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Short communication

Analysis of traditional Chinese anticancer drugs by capillary electrophoresis[1]

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Abstract

The results reported in this communication demonstrate that capillary zone electrophoresis (CZE) can be used easily for the quantitative determination of the potential anticancer drugs berberine and isoguanosine in the extract of the traditional Chinese medicinal herb. Isoguanosine and berberine can be monitored selectively and sensitively at 254 nm within 14 min in the plant extract using a 100-m*M* sodium citrate running buffer (adjusted to pH 2.7; applied voltage 12 kV). The concentration range of 0.1–50 μg ml^{-1} proved to be sufficient for exact quantification and the peak profile showed good reproducibility [relative standard deviations ($n=32$): for the migration time 0.22% (isoguanosine) and 1.32% (berberine); for the peak area 2.8% (isoguanosine) and 3.2% (berberine)]. The measured concentrations in the crude extract were 1.3 μg ml^{-1} for isoguanosine and 8.7 μg ml^{-1} for berberine. In addition to the better separation performance, CZE shows several other remarkable advantages over high-performance liquid chromatography (HPLC), such as rapidity of analysis, small sample volume, no requirement of organic solvent in the running buffer and low cost of reagents. © 1998 Elsevier Science B.V.

Keywords: Isoguanosine; Berberine; Nucleosides; Alkaloids

1. Introduction

Traditional Chinese drugs have been used in China for the treatment of cancer for many years. Remarkable anticancer activities have been reported, for instance, for *Coptis chinensis* F. and *Croton tiglium* L. We have isolated and identified the active fractions from these two plants using bioassay-guided fractions prepared by preparative HPLC. The purine nucleoside isoguanosine, the alkaloid berberine, with other protoberberine alkaloids as minor components, were determined to be the effective chemical agents [1,2]. Subsequently we started our anticancer experiments using the purified isoguanosine and berberine in various tumour cell lines, in P-388 leukaemic mice and in mice bearing S-180 solid tumours. Each substance alone could not achieve the same promising results as a mixture of isoguanosine and berberine. We observed a three- to eight-fold antiproliferative effect in our cell cultures. In solid-tumour-

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bearing mice, a growth inhibition of 70% was found, and in leukaemic mice the mean survival rate was 1.63-fold increased [1].

The isolation and purification of isoguanosine and berberine currently entail labour-intensive and time-consuming procedures (Fig. 1). To avoid this, the use of pure plant extracts of *Coptis Chinensis* F. and *Croton Tiglium* L. could be an attractive alternative, provided that they show the same effectiveness as the purified components. For this reason it became important to determine and control the amount of isoguanosine and berberine in the anticancer plant extract. In this communication high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) were compared with regard to find a fast solution of this analytical problem.

2. Experimental

2.1. Reagents and material

All chemicals were of analytical grade and were purchased from Sigma (Deisenhofen, Germany). The purified substances were prepared as shown for isoguanosine in Fig. 1 (for details see Ref. [1]) and dissolved in methanol–water (50:50, v/v).

2.2. Capillary electrophoresis

Fused-silica capillaries (Grom, Herrenberg, Germany), 44 cm×75 μm I.D., were used for all electrophoretic runs. All separations were performed on a capillary electrophoresis system CES I (Dionex,

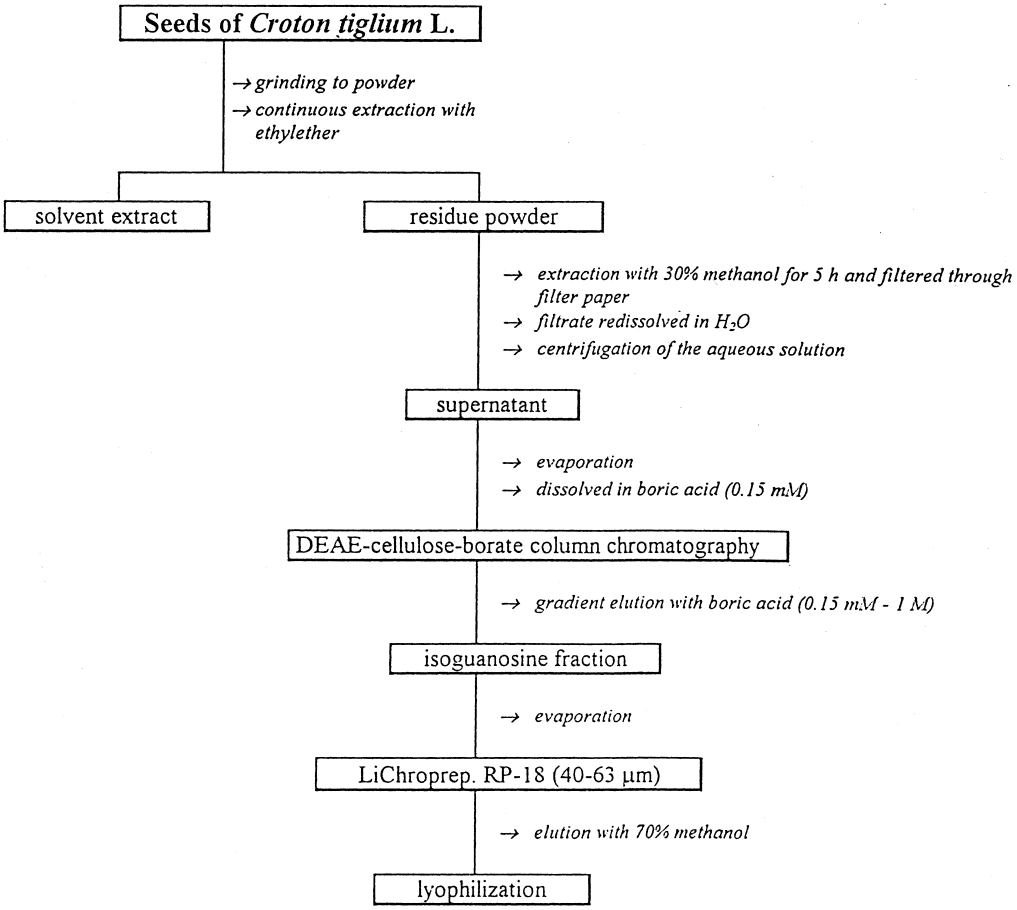

Fig. 1. Isolation procedure of isoguanosine from seeds of *Croton tiglium* L.

Table 1
Gradient composition for the HPLC separation

Time (min)	0	1.0	5.0	40.0	55.0	60.0	65.0
KH_2PO_4 (%)	0	1	1	80	80	0	0
MeOH/H_2O (%)	100	99	99	20	20	100	100

Idstein, Germany), equipped with an automatic constant volume sample injection system, a high sensitivity UV–Vis detector with wavelength programming and a dedicated computer system with a Dionex AC interface and the Dionex AI 450 software. The separations were performed under alkaline and acidic conditions. We tried three different buffer systems: (1) 20 m*M* sodium borate, pH 9.4, applied voltage 12 kV; (2) 20 m*M* 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), pH 10.4, applied voltage 25 kV; (3) 100 m*M* sodium citrate, adjusted to pH 2.7 with 1 *M* HCl, applied voltage 12 kV. The capillary was rinsed for 60 s with water, 60 s with 0.1 *M* NaOH, 60 s with water, followed by 90 s with buffer after each run. Samples were introduced hydrodynamically and detected at 254 nm.

2.3. High-performance liquid chromatography

The HPLC system (Merck–Hitachi, Darmstadt, Germany) was composed of a L 6200 gradient pump, a photodiode array detector L-3000, a column oven 655 A-40, an interface D-6000 and the Merck–Hitachi HPLC manager software. The separation of the seed extract of *Croton tiglium* L. was performed on a 250×4 mm, 5 μm LiChrospher 100 C_{18} column (Merck, Darmstadt, Germany) at 30°C using a gradient composed of 25 m*M* KH_2PO_4 buffer, pH 4.7 and methanol–water (60:40, v/v) (Table 1) at a constant flow-rate of 1.5 ml min^{-1}. The injection volume was 10 μl and the components were detected at 254 nm.

3. Results and discussion

To avoid the time-consuming and labour-intensive isolation procedure, we started to separate isoguanosine and berberine by HPLC, but so far we have not been satisfied with the results achieved (Fig. 2). An alternative powerful tool for the quantitative analysis of pharmaceuticals and drugs is CZE. Since the relative amounts of the two components of the plant extract are of great importance, we developed a CZE approach. Bearing in mind the chemical structure of isoguanosine and berberine, we checked out different buffer systems and pH values (Fig. 3). The best and very fast separation for the isolated substances was achieved under basic conditions in a 20 m*M* CAPS buffer at pH 10.4 (Fig.

