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P03 DIAGNOSIS OF CIRRHOSIS BY GC/MS

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Introduction

Caffeine test consists in caffeine oral intake followed by measurements of blood, saliva, labelled CO_2 in the exhalation air, urine caffeine or metabolites[1,2]. The pharmacokinetic parameters of caffeine, clearance and half-life time, were usually studied by HPLC and immunoassay methods and by GC/MS[3]. Clearance of caffeine is a quantitative test of hepatic function, because caffeine is metabolized by the hepatic P-450 cytochrome oxidase system. Caffeine, 1,3,7-trimethylxanthine, has been introduced as a compound for measuring the metabolic capacity of the liver, being well tolerated when administrated orally.

The aim of the present investigation was to validate a rapid GC/MS method for plasma caffeine level determination for the characterization of some pharmacokinetic parameters in children. The application of the method on hepatitis and cirrhosis is tested.

"Comchim, Bucuresti" in Culea2007

Experimental

Chemicals and Reagents

Caffeine as a sterile caffeine sodium benzoate solution in water for injection use containing 125 mg of caffeine and 125 mg of sodium benzoate per 1 ml ampoule was obtained from pharmacy. All other reagents were from Merck (Germany). ^{15}N-theophylline, 74,2 atom % ^{15}N, labeled at the nitrogen in the position 7, synthesized in the National Institute for Research and Development for Isotopic and Molecular Technology Cluj-Napoca, was used as internal standard

Equipment

A Hewlett Packard (Palo Alto,CA, USA) 5989B mass spectrometer coupled to a 5890 gas chromatograph were used in the conditions: EI mode, electron energy 70 eV, electron emission 300 µA and ion source temperature 200 °C, selected ion monitoring (SIM) mode. The GC/MS interface line was maintained to 280 °C, and quadrupol analyser at 100 °C. The gas chromatograph-mass spectrometer (GC/MS) assay used a HP-5MS fused silica capillary column, 30m × 0.25 mm, 0.25 µm film-thickness, programmed from 200 °C to 250 °C at a rate of 10 °C min^{-1}, the flow rate 1 ml min–1, with helium 5.5 as carrier gas. Injector temperature was 200 °C.

Extraction Procedure "1 ml" in Culea2007

0.5 ml of plasma containing caffeine was placed into a 5 ml screw-cap vial and 5 µl of internal standard ^{15}N-theophylline, 1 ml of the extraction solvent, chloroform: isopropanol 20 : 1 (v/v) and 0.2 g NaCl were added. After mechanical mixing for 1 min, the sample was centrifuged for 3 min. 3 µl of the organic layer (lower layer) were injected into the GC.

"2 ml" in Culea2007 "10 l" in Culea2007 "5 g" in Culea2007

Method validation

The method was validated in the range 0–20 µg ml^{-1} caffeine. Known amounts of caffeine 3, 5, 10, 15, 20 µg ml^{-1} and 10 µg of ^{15}N-theophylline were taken through above procedure. The regression curve, plotted as peak-area ratio of m/z 194 to m/z 181 versus caffeine concentration, gave the following linearity parameters: slope 0.5082, intercept –0.0528, r = 0.98.

Table I
Precision and accuracy of the method

Concentration added [µg ml^{-1}]	n	Concentration measured [µg ml^{-1}]	RSD [%]	Accuracy [%]
3	5	3.1	2.96	3.36
5	7	5.5	5.06	10.0

Precision gave R.S.D values lower than 5% for 5 µg ml^{-1} (n = 7) and lower than 3 % for 3 µg ml^{-1} (n = 5). Accuracy showed values lower than 10 % (Table I). Each value was obtained as an average between two measurements of the same sample. The limit of detection was 0.1 µg ml^{-1} caffeine in blood sample, signal to noise ratio 4 : 1.

"31" in Culea2007 "4" in Culea2007

Population

Caffeine concentration measurements were performed in 32 hospitalized children suffering of hepatic dysfunctions and controls. Three different groups were studied: A, formed by 19 children with hepatitis aged 3–15 years old, B, consisting from 5 children with cirrhosis, aged between 5–12 years old, and C, 8 children as control aged between 5–15 years old. The main dose was 4 mg kg^{-1}, p.o., for all groups. Blood samples were taken, at 0, 30 min, 1, 3, 6, 9 and 12 h. Blood samples were drawn into heparinized plastic tubes and immediately centrifuged. Plasma was stored at –20 °C. Written informed consents were obtained from each subject parent prior to this study.

