PART II
DURATION AND FREQUENCY OF DIALYSIS
Chairman: Professor L Migone
Removal by Dialysis of Methylguanidine from Body Fluids

G BARSOTTI, S GIOVANNETTI
Semieiotica Medica Università, Pisa, Italy

Summary

The dialysis clearance of methylguanidine (MG) was found to be lower ‘in vivo’ than ‘in vitro’ and to decrease during a haemodialysis. Its protein binding, which rises as the plasma pH rises during haemodialysis, accounts for its dialysis behaviour. The low dialysis clearance of MG ‘in vivo’ explains why the post dialysis percentage decrease in its plasma levels is lower than that of urea (U) and of creatinine (CR).

The slow transfer of MG from tissue during dialysis, shown by direct measurements on plasma and muscle tissue of anuric dogs, accounts for its high plasma rebound level after haemodialysis. Rebounds after peritoneal dialysis were lower and the plasma MG levels 12 hr after the termination of the two dialysis procedures were not different.

The dialytic behaviour of MG is different from that of U and of CR which have similar molecular weights and the conclusions drawn from the behaviour of the latter two metabolites cannot be applied to MG. On the contrary, the removal of MG from body fluids of uremic patients by dialysis is equal with peritoneal dialysis and with the various haemodialysis schedules that ensure good clinical results, while leaving plasma U and CR concentrations in a high range.

Introduction

On the basis of the clinical results obtained with chronic uremic patients maintained on various dialysis schedules and, particularly, on the basis of the good condition of patients on regular peritoneal dialysis, the hypothesis was advanced that uremic intoxication is caused by the retention of substances with a molecular weight ranging between 300 and 1,000 (Babb et al, 1971). This is in contrast with our hypothesis that methylguanidine (MG) having a molecular weight of 73, is an important uremic toxin (Giovannetti et al, 1973). The present study aims to determine whether the dialysis behaviour of MG is compatible with our hypothesis.
MATERIALS AND METHODS

The in vitro measurements of the dialysis clearance of urea (U), creatinine (CR) and methylguanidine (MG) (6 experiments) were performed on a solution of U (250 mg/100 ml), CR (15 mg/100 ml) and MG (250 µg/100 ml), using a standard two-layer Kiil dialyser. Both the test solution and dialysate (tap water) were circulated in single pass and maintained at 38°C during the experiments. In all cases the formula \( \text{Cl} = \frac{Q_D}{C_D - C_B} \) was employed where \( C_D \) and \( C_B \) are the concentrations of the examined metabolite in dialysate and test solution, \( Q_D \) is the dialysate flow rate in millilitres per minute, and Cl is the clearance.

The pre- and post-dialysis concentrations of U, CR and MG were measured:

1. on 40 l of the test solution recirculating for 8 hr in a multi-point dialyser at \( Q_B \) 250 and \( Q_D \) 750 (5 experiments);
2. on the plasma of 28 uraemic patients on a chronic dialysis programme of 2 dialyses per week performed in the same conditions as the in vitro experiments;
3. on the plasma of 8 uraemic patients receiving 2 dialyses per week of 12 hr duration carried out with a standard two-layer Kiil dialyser at \( Q_B \) 200 and \( Q_D \) 500;
4. on the plasma of 11 uraemic patients on twice weekly peritoneal dialyses of 18–20 hr duration, with a dialysate exchange of 80–100 litre;
5. on the plasma of 6 uraemic patients receiving 1 dialysis of 90 min duration every other day performed with a coil dialyser of 1.5 m² dialysing surface;
6. on the plasma and muscle tissue of 6 dogs on their third day of anuria following bilateral ureteral ligation. These animals were dialysed for 6 hr with a standard two-layer Kiil dialyser utilising femoral artery and vein for blood circulation. These experiments were performed under general anaesthesia induced and maintained with intravenous pentabarbital.

In 8 patients on maintenance peritoneal dialysis and in 12 of those on a haemodialysis programme based on two dialyses per week of 8 hr duration performed with a multi-point artificial kidney, the concentrations of U, CR and MG were measured immediately pre-dialysis, immediately post-dialysis and 12 hr later, to evaluate their plasma rebounds rates.