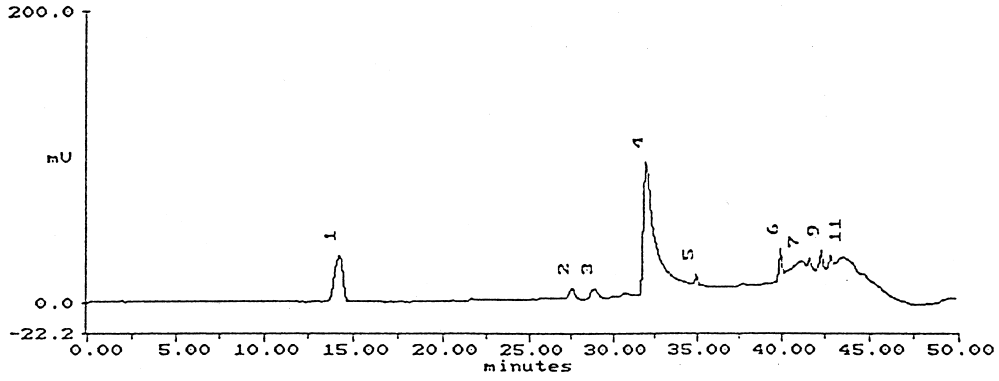

Fig. 2. HPLC separation of the seed extract of *Croton tiglium* L. The analysis was performed on a 250×4 mm, 5-μm LiChrospher 100 C_{18} column at 30°C using a gradient composed of 25 m*M* KH_2PO_4 buffer, pH 4.7 and methanol–water (60:40, v/v) at a constant flow-rate of 1.5 ml min. The components were detected at 254 nm. Peaks: 1=isoguanosine; 4=berberine.

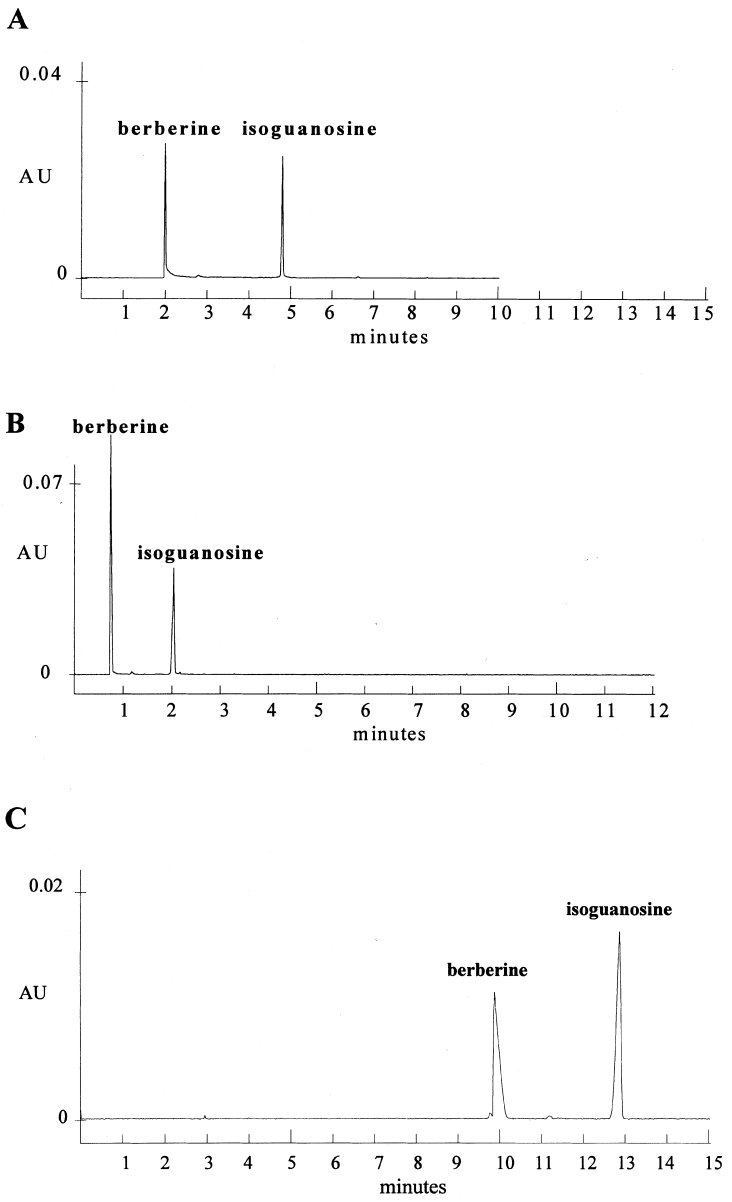

Fig. 3. Capillary electropherogram of purified berberine and isoguanosine. Electrophoretic conditions: capillary, uncoated fused silica (44 cm×75 μm); loading, 60 mm for 40 s; running conditions, 12 kV; detection, UV at λ=254 nm. Buffers: A, 20 m*M* sodium borate, pH 9.4; 20 m*M* CAPS, pH 10.4; C, 100 m*M* sodium citrate, pH 2.7.

