ERYTHROCYTE TRANSPORT OF MIDDLE MOLECULAR SUBSTANCES

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Summary

Middle molecule (MM) fractions were isolated from the erythrocytes of patients in chronic renal failure on conservative treatment, and healthy subjects and fractioned on Sephadex G 15. Most MM fractions were found in markedly higher concentrations in erythrocytes than in plasma. A model inhibitor of gluconeogenesis found previously in plasma and urine of the uraemic subjects was found also in the erythrocytes. Repeated washing of the erythrocytes with isotonic solution of NaCl did not wash out the inhibitor of gluconeogenesis from erythrocytes.

Introduction

Middle molecular substances were studied primarily from the point of view of their elimination, particularly in dialysed patients. However, it soon became evident that some of the findings could have been caused not only because of inefficiency of the used dialysing membranes but also by the increased generation or altered compartmentalisation of middle molecules. Consequently the interest in MM substance synthesis has increased recently. It was found that various tissues released MM substances into the incubation media in vitro [1] and the corresponding homogenates contained MM fractions eluted similarly to those of plasma. There appeared to be a relationship between tissue and plasma MM substances: Brain, namely its hypothalamic region, contained the inhibitor of gluconeogenesis (IGN) and muscle the inhibitor of glucose utilisation [2].

As a further step it was necessary to study the transport of MM substances in blood and to exclude the possibility that erythrocytes participate in this transport. However, highly surprising data were obtained, which made it obligatory to investigate MM fractions in the erythrocytes and the paper summarises the data obtained.
Material and methods

Patients

Blood was obtained from patients in chronic renal failure (CRF) on conservative treatment and with serum creatinine levels between 500–800 μmol/L after overnight fasting, and similarly from a group of healthy volunteers. It was collected into tubes containing EDTA as an anticoagulant.

Determination of MM fractions

Plasma MM fractions were determined in ultrafiltrates (Amicon cone filters CF 50) as described elsewhere [3]. Erythrocyte MM fractions were determined in haemolysates prepared by 1:2 dilution of whole blood with H₂O and freezing at –20°C. After thawing haemolysates were processed the same as plasma samples. Erythrocyte MM fraction concentrations were calculated according to the usual formula [4].

IGN investigation

To 1 part of erythrocytes 1.5 parts of distilled water and 2.5 parts of 0.6 N perchloric acid were added. The proteins were removed by centrifugation and the supernatant adjusted by adding KOH to pH 4.4. After precooling the samples were centrifuged, supernatant freeze dried and fractionated on a 100 x 1.5cm column of Sephadex G 25 eluted with 0.02 mol/L formic acid. The void volume was collected and filtered through the UM 10 Amicon membrane. Retentate was further fractionated on a column of Dowex 50 WX8 as published elsewhere [5].

Results

Erythrocyte MM fractions

MM fractions were found to be higher in erythrocytes than in plasma (Figure 1) both in healthy subjects and patients in CRF in the case of fractions 1–4, while the accumulation of fractions 5 and 6 was small. The concentration of fraction 7 was smaller in the erythrocytes than in plasma. The relatively small significance of the accumulation was caused by the large variability and relatively preserved renal function of the patients.

The erythrocyte/plasma ratio in patients with CRF was lower than in control subjects because of the accumulation of MM fractions in plasma which did not reflect in the accumulation of MM substances in the erythrocytes.

Similar elution patterns of erythrocytes and plasma fit well with the transport function of the erythrocytes. However, it is inadequate evidence of their identity, which is the prerequisite for the acceptance of the transport function of erythrocytes. Because of the complexity of any fraction no further subfractionation would be of significant help. To get reliable evidence it was necessary to test at least one model MM substance.
Inhibitor of gluconeogenesis

The isolation procedure was used with the erythrocytes and the inhibitory gluco-
neogenic activity was found to be present in the erythrocytes (Table I). Gluconeo-
genesis did not take place in the erythrocytes. Thus the erythrocytes serve as a trans-
port cell in the case of IGN.

Next the binding of IGN to the erythrocytes was studied: They were repeat-
edly washed with isotonic NaCl solution to remove all the weakly bound sub-
stances. No loss of IGN was found (Table I).

Discussion

Erythrocyte transport function

In the past, erythrocyte transport function was limited just to O₂ and CO₂ trans-
port. However, it was found that they participate in the transport of various drugs,
TABLE I. Effect of erythrocyte IGN on kidney cortex gluconeogenesis and the effect of erythrocyte washing with isotonic saline

<table>
<thead>
<tr>
<th>Washing</th>
<th>Control</th>
<th>IGN</th>
<th>Inhibition</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.06 ± 0.26 /6/</td>
<td>10.57 ± 1.07 /6/</td>
<td>34%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6.00 ± 0.38/6/</td>
<td></td>
<td>52%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12.44 ± 0.37 /6/</td>
<td>5.54 ± 0.21/6/</td>
<td>55%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

for instance pethidine. Moreover, it was calculated that plasma concentrations of various amino acids could not explain their turnover rate and it was suggested that erythrocytes participated in amino acid transport. It was later found that the erythrocyte concentrations of most amