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CHROMATOGRAPHIC EVALUATION OF PERFUSION ON CHARCOAL IN URAEMIA

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SUMMARY

Chromatography on Bio-Gel P-2 and high-performance liquid chromatography on RP-18 columns monitored with UV, fluorescence and electrochemical detectors have been used to evaluate the efficiency of granular, uncoated, active charcoal to remove from the ultrafiltrates of uraemic patients those organic substances accumulated in the blood that are not easily removed by dialysis. Chromatography on Bio-Gel P-2 and high-performance liquid chromatography on RP-18 columns carried out isocratically and monitored with an electrochemical detector seem very useful for clinical investigation as they increase the information obtained from routine haematochemical analyses such as blood urea nitrogen, creatinine, uric acid and electrolytes (calcium, phosphorus, sodium and potassium).

INTRODUCTION

Numerous organic substances are known to accumulate in the blood during chronic renal failure. Patients with chronic renal failure have many metabolic

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abnormalities and their clinical state can be improved by various techniques in which artificial systems remove those toxic metabolic products that would normally be excreted by the kidney. The resulting improvement in clinical state suggests that the uraemic symptoms are attributable to the presence of so-called toxins in the body fluids at concentrations greater than those encountered in healthy subjects [1–6].

It has been widely demonstrated that the use of active charcoal as a depurative in acute intoxication and poisoning brings about a substantial improvement of the clinical state of patients. This confirms the hypothesis that the toxic state, at least partly, is determined by substances which are not easily eliminated by traditional dialysis, either extra- or intracorporeal, but which are easily removed by active charcoal [7—9]. The use of charcoal, supplemented by dialysis, has been successful also in removing toxins produced by metabolism in patients with chronic renal failure [10—12]. Most data reported refer to the use of charcoals coated with polymers to prevent contact with the particulate fraction of the blood. The high cost of this material limits its use in the treatment of chronic renal failure.

The aim of this work is to evaluate the efficiency of an uncoated and inexpensive active charcoal in the treatment of chronic renal failure. This evaluation has been accomplished, in addition to traditional haematochemical and chemical tests, by applying various chromatographic techniques in order to acquire a large number of quantitative data on the adsorption efficiency of this treatment.

EXPERIMENTAL

Routine haematochemical analyses

Blood urea nitrogen (BUN, urease method), creatinine (picric acid method), uric acid (uricase method), calcium and phosphorus (Technicon original methods) analyses were performed with a Technicon RA 1000 autoanalyser (U.S.A.). Sodium and potassium were measured with a Corning 435 flame photometer (U.S.A.).

Uraemic ultrafiltrate

Samples were taken from patients with serious renal failure (creatinine clearance less than 10 ml/min, serious general weakening, blood arterial pressure between 170/110 and 240/130 in spite of hypotensive treatment) and treated with Pan Asahi 150 filters.

Normal ultrafiltrate

Samples were obtained from volunteers with normal renal function, detected via routine haematochemical analyses and by the clearance of creatinine and of urea; a 300-ml blood sample was drawn and ultrafiltered with a filter identical to the one used for uraemic patients.

Gel chromatography

A 2-ml aliquot of ultrafiltrate was analysed on a K-16 column (100 × 1.6 cm I.D.; Pharmacia, Sweden) filled with Bio-Gel P-2, particle size < 40 μm (Bio-Rad Labs., U.S.A.), to give a bed height of 83 cm. Elution was performed using a peristaltic pump (LKB, Sweden) with 50 m*M* of ammonium bicarbonate, pH 8.0, at a flow-rate of 0.5 ml/min (linear flow-rate, 14.92 cm/h), monitoring at 254 nm with a Uvicord S detector (LKB) equipped with a cell of pathlength 2.5 mm.

High-performance liquid chromatography (HPLC)

A Perkin-Elmer Series 3B liquid chromatograph equipped with an ultraviolet detector LC-75, a fluorescence detector LS-3 and an amperometric detector LC-4B (all from Perkin-Elmer, U.S.A.) was used. The detector cell of the electrochemical detector includes a recently developed [13] tubular working electrode (polyethylene-graphitized carbon black), an auxiliary and a silver/silver chloride reference electrode.

The HPLC experiments using UV and fluorescence detectors were performed by injecting 20 μl of ultrafiltrate on a 4.6 × 250 mm column filled with 5-μm spherical beads of Hypersil C_{18} (Policonsult Scientifica, Rome, Italy). The elution programme at a flow-rate of 1 ml/min was as follows: 10 min with a solution composed of 0.02 *M* potassium dihydrogen phosphate (brought to pH 4 with orthophosphoric acid)—methanol (99.9:0.1); then a 50-min linear gradient was carried out until a final elution solution containing potassium dihydrogen phosphate—methanol (30:70) was reached; experiments were performed at room temperature, monitoring at 254 nm (0.04 a.u.f.s.) and with fluorescence detection as reported by Swan et al. [14] (295 nm excitation; 405 nm emission; f.s.d. = 0.4 μA). All solutions were filtered through a 0.2-μm Millex 500 filter (Millipore, U.S.A.) and degassed with helium before use.

The HPLC experiments with an electrochemical detector were performed using a 4.6 × 250 mm column filled with 10-μm spherical beads (Erbasyl, Carlo Erba, Italy). Elution was carried out isocratically at 2 ml/min with a solution of methanol and 0.02 *M* potassium dihydrogen phosphate brought to pH 4.0 with phosphoric acid (7:93). The electrochemical detector was fitted at a positive potential (1.0 V) with respect to the silver/silver chloride electrode (f.s.d. = 20 nA). The sample was filtered through a 0.2-μm Millex 500 filter and 20-μl aliquots were injected into the column.

Charcoal cartridge

Cartridges were prepared with active charcoal treated using the following procedure. Zenith charcoal (surface area 1400 m^2/g; Filtrati, Rovereto, Italy) was mechanically shaken with 6 *M* hydrochloric acid for 12 h and then washed with distilled water to neutrality. The charcoal was heated in an oven up to 700°C and thereafter was used to fill up a sterile cartridge. Usually, about 0.5 kg of material was used for a single treatment. The eluent from the cartridge was made to flow on a 0.2-μm Nylon filter to trap the fines of the charcoal. Other charcoals used included Sorbabil BT 905 cartridges (Dideco, Mirandola, Italy), with which similar results were obtained.

The experimental set-up used for the charcoal treatment is shown in Fig. 1.

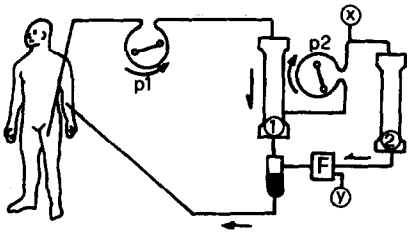

Fig. 1. Scheme of the experimental set-up. Peristaltic pump (p1) gives a blood flow of 250 ml/min and a transmembrane pressure through ultrafilter 1 (Pan Asahi 150) of 450 mmHg with the production of 100—110 ml/min ultrafiltrate which is now pressed by pump p2 through charcoal filter 2; X and Y are sampling sites, F is Nylon filter(0.2 μm).

Using peristaltic pump p1, the blood was made to flow at 250 ml/min in ultrafilter 1 (Pan Asahi 150), where a transmembrane pressure of about 450 mmHg was obtained which gave an ultrafiltrate production of 110 ml/min pushed by pump p2 through the cartridge containing the charcoal filter. After percolation through charcoal, the ultrafiltrate was added to the particulate fraction of the blood and the recombined blood was injected back into the patient. The sampling for chromatographic and haematochemical analysis was performed at points X and Y of Fig. 1, located before and after the charcoal filter.

RESULTS AND DISCUSSION

A selection of chromatographic techniques was used in order to obtain an overall picture of their performance for the evaluation of active charcoal treatment. HPLC with a UV detector yields a general overview of species that are found in a uraemic ultrafiltrate, whereas the fluorescence detector characterizes indolic group substances as focusing has been set on two typical wavelengths [14]. HPLC with electrochemical detection, according to the procedure employed, supplies information on the content of certain amino acids and metabolites found in uraemic patients [15].

Gel chromatography on Bio-Gel P-2 has been performed, as this simple proce-

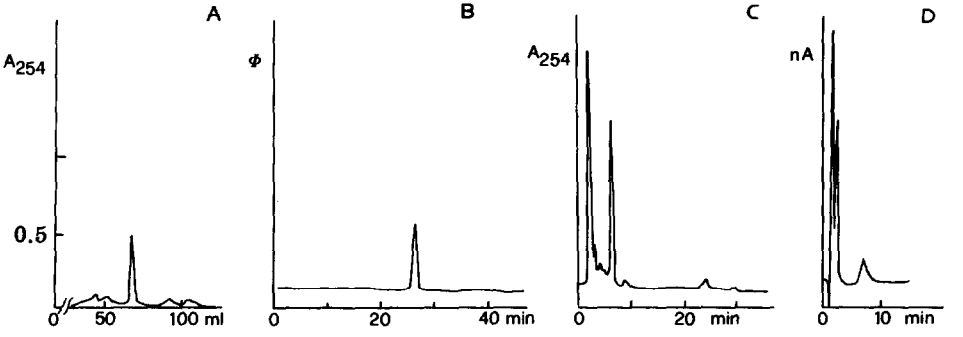

Fig. 2. Chromatographic profiles of the ultrafiltrate of a healthy subject. (A) Bio-Gel P-2; (B) RP-18 column monitored in fluorescence (295 nm excitation; 405 nm emission; f.s.d. = 0.4 μA); (C) RP-18 column monitored at 254 nm (0.04 a.u.f.s.); (D) RP-18 column monitored with electrochemical detector: f.s.d. = 20 nA. The sample was taken at point X of Fig. 1.

dure is able to give a very good separation of species present in uraemic ultrafiltrates [16]. As previously demonstrated, fractionation is not based upon molecular weights but on the formation of hydrogen bonds and, in some cases, of hydrophobic bonds [17]. In addition to these interactions, we have recently demonstrated that Bio-Gel P-2 should also be considered as a weak ion exchanger [18].

A chromatogram of ultrafiltrate from a healthy subject (sampled at point X of Fig. 1) is shown in Fig. 2; chromatograms of a uraemic subject before (line a) and after (line c) the treatment, which was carried out for 3 h by flowing the ultrafiltrate through the charcoal cartridge, are shown in Fig. 3.

It is worth observing that the chromatograms of a uraemic ultrafiltrate examined either with a fluorescence or with a UV detector (A, B and C of line a) consist of a large number of peaks, whereas in the chromatograms after the charcoal filter no peaks are detected (line b), with the exception of a few absorbing compounds detected in HPLC by using the UV detector (C).

As regards the use of the electrochemical detector under the reported experimental conditions, the ultrafiltrate of a healthy subject shows the presence of only a few amino acids; of these, tryptophan, eluting with a K of 5 [where K =

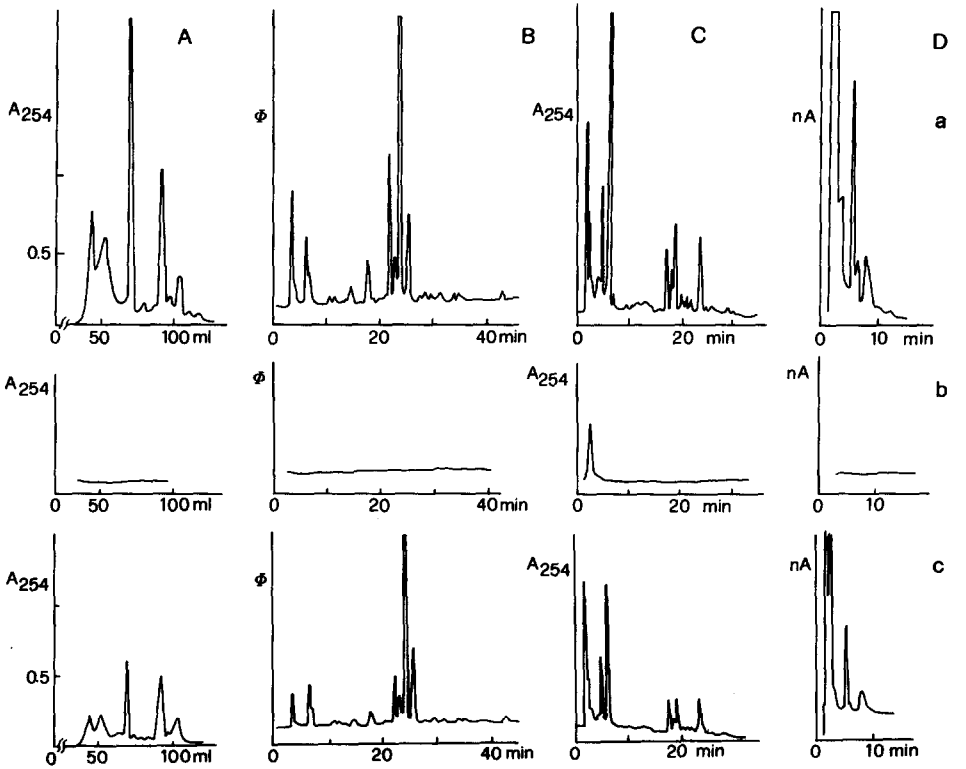

Fig. 3. Chromatographic profiles of the ultrafiltrate of a uraemic patient: line a, sample taken at point X of Fig. 1 at zero time; line c, sample taken at point X after 3 h of perfusion on charcoal filter; line b, sample taken at point Y of Fig. 1 after 3 h of perfusion on charcoal filter. A, B, C and D: same as in Fig. 2.

$(t_R - t_M)/t_M]$, has been identified (Fig. 2D), none of their metabolites have been detected. In uraemic patients, other species are present and, apart from tryptophan, some of their metabolites are detected. Indican, with a K of 3.3 (Figs. 3D and 4D), is always present in different concentrations and can be used to evaluate the severity of the uraemic state. After charcoal treatment, the chromatogram does not show the presence of any peak.

These experiments indicate that a large number of species, related to uraemia, are present in the ultrafiltrate and that most of them are removed by charcoal treatment.

It is important to outline the fact that as a large number of species are present in significant amounts in the interstitial fluids, when the ultrafiltrate after charcoal treatment is added to the particulate fraction of the blood and is returned to the patient, most species rapidly re-equilibrate. This behaviour can be followed experimentally. The chromatogram of the recombined blood shows the presence of species that were removed by charcoal treatment. Chromatograms of the ultrafiltrate of the recombined blood after 3 h of treatment with charcoal are reported in Fig. 3 (line c). As all chromatograms have been carried out under the same experimental conditions, it may be estimated that a decrease of about 50% of the absorbing species occurs in the recombined blood, whereas in the ultrafiltrate at the exit of the charcoal filter no elution peaks were detected (Fig. 3, line b).

It is worth noting that when patients were treated every day for fifteen days, analyses performed every five days demonstrated a progressive decrease of chromatographic profiles of the ultrafiltrate at the entrance to the charcoal

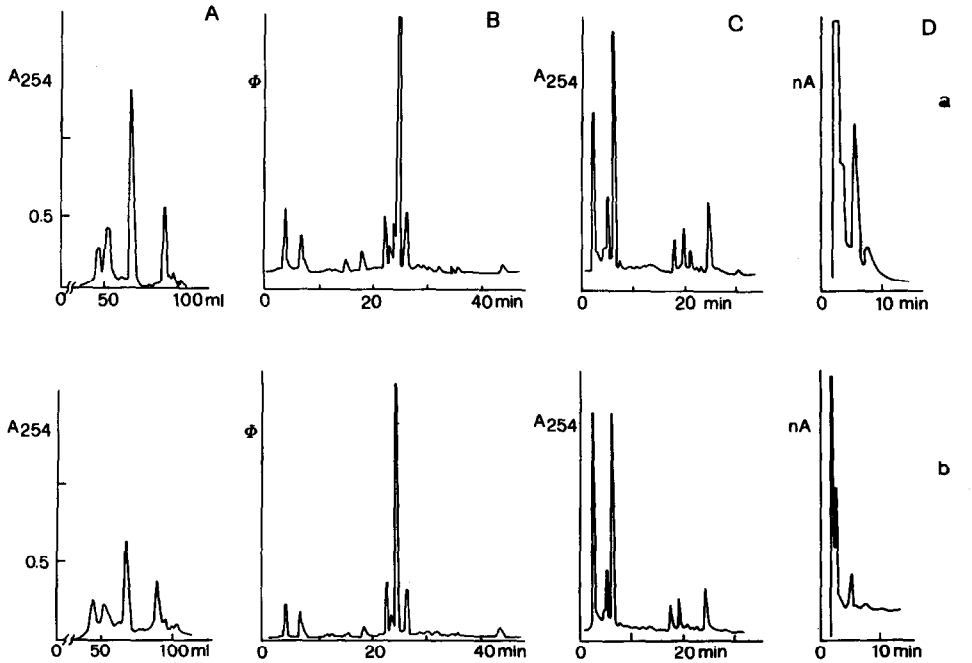

Fig. 4. Chromatographic profiles of the ultrafiltrate of the uraemic subject reported in Fig. 3, after ten (line a) and fifteen (line b) consecutive perfusions on charcoal. Samples were taken at point X of Fig. 1. A, B, C and D: same as in Fig. 2.

cartridge. In order to have almost complete elimination of the absorbing compounds reaching values similar to those of healthy subjects, at least fifteen consecutive treatments of 3 h/day on charcoal cartridges are required, each followed by 1 h of dialysis as demonstrated by the chromatograms of Fig. 4. After this treatment, the patients improve; the typical greyish colour of the skin disappears, and predialysis arterial pressure falls to values between 140/85 and 150/90 without the aid of any hypotensive drug. In order to determine whether stopping the charcoal perfusion treatment results in a worsening of the clinical state, a patient after fifteen consecutive perfusions was used in the standard haemodialytic programme and chromatographic controls were performed after one and three months of haemodialysis. Chromatograms on Bio-Gel P-2 are reported in Fig. 5. A slow but progressive increase in peak height is evident in the chromatograms, which further confirms that the daily perfusion on charcoal for at least two weeks depletes the body fluids of the uraemic patient of all those compounds which, not being eliminated by dialysis, slowly accumulate in the interstitial fluids. Furthermore, it is important to note that some clinical symptoms such as the general disposition, the greyish colour of the skin and hypertension are slowly restored after stopping the perfusion on charcoal.

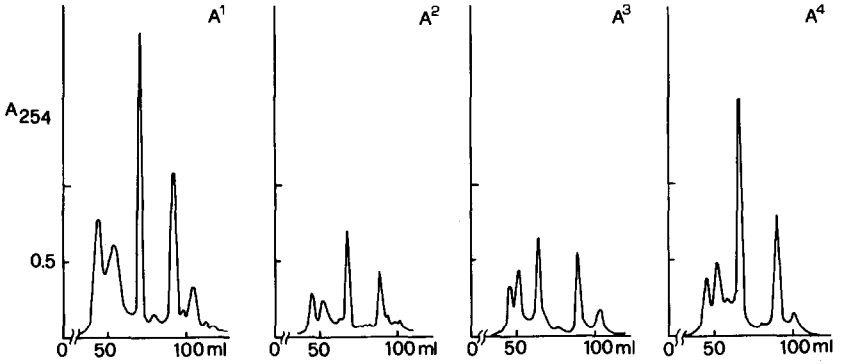

Fig. 5. Chromatographic profiles on Bio-Gel P-2 of the ultrafiltrate of a uraemic subject before treatment with charcoal (A^1) and after fifteen consecutive treatments on charcoal (A^2). The patient was then put back into the standard programme of haemodialysis and after one month (A^3) and three months (A^4) the ultrafiltrates were analysed.

CONCLUSION

All the experimental results reported show that in chronic renal failure there is a slow accumulation in the interstitial fluids of catabolites and various other compounds. Some of them are only partially eliminated through dialysis, but are completely removed by charcoal. Some clinical symptoms dramatically decrease upon charcoal treatment and are slowly restored if this treatment is stopped. These symptoms are ascribable to the compounds that are not eliminated by dialysis.

This investigation outlines the efficiency of active charcoal treatment in uraemia and the possibility of using chromatographic techniques to monitor

the state of the patient before, during and after the application of the depurative process.

Though many chromatographic techniques may be used, it seems that either Bio-Gel P-2 chromatography under the described conditions or HPLC with electrochemical detection are very simple methods which permit a more complete evaluation of the humoral situation of the patient, supplementing and increasing information from standard haematochemical analyses (BUN, creatinine, uric acid and electrolytes).

The efficiency of charcoal in blood depuration of the uraemic patient using the chromatographic procedures described has been documented in the present work for the first time in a complete way.

The charcoal treatment seems, then, to be a very good coadjuvant of the depurative processes in chronic uraemia. However, dialysis should always be applied to remove compounds such as urea and phosphates, which are poorly adsorbed on charcoal [12, 19, 20]. The final result of the present work is the demonstration that in chronic renal failure, an uncoated and therefore inexpensive, granular charcoal may be used in addition to or at intervals during dialysis treatment.

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