3B). Therefore we tried the CAPS buffer for the pure plant extract as well, but the separation profile was disappointing. With both basic buffer systems, a clear-cut separation for berberine was not possible (Fig. 4A and Fig. 4B). Contrary to this, the acidic buffer (100 m*M* sodium citrate, adjusted to pH 2.7)

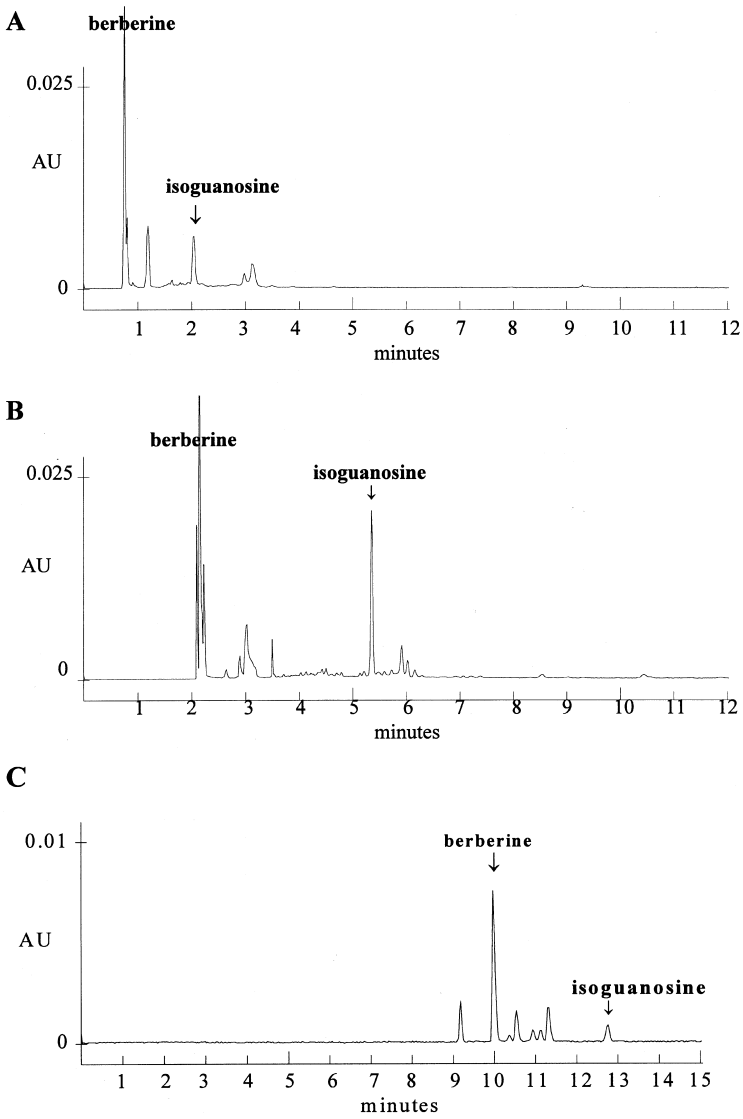

Fig. 4. Capillary electropherogram of the seed extract of *Croton tiglium* L. For separation conditions see legend of Fig. 3. Results of quantitative analysis: isoguanosine 1.3 μg ml^{-1}; berberine 8.7 μg ml^{-1}.

gave satisfactory results with acceptable migration times (Fig. 4C). Isoguanosine and berberine can be selectively monitored at 254 nm within 14 min in the plant extract. The concentration range of 0.1–50 μg ml^{-1} proved to be sufficient and showed good reproducibility [relative standard deviations (n=32): for the migration time 0.22% (isoguanosine) and 1.32% (berberine); for the peak area 2.8% (isoguanosine) and 3.3% (berberine)]. We measured concentrations of 1.3 μg ml^{-1} for isoguanosine and 8.7 μg ml^{-1} for berberine in our pure seed extract of *Croton tiglium* L.

The results reported in this communication demonstrate that CZE can be used easily for the quantitative determination of the potential anticancer drugs berberine and isoguanosine in an extract of the traditional Chinese medicinal herb. In addition to the higher separation performance, CZE shows several

other remarkable advantages over HPLC, such as rapidity of analysis, small sample volume, no requirement for organic solvent in the running buffer and low cost of reagents. Analysing the plant extract by CZE, we now can proceed with investigating the anticancer effects of this traditional Chinese medicinal herb.

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