Journal of Chromatography, 222 (1981) 156—159
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 718

Note

Rapid method for the high-performance liquid chromatographic determination of bredinin in human serum

KANJI TAKADA* , HIROKO NAKAE and SHOZO ASADA

Kobe Women's College of Pharmacy, Higashinada-ku, Kobe 658 (Japan)

YASUJI ICHIKAWA and TAKANOBU FUKUNISHI

Renal Transplantation Center, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya-city, Hyogo-ken 662 (Japan)

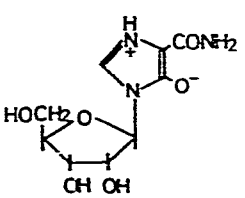
and

TAKAO SONODA

Department of Urology, Osaka University Medical School, Fukushima-ku, Osaka 553 (Japan)

(Received June 26th, 1980)

Bredinin® (4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate) has been isolated from the culture filtrate of *Eupenicillium brefeldianum* M-2166 [1] and has a potent immunosuppressive activity [2]. However, adequate



information on dosages of bredinin in patients undergoing renal transplantation is now required. The best way to overcome this problem is to monitor the drug in the serum of patients. However, until now no specific method for

0378-4347/81/0000—0000/$02.50

the determination of bredinin has been described in the literature. The animal study concerning its absorption, distribution, metabolism and excretion using the ^{14}C-labelled compound shows that bredinin is excreted mainly unchanged in the urine (81% of the unchanged radioactivity was found in the 24-h rat urine) [3].

As bedinin has a characteristic UV absorption spectrum (Fig. 1), a high-performance liquid chromatographic (HPLC) procedure for the determination of

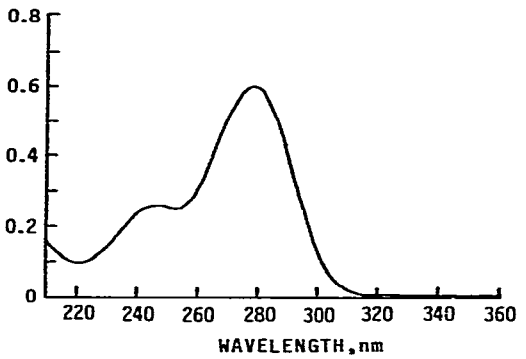


Fig. 1. The UV absorption spectrum of bredinin at a concentration of 10 μg/ml in 30% (v/v) 0.1 *M* imidazole · HCl buffer (pH 7.0) in acetonitrile.

bredinin to study its absorption and pharmacokinetics in serum has been developed. Proteins are removed by precipitation with perchloric acid. The supernatant is neutralized by potassium carbonate. Excess perchloric acid is thus precipitated as potassium perchlorate.

PROCEDURE

A 0.5-ml serum sample was pipetted into a tube containing 50 μl of 70% perchloric acid. After mixing for 30 sec on a Vortex mixer, the tube was centrifuged to precipitate the denatured proteins. This was followed by the addition of 100 μl of a saturated potassium carbonate solution and further mixing. The potassium perchlorate was precipitated by centrifuging at 8000 *g* for 2 min using an Eppendorf centrifuge Model 3200. Suitable volumes, usually 25 μl, of the clear supernatant were injected on to the column.

Analyses were performed using a constant-volume high-pressure liquid chromatograph (Hitachi, Tokyo, Japan) containing a Model 635A pump, a sample injection valve for high pressure and Model 635M UV detector. The column (25 cm × 4 mm I.D., stainless steel) was packed with nominal 10-μm ODS-silica gel with NH_2 groups (LiChrosorb NH_2, manufactured by E. Merck, Darmstadt, G.F.R.) using a balanced density slurry packing procedure similar to that described by Majors [4]. The mobile phase, consisting of 30% (v/v) 0.1 *M* imidazole · HCl buffer (pH 7.0) in acetonitrile, was prepared fresh daily. A flow-rate of 1 ml/min (20 kg/cm^2) was used. Absorbance was monitored at 280 nm. The detector was operated at a sensitivity of 0.01 a.u.f.s. Peak heights were used for quantitation.

RESULTS AND DISCUSSION

Chromatograms of serum samples (Fig. 2) demonstrate that the bredinin peak is sharp and well resolved with no interference from endogenous compounds. The retention time of bredinin is 8 min and a sample could be injected every 18 min. Decreasing this time by increasing the acetonitrile concentration in the eluent causes the serum and bredinin peaks to merge. The standard curve of bredinin added to serum was linear over the range 0.25—10 μg/ml and passed through the origin. The correlation coefficient was $r = 0.968$, and the standard deviation was 3.2% ($n = 6$ at 2.5 μg/ml). The lower detection limit was 0.25 μg/ml of serum. The standard curves were reproducible even though no internal standard was used in this assay. This can probably be attributed to the stability of the column and the simplicity of the procedures.

This HPLC method is easy to perform, involves no extraction or derivatization, and can be successfully performed with 25 μl of serum. The standards were prepared in serum, so the recovery was 100%.

Using this assay, an absorption and disposition kinetics study of bredinin is now in progress with patients undergoing renal transplantation.

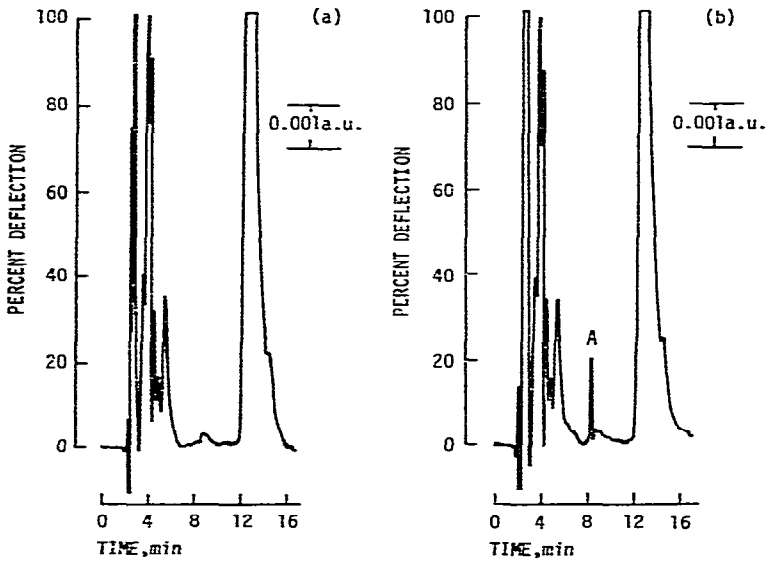


Fig. 2. (a) Chromatogram of a blank human serum. (b) Representative HPLC chromatogram of bredinin in human serum (1 μg/ml). Peak A = bredinin.

ACKNOWLEDGEMENT

We are much indebted to Toyo Jozo Co. Ltd., Ohito, Shizuoka, Japan, for providing us with bredinin.

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