The binding of MG to the plasma proteins was measured on 8 samples of normal human plasma to which MG had been added at concentrations of 100 and 200 µg/100 ml. The pH of an aliquot of this plasma was adjusted to about 7.3 and that of another aliquot to about 7.5, by adding a concentrated solution of NaHCO₃. Each aliquot was then poured into a cuprophane bag which was placed in the upper half of a glass tube with a central porous septum. These were then centrifuged for 6 hr at 6,000 rpm at room temperature. The MG concentrations were then measured on both the plasma and the protein-free ultrafiltrate from the bottom of the centrifuge tubes.
Determinations of U and CR on fluids and on water extracts of dog muscle tissue were performed with an Auto-Analyser. Measurements of MG in fluids were made with a cation-exchange chromatography method (Menichini et al, 1971) and, in the muscle tissue samples of dogs, by applying this method to the water extracts, as previously described (Giovannetti et al, 1973).

RESULTS

The in vitro dialysis clearance rate of MG of $73 \pm 2 \text{ ml/min at } Q_B \text{ 200 and } Q_D \text{ 500}$ between those of U ($82 \pm 2 \text{ ml/min}$) and CR ($63 \pm 2 \text{ ml/min}$) and the percentage decrease of MG, U and CR concentrations in a 40 L reservoir of test solution was in the same range, with values of MG, $79 \pm 3$, U, $84 \pm 2$, and CR, $73 \pm 4$.

The in vivo U and CR dialysis clearances were not significantly different from those performed in vitro. However, the rate of MG clearance in vivo ($65 \pm 6 \text{ ml/min}$) was lower ($P < 0.02$) than that in vitro and showed a tendency to decrease during the course of dialysis. The percentage of the plasma concentration of MG after dialysis was lower than that of U and of CR, whatever procedure was employed (Fig. 1).

The percentage of the non-ultrafiltrable MG, assumed to correspond to the amount bound to proteins, was significantly higher ($P < 0.001$) at pH $7.51 \pm 0.04$ ($29.6 \pm 5.9$) than at pH $7.31 \pm 0.03$ ($15.2 \pm 5.8$).

The muscle tissue content of U of anuric dogs decreased after dialysis by a percentage ($67 \pm 6\%$) not significantly different from that of plasma ($67 \pm 7\%$). On the contrary, those of CR and MG decreased much less in muscle tissue than in plasma ($p < 0.01$ for CR and $p < 0.001$ for MG); muscle tissue CR fell by $31 \pm 11\%$ and plasma CR by $54 \pm 11\%$; muscle tissue MG fell by $24 \pm 9\%$ and plasma MG by $47 \pm 15\%$ (Fig. 2).

Twelve hours after the termination of haemodialysis plasma U concentrations were $53 \pm 7\%$ lower than pre-dialysis concentrations, and after peritoneal dialysis they were only $27 \pm 9\%$ lower ($p < 0.001$). The plasma CR levels 12 hr after haemodialysis were lower than predialysis by $37 \pm 7\%$ and, after peritoneal dialysis, by $26 \pm 8\%$ ($p < 0.01$). The mean decrease of plasma MG levels 12 hr after haemodialysis, $27 \pm 8\%$, was not significantly different from that found 12 hr after peritoneal dialysis, $25 \pm 9\%$.

The pre-dialysis concentrations of U and CR in 28 uraemics receiving 2 dialyses per week of 8 hr duration, using a 1.0 m$^2$ surface area multi-point dialyser, were not significantly different from those of 6 other patients dialysed every other day for 90 min with a coil kidney with a dialysing surface of 1.5 m$^2$ (Table 1). However, the post-dialysis plasma levels of U and CR in the patients of the former group were lower ($p < 0.001$) for U and for CR ($p < 0.001$) and consequently, the inter-dialysis concentrations were estimated to be lower. The pre-dialysis plasma
Figure 1. The percentage decrease of plasma concentration of U, CR and MG after peritoneal dialysis and haemodialyses of 8 hr duration performed with multi-point dialyser and haemodialyses of 12 hr duration performed with a two-layer Kiil dialyser.

Figure 2. The percentage decrease of the plasma and muscle tissue contents of U, CR and MG in 6 anuric dogs after haemodialysis of 6 hr duration performed with a two-layer Kiil dialiser.
TABLE I. The Pre- and Post-dialysis Plasma Concentrations of U, CR and MG in Two Groups of Patients.

One group is on a haemodialysis programme of two dialyses per week of 8 hr duration using a multi-point dialyser, and the other group is on a programme of one dialysis every other day of 90 min duration using a coil kidney with a dialysing surface of 1.5 m².

<table>
<thead>
<tr>
<th></th>
<th>Urea</th>
<th>Creatinine</th>
<th>Methylguanidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-d mg/100 ml</td>
<td>Post-d mg/100 ml</td>
<td>Pre-d µg/100 ml</td>
</tr>
<tr>
<td>Multi-point (28 cases)</td>
<td>240 ± 41</td>
<td>87 ± 27 - 64%</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Coil (6 cases)</td>
<td>248 ± 32</td>
<td>165 ± 19 - 33%</td>
<td>15 ± 4</td>
</tr>
</tbody>
</table>

concentrations of MG were, instead, significantly lower \( (p < 0.01) \) in the patient dialysed more frequently and, although they decreased less than in those of the other group during each dialysis, the inter-dialysis concentrations were estimated not to be different in the two groups of patients (Table 1).

**CONCLUSIONS**

The following conclusions may be drawn from these findings:

1. The dialysis clearance of MG in vitro is intermediate between that of U (molecular weight 60) and that of CR (molecular weight 113) as expected for its intermediate molecular weight (73). The dialysis clearance of MG is lower in vivo because of its binding to plasma proteins. Since such binding increases as the plasma pH rises, the in vivo clearance of MG falls during dialysis. These obstacles to its dialysis removal account for the fact that the percentage decrease of the MG plasma levels after dialysis are lower than those of U and of CR whatever the procedure employed.

2. MG accumulates preferentially in the intracellular fluid compartment and transfers slowly, as indicated by the direct measurements in plasma and muscle tissue of anuric dogs, before and after dialysis. The preferential distribution of MG (Giovannetti et al., 1973) inside the cells hinders its dialysis whatever procedure is employed. Its slow transfer further limits it in the case of efficient dialysis procedures, and causes high postdialysis rebounds of its plasma concentration. With a low-efficiency procedure such as peritoneal dialysis, a more stable equilibrium exists between intracellular and extracellular MG and its slow transfer from the intracellular compartment does not limit its removal by dialysis. The postdialysis rebound is therefore low.

3. These obstacles to dialysis transfer do not exist for U and CR which are removed from the body fluids of uraemic patients in amounts which are more strictly proportional to the dialyser clearance rates and to the duration of dialysis. The conclusions drawn from their dialysis behaviour cannot therefore be applied to MG.

4. The ideal haemodialysis schedule to remove MG from body fluids of uraemics would seem to consist of short, highly efficient and frequent dialyses. The high frequency allows the utilisation of postdialysis rebound, which increase the extracellular MG concentrations and makes possible rapid removal per unit of dialysis time and dialyser clearance. Low-efficiency procedure such as peritoneal dialysis may also be adequate, provided the duration is long enough (18–20 hr each) and at least 2 dialyses per week are performed.

It is evident that factors other than molecular weight may affect the dialysis of metabolites: MG behaves, indeed, quite differently from U and CR though the
molecular weights of the three substances are of the same order of magnitude. All the dialysis procedures and haemodialysis schedules which ensure good clinical results remove U and CR poorly, and allow the body fluids of uraemic patients to be adequately cleared from MG. These findings do not demonstrate that MG is an important uraemic toxin, since other metabolites may have similar distribution in the body fluid compartments and similar dialysis behaviour: they show, however, that the observations supporting the ‘middle molecule’ hypothesis do not argue against a role for methylguanidine as a uraemic toxin.

Acknowledgements

We wish to express our gratitude to Dr U Buoncristiani of the University of Perugia for having supplied us with samples of blood from patients on regular peritoneal dialysis treatment, and Dr V Cambi of the University of Parma, for samples of blood from patients on a haemodialysis programme of short and frequent dialyses.

This investigation was supported in part by Public Health Service Research Contract PH-43-68-1966.

References


Giovannetti, S, Balestri, P L and Barsotti, G (1973) Archives of Internal Medicine 131, 709.