Blood Level of Middle Molecular Substances During Uraemia and Haemodialysis

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Middle molecular substances have been held to be responsible for uraemic toxicity in dialysed patients (Babb et al, 1971; Cambi et al, 1972; Dzúrik et al, 1971). On the basis of indirect evidence middle molecular substances with molecular weight 300-1500 (MMS-1500) are most important (Babb et al, 1972).

This group of substances is heterogeneous and no simple chemical method can be used for their determination. However, two separation methods are available. 1, Ultrafiltration with the use of suitable membranes. 2, Gel filtration on Sephadex G-15 which separates both large and small molecules. The specificity can be increased by the UV absorbance, which abolishes the interference of most low molecular substances. The second method was used in this paper because of the good results of others (Dall'aglio et al, 1972) in the separation of other groups of middle molecular substances.

MATERIAL AND METHODS

Patients: Blood and urine were obtained from both dialysed and undialysed patients suffering from renal failure, and subjects with normal kidney function. Blood was centrifuged and the separated serum and urine were kept at -20°C until chromatography.

Clearance of MMS-1500: The tests were performed in fasting subjects in the morning. A three hour period was used for the collection of urine and blood was taken at the mid point.

Gel filtration: 1-3 ml of serum or 0.1-0.2 ml of urine, depending on the concentration of MMS-1500 were fractionated on a 120 x 2 cm column of Sephadex G-15 which was eluted with 0.03 M-NaCl at a flow rate of 0.6 ml/min. 10 ml fractions were collected and their absorbance measured at 240 nm.
RESULTS

Serum fractionated on a column of Sephadex G-15 and detected by measuring absorbance at 240 nm, separated into several fractions (Figure 1): (1) Fraction 1, consisting of proteins and peptides with the molecular weight over 1500. (2) Fraction 2, with a flat peak. (3) Fraction 3, giving the main peak. (4) Fraction 4, also a small flat peak.

Peaks 2 and 4 are often absent in the serum of healthy subjects. The main changes are seen in fraction 3, which will be described in the presented paper and will be called MMS-1500. Even this fraction is not homogeneous and can be evaluated only in comparison with a standard. For simplicity we have used tryptophan as an arbitrary standard.

Figure 2 presents the mean values of MMS-1500 in normal subjects and patients in renal failure. Some of them were on conservative treatment, others were dialysed intermittently. It was found that the concentration of MMS-1500 was increased in the serum of patients with renal failure. Moreover, intermittent dialysis did not decrease their serum concentration but only helped to keep it at the level found in patients on conservative treatment.

The effect of single dialysis: Serum was obtained before and after dialysis in a group of patients dialysed for varying numbers of hours (Figure 3).
There was great variability in the serum concentration of MMS-1500 before dialysis; the concentration was normal in two patients. Dialysis tended to decrease the concentration of MMS-1500 in the serum by an average of about 40%. In several cases, however, the concentration of MMS-1500 during dialysis increased, although the serum creatinine and urea concentration fell as expected. These findings may indicate that MMS-1500 may be synthesised in or released from the tissues very quickly.

The results presented in Figures 2 and 3 show that conventional intermittent dialysis is unsatisfactory from the point of view of removing MMS-1500. Though dialysis decreases their concentration, they reappear in the serum so quickly that their concentration is restored by the next dialysis.

**Comparison of two membranes:** The method can be used for evaluation of the effectiveness of dialysis membranes. An example is shown in Figure 4 where Cuprophan and Nephrophan membranes were compared. The slopes of lines indicate that there is no difference in the efficiency of these membranes in removing MMS-1500 from the serum.

**Physiological studies:** The results presented are so unpredictable that several endogenous as well as exogenous factors must be presumed to participate. It is our opinion that though attention should be paid to the technical problems of removing MMS-1500 from the body, the principal task is to study physiological, metabolic and clinical relationships.
The relationship between GFR measured by creatinine clearance ($C_{cr}$) and MMS-1500 serum concentration was evaluated next (Figure 5). An exponential correlation was found, which indicated that the serum concentration of MMS-1500 depended on GFR. However, this result gave no information on the mechanism of the excretion of MMS-1500. In order to determine the physiological mechanism of MMS-1500 was compared with $C_{cr}$. It was found that MMS-1500 were reabsorbed in the renal tubules. The relationship between the reabsorption and GFR is illustrated in Figure 6. In normal subjects about 80–90% of the filtered load is reabsorbed by the tubules. With decreasing GFR MMS-1500 reabsorption also decreases. This decreased reabsorption may be a fundamental mechanism enabling more effective removal of MMS-1500 from the body.

There are two mechanisms which could account for decreased tubular
Figure 4. Comparison of cuprophane and nephrophane membrane in the dialysis of MMS-1500

Figure 5. The relationship between the GFR and serum concentration of MMS-1500

reabsorption: First decreased metabolic activity of the tubular cells. In this case regulatory mechanisms probably participate, as in decreased reabsorption of P in secondary hyperparathyroidism. Second, overload of the tubular cells, due to increased tubular fluid concentration. In order to decide between these possibilities, the reabsorption of MMS-1500 was
calculated per ml of glomerular filtrate, (vertical bars, Figure 6). Significant increase of tubular activity was found, which suggests that the decrease of reabsorption is relative, due to tubular overload.

DISCUSSION

Relatively high tubular reabsorption of MMS-1500 in healthy subjects indicates that they are not waste products. They resemble amino acids or peptides and in fact peptides were shown to be present. The increase of the serum concentration of MMS-1500 in response to decreased GFR makes it probable that the kidney resorbs MMS-1500 from the tubular fluid and breaks them down to their fundamental constituents.

We are speaking not of a substance, but of a group of substances. In the strict physiological sense it is difficult to accept these calculations and we are aware that it will be necessary to identify the individual substances involved. Nevertheless we are sure it was worthwhile to perform this study.

CONCLUSIONS

1. There is an exponential inverse correlation between the serum concentration of MMS-1500 and $C_{cr}$.  

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2. MMS-1500 are filtered in glomeruli but in healthy subjects about 80% of them are reabsorbed in the tubules.
3. Their reabsorption decreases with decreasing GFR possibly due to overload of the remaining tubules.
4. The serum concentration of MMS-1500 rises in renal failure.
5. The effect of dialysis is transitory. In the predialysis period serum MMS-1500 concentration in patients on intermittent dialysis is usually as high as in undialysed patients.

REFERENCES


OPEN DISCUSSION

MIGONE (Parma): We have a good demonstration of middle molecules in renal failure which is in agreement with our results, obtained with the same methods. We also used ultrafiltration in order to separate small from large molecules. But can you be sure you can eliminate the possibility that in your peak of middle molecules after filtration with G 15 Sephadex, that small molecules such as creatinine, uric acid, urea are not present.

DZÚRIK: Yes, by measuring the absorbance at 235 nm they do not interfere.

MIGONE: We found urea and creatinine in the main peak, but we could eliminate them.

F BRENTANO (Paris): My comment is that it is very difficult to know what kind of middle molecules you have when you use different chromatographic techniques. We have personally used these techniques to look at the dialysate obtained through a membrane highly permeable to the middle molecule, from patients with polyneuritis. In these patients we have seen, after different kinds of chromatography of the dialysate that a peak appears in the range of middle molecules (MW 1,700). In patients with motor nerve
paralysis, the peak seemed to decrease with repeated dialysis with concomitant improvement of polyneuritis over one month, as measured clinically and by nerve condition velocity.

DZÚRIK: Yes, the result seems to depend on the technique used. We would have to standardise our protocols to get comparable results.

I would like to ask whether your MMH-1500 clearance values remain the same in different nephropathies. Isn't it possible that in tubular disease eg pyelonephritis, tubular absorption changes or increases.

BRENTANO: We haven't measured these. Ours are mainly patients with glomerulonephritis and a few with pyelonephritis. But mostly they were in renal failure and I don't think at that time there is a fundamental difference between them. In the future we shall probably concentrate on the group with a glomerular filtration rate between 30 and 80ml/min.