Calculation

Regression curves obtained by the GC/MS method in the SIM mode were used for pharmacokinetic parameters study. Caffeine elimination constant was calculated as follows:

$$k_{el} = (\ln C_1 - \ln C_2)/\Delta t, \quad (1)$$

where C_1 = higher caffeine blood concentration, C_2 = lower caffeine blood concentration and Δt = the time elapsed between venous blood samples

Two points caffeine clearance was calculated as $Cl = k_{el} \cdot V_d$ and caffeine half-life as $t_{1/2} = \ln 2/k_{el}$, using a constant volume of distribution (V_d) of 0.6 liters per kg body weight.

Clearance values calculated as dose/area under curve (AUC) were compared with the two-points values.

"four" in Culea2007

Results

Caffeine clearance, measured in patients with cirrhosis and chronic hepatitis, was reduced and half live time was increased in children with liver disease as compared with control. The decreased metabolism observed in patients with various forms of liver disease was correlated to the disease status. Plasma concentrations of caffeine were measured in 19 patients with chronic hepatitis and 5 patients with cirrhosis and in 8 healthy subjects after caffeine (4 mg kg^{-1} p. o.) loading. The correlations of total body clearance between two-point study (sampling times 1 h and 9 h) and seven-point study (sampling times 0, 0.5, 1, 3, 6, 9, 12 h) were highly significantly, r = 0.94, p less than 0.001. These findings suggest that caffeine pharmacokinetic parameters can be estimated using two-point blood sampling procedure and GC/MS determination, following a single load. The elimination half-life ($t_{1/2}$) of caffeine was significantly longer in cirrhotic patients than in the other two groups and clearance was substantially reduced in these patients. The higher concentrations of caffeine observed in the first hour after caffeine loading in hepatitis (Fig. 1.) compared with controls could be a possible test for hepatitis when very precise and accurate methods as isotopic dilution GC/MS are used. Significant changes (Student's paired t-test $p < 0.01$) were observed in caffeine metabolism in children with decompensate cirrhosis.

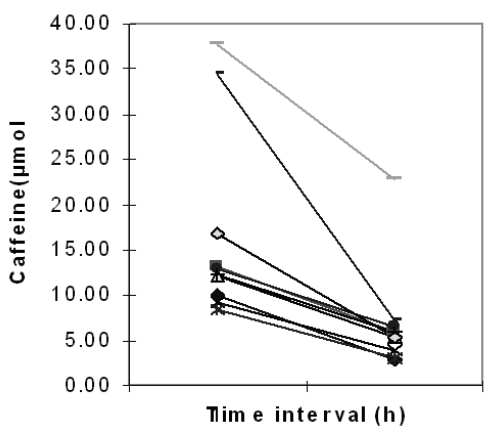

Fig. 1. **Caffeine plasma concentrations at time interval (1 h and 9 h) in hepatitis (n = 19)**

The clearance values of 0.55 ± 0.41 ml min^{-1} kg^{-1} and half-life times of 19.11 ± 14.9 h are changed because of the reduction in "functioning hepatocyte mass". The control values for clearance and half-life time were of 1.36 ± 0.23 ml min^{-1} kg^{-1} and $t_{1/2} = 5.23 \pm 0.85$ h (n = 8). Patients with noncirrhotic liver disease showed intermediate values(Cl = 1.19 ± 0.45 ml min^{-1} $kg^{-1}$and$t_{1/2} = 6.62 \pm 2.37$ h) but higher values of caffeine plasma concentrations especially in the first hour after dose.

Conclusions

The method is simple, precise and rapid, useful in the analysis of xanthines. Isotopic labeled internal standard used avoids metabolites overlapping. The elimination half-life ($t_{1/2}$) of caffeine was significantly longer in cirrhotic patients and clearance was substantially reduced than in control. Caffeine pharmacokinetic parameters can be estimated using two-point blood sampling procedure by GC/MS determination, following a single load. The higher concentrations of caffeine observed in the first hour in hepatitis compared with controls could be a possible test for hepatitis.

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