DETERMINATION OF THIOPROLINE IN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The determination of thioproline in plasma of cancer patients, using high performance liquid chromatography (HPLC) is reported. As column support, a silica-bonded cation exchanger was used. Detection was performed at 205 nm. The detection limit of the method was 5×10^{-6} M and the linear dynamic range was over 500. No sample clean-up procedure was necessary other than deproteinization of the plasma. The method was applied to the measurement of plasma drug levels in 3 patients, part of a clinical trial testing the effectiveness of thioproline as an anti-cancer agent.

INTRODUCTION

Thioprolin (thiazolidine-4-carboxylic acid, Norgamen, NSC 25855) was recently introduced as a new anti-cancer agent [1] and is currently being investigated in clinical trials [2]. Different dosage schedules have been proposed. However, until recently, plasma concentrations of thioproline in treated patients have not been available. Unlike other antineoplastic agents, the drug is administered in a multiple dose schedule for months [5]. Data on plasma drug concentration may be necessary in assessing toxicity or antitumor effect for optimization of treatment schedule.

MATERIALS AND METHODS

Chemicals

Thioprolin was synthesized by Dr. Gosálvez in the Clinica Puerta de

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Hierro (Madrid, Spain) and was lyophilized and sterilized by Davus S.A. (Madrid, Spain). Acetonitrile (Uvasol quality) was obtained from Merck (Darmstadt, F.R.G.). Deionized water was used in the preparation of the eluent. All other chemicals used were of analytical grade.

HPLC apparatus

The chromatographic system consisted of a high pressure pump (Orlita, type DMP AE 10.4), a flow-through pressure gauge, a high pressure injection valve (Valco, type CV-6-UHPa-N60) and a variable wavelength detector (Perkin Elmer, type LC-75). Separation was carried out in a microparticulate strong cation exchange column (Partisil SCX, Whatman) with column dimensions: length 25 cm, i.d. 4.6 mm, and particle diameter of 10 μm . Column temperature was ambient.

Blood samples

Blood samples were obtained from patients with head and neck cancer who were participating in a clinical trial of the Early Clinical Trials Group of the European Organization for Research on the Treatment of Cancer. Thioprolone was given intramuscularly at a daily dose of 40 mg/kg, divided into 4 equal doses. This schedule was maintained for a minimum of 6 weeks. No other anti-cancer agents were administered during thioprolone treatment. Heparinized blood (4 ml) was centrifuged at $2000 \times g$ for 5 min. Plasma (0.8 ml) was deproteinized by addition of 2 N perchloric acid (0.2 ml). Precipitated protein was removed by centrifugation and 50 μl of the supernatant was injected onto the column.

RESULTS AND DISCUSSION

The UV spectrum of thioprolone showed a maximum at a wavelength of 205 nm with a molar extinction coefficient of $280 \text{ AU} \cdot \text{mol}^{-1} \cdot \text{l} \cdot \text{cm}^{-1}$. Absorption at wavelengths above 230 nm was negligible. The use of low wavelength detection puts serious limitations on the choice of the mobile phase constituents. Thioprolone was separated from UV-absorbing plasma components by cation exchange chromatography.

The mobile phase consisted of phosphoric acid (10^{-2} M , pH 2.2)/acetonitrile, 9 : 1 (v/v). The eluent flow rate was 2.7 ml/min at a pressure of 8.6 MPa. The capacity factor for thioprolone was 3.5. Figure 1 shows a chromatographic run of 50 μl of deproteinized plasma, spiked with thioprolone (T). A new sample could be injected every 20 min. The detection limit was $5 \times 10^{-6} \text{ M}$ and the recovery of thioprolone from plasma was 87.7%. Peak height versus concentration was linear up to at least 10^{-3} M , indicating a linear dynamic range of more than 500. For a plasma concentration of 10^{-5} M the standard deviation in peak height was 3.2% ($n = 6$), and 1.6% ($n = 6$) for 10^{-3} M thioprolone in plasma. The addition of acetonitrile to the mobile phase reduced 'tailing' of the peaks. Plasma samples were stable at

Fig. 1. HPLC of 50 μ l deproteinized plasma, spiked with thioproline (5.5×10^{-5} M). The dotted line represents the base line absorption for an unspiked sample.

Fig. 2. Plasma concentrations of thioproline after i.m. injection.

ambient temperature for at least 5 h. The limit of detection of thioprolone was dependent more on interference by the surrounding peaks than on the noise of the detector. An injection volume of more than 50 μ l of deproteinized plasma was avoided because of too much interference of plasma peaks. In an attempt to use anion exchange chromatography, thioprolone could be retained on an Aminex type anion exchange resin (Biorad, type A28). However, due to a low flow rate and an analysis time of about 1 h, the system described above was preferred.

Clinical application

Figure 2 shows the plasma concentration of thioprolone after the 16th i.m. injection of 710 mg in a patient. Figure 3 gives the plasma drug concentrations at various stages in the course of treatment of 2 patients. I, II and III correspond to serum lactate dehydrogenase levels presented in Table 1. By the use of linear pharmacokinetic models [4] for patient Sm, the plasma concentration of thioprolone as a function of time could roughly be described by:

$$C_p = 3 \cdot 10^{-4} \left(\frac{1 - e^{-1.2n\tau}}{1 - e^{-1.2 \cdot \tau}} \right) e^{-1.2t} + 4 \cdot 10^{-6} \left(\frac{1 - e^{-0.04n\tau}}{1 - e^{-0.04 \cdot \tau}} \right) e^{-0.04t} - 3.04 \cdot 10^{-4} \left(\frac{1 - e^{-3n\tau}}{1 - e^{-3 \cdot \tau}} \right) e^{-3t} \quad (1)$$

where: C_p = plasma concentration;
 n = number of foregoing injections;
 t = time after injection in hours;
 τ = dose interval in hours

and the coefficients of the exponential terms refer to intercepts at the ordinate.

Generally, after multiple doses the plasma concentration disappearance curves show a decreasing slope and an increasing absolute plasma concentration of drug, with increasing number of doses. Assuming a higher excretion rate with increasing plasma concentration, after a certain number of doses the dose equals the excretion during the dose interval and the plasma concentration profile does not change any more. The effect of increasing number of injections on plasma concentrations of patient Sm, simulated by Eqn. (1) is presented in Fig. 4. A considerable change in either one of the intercepts or slopes of the curves makes the data fit worse. This figure shows that when the number of injections (n) increases, the change of the shape of the curve decreases. After 10–100 injections, a change will be invisibly small. For patient Sm, the change between curve

Fig. 3. Plasma concentrations of thiopropine during the course of treatment in 2 patients. For patient Sm the thiopropine concentration in cerebrospinal fluid is indicated (measured after 170th dose).

TABLE 1
SERUM LIVER ENZYME LEVELS IN PATIENTS Sm AND Su

	Patient Sm (I.U./l)	Patient Su (I.U./l)
Alkaline phosphatase	80	110
Glutamic-oxalacetic transaminase	8	20
Glutamic-pyruvate transaminase	9	15
γ -Glutamyltransferase	10	100
Lactate dehydrogenase	120	110 (I) 185 (II) 285 (III)

I, II, III refer to curves in Fig. 3.

I and II and the similarity of curves II and III is consistent with Eqn. 1. However, for patient Su, the difference between curves II and III is more than attributable to accumulation for multiple doses, calculated similarly to Eqn. 1. Patient Su was known to have liver dysfunction during the last months before thioproline therapy started, as indicated by high serum liver enzyme levels (Table 1). The enzyme levels remained the same after starting

Fig. 4. Simulation of the plasma concentrations of patient Sm, described by Eqn. (1), after the 1st, 2nd, 5th, 10th and 100th dose.

thioproline treatment, except lactate dehydrogenase, which was highest at the time of the 170th dose (Table 1). The enzyme levels of patient Sm were normal 3 days before thioproline treatment (Table 1). It has previously been suggested that thioproline is metabolized in rat liver [3]. In all 3 subjects, the occurrence of drowsiness increased during the therapy. The drug level in the cerebrospinal fluid was 3×10^{-5} M at 1.25 h after the 170th dose, about half of the plasma concentration (see Fig. 3). Patient Su had severe mental disturbances, which can be explained from the higher plasma concentrations and possibly high concentrations in the cerebrospinal fluid. The sleep-inducing effect of thioproline in all 3 patients examined, warrants studies of spinal fluid drug levels.

Conclusions

To date, plasma concentrations of thioproline have been determined by HPLC in 3 treated patients. The method presented here is useful for measurement of thioproline in plasma of patients receiving the drug against malignancies. Although the aim of this study was not the estimation of pharmacokinetic parameters, a rough pharmacokinetic calculation showed that a change in the shape of the plasma concentration versus time curve was not expected after 10–100 injections. This was confirmed in 1 patient with normal liver function. The importance of measurement of thioproline levels during liver dysfunction, is indicated in 1 patient, showing a change in the plasma concentration curve after 100 injections.

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REFERENCES

- 1 Brugarolas, A. and Gosalvez, M. (1980) Treatment of cancer by an inducer of reverse transformation. *Lancet*, 12, 68–70.
- 2 Brugarolas, A. and Alberto, P. (1980) Norgamen (NSC 25855) initial clinical studies in epidermoid carcinoma of the head and neck. *Proc. Am. Assoc. Cancer Res.*, 21, 155.
- 3 Geelen, G. and Lier, A. (1972) Sur le métabolisme d'un médicament hépatoprotecteur: l'acide thiozolidine-4-carboxylique. *Gazz. Med. Ital.*, 1311, 256–264.
- 4 Gibaldi, M. and Perrier, D. (1975) *Pharmacokinetics*, Vol. 1. Marcel Dekker Inc., New York.
- 5 Protocol No. 16801. Early clinical trials cooperative group. European Organization for Research on the Treatment of Cancer. Study chairman, P. Alberto.