DETERMINATION OF THIOPROLINE IN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

JAN LANKELMA*, PAUL G. M. PENDERS, ALBERT LEYVA and HERBERT M. PINEDO

Section of Experimental Chemotherapy, Antoni van Leeuwenhoekhuis, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam (The Netherlands)

(Received 23 September 1980)
(Revised version received 19 November 1980)
(Accepted 20 November 1980)

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 $5 \times 10^{-6}$ 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

Thioproline (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

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

*To whom correspondence should be addressed.
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. Thioproline 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 thioproline treatment. Heparinized blood (4 ml) was centrifuged at 2000 × 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 thioproline showed a maximum at a wavelength of 205 nm with a molar extinction coefficient of 280 AU · mol⁻¹ · 1 · 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. Thioproline 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 thioproline was 3.5. Figure 1 shows a chromatographic run of 50 µl of deproteinized plasma, spiked with thioproline (T). A new sample could be injected every 20 min. The detection limit was 5 × 10⁻⁶ M and the recovery of thioproline 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 thioproline 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 μl 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 thioproline was dependent more on interference by the surrounding peaks than on the noise of the detector. An injection volume of more than 50 µl of deproteinized plasma was avoided because of too much interference of plasma peaks. In an attempt to use anion exchange chromatography, thioproline 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 thioproline 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 thioproline as a function of time could roughly be described by:

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

(1)

where: \(C_P\) = plasma concentration;
\(n\) = number of foregoing injections;
\(t\) = time after injection in hours;
\(\tau\) = 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 thioproline during the course of treatment in 2 patients. For patient Sm the thioproline concentration in cerebrospinal fluid is indicated (measured after 170th dose).
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 thiopropionle therapy started, as indicated by high serum liver enzyme levels (Table 1). The enzyme levels remained the same after starting

![Graph of plasma concentrations](image)
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 $3 \times 10^{-5}$ 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 concen-
trations 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 measure-
ment of thioproline in plasma of patients receiving the drug against malign-
nancies. 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.

ACKNOWLEDGEMENTS

The authors are grateful to Drs. J.B. Vermorken and W.W. ten Bokkel
Huinink for collecting the patient samples.

REFERENCES

transformation. Lancet, 12, 68–70.
2 Brugarolas, A. and Alberto, P. (1980) Norgamen (NSC 25855) initial clinical studies in
3 Geelen, G. and Lier, A. (1972) Sur le métabolism d'un médicament hépatoprotecteur:
New York.
5 Protocol No. 16801. Early clinical trials cooperative group. European Organization for