the blank urine samples. Since the subjects used for oxidation phenotyping should be drug-free volunteers, the possible interference from external sources can also be excluded.

Tab 1. Coefficient of variation (%) in determination of metoprolol (M) and α-hydroxymetoprolol (HM) in spiked urine.

Added/ $mg \cdot L^{-1}$	Intra-day ($n=5$) M	Intra-day ($n=5$) HM	Inter-day ($n=10$) M	Inter-day ($n=10$) HM
2.5	5.38	6.32	6.06	6.93
10.0	4.14	5.94	5.46	7.72
25.0	4.28	2.23	5.06	4.85

Tab 2. Recovery of metoprolol (M) and α-hydroxymetoprolol (HM) from spiked urine. $n=10$, $\bar{x} \pm s$.

Compound	Added/ $mg \cdot L^{-1}$	Found/ $mg \cdot L^{-1}$	Recovery/%
M	2.5	2.6±0.2	102.2±6.2
	10.0	9.9±0.5	98.9±5.4
	25.0	24.4±1.2	97.5±4.9
HM	2.5	2.6±0.2	101.9±7.1
	10.0	10.2±0.8	101.8±7.9
	25.0	25.2±1.2	100.9±4.9

Applications of the method Formation of HM is impaired in poor metabolizers (PM) of M because of its defective oxidation[2,3], whereas normal subjects of the M oxidative ability are defined as extensive metabolizers (EM). In this study, M and HM concentrations in postdosing 8 h urine from one subject were 6.87 and 25.82 $mg \cdot L^{-1}$, respectively. The subject was classified as EM with a lg MR being — 0.58 according to criteria (PM: lg MR > 1.09) established for Chinese population[5,7]. If the HM level in urine is < 0.2 $mg \cdot L^{-1}$, the dilution times of sample preparation should be reduced[7].

The present devised procedure was proven much simpler (direct injection) and more rapid ($t_r < 7$ min) than those[7,9-11] reported previously (t_r 15 – 30 min). It makes the use of technology readily available in most laboratories, and is well suited for pharmacogenetic studies.

ACKNOWLEDGMENTS Dr Roger SIMONSSON of Astra Hässle AB, Sweden for supplying HM as HPLC standard chemical, and Dr ZHOU Ping from Analytical and Testing Center of Hu-nan Medical University for fluorescence scanning.

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反相液相色谱直接进样分析人尿中美多洛尔和α—羟基美多洛尔

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目的： 建立一个 HPLC 直接进样法以同时检测人尿中美多洛尔（M）和 α-羟基美多洛尔（HM）浓度. **方法**： 量取小体积尿样（200 μl），用流动相稀释直接进样；M 和 HM 在 ODS 柱上以等强度洗脱方式分离，并用荧光检测法监测. **结果**： 未发现潜在干扰峰，M 和 HM 获得快速洗脱与基线分离，其线性范围为0.2–100 mg·L^{-1}，检测灵敏度约为0.1 mg·L^{-1}，其变异系数小于8 %. 应用该法对一口服美多洛尔100 mg 的健康志愿者进行了氧化分型. **结论**： 本法因快速、简便和灵敏，可用于大样本人群 M 氧化能力的分型研究.

关键词 美多洛尔；α—羟基美多洛尔；高压液相色谱法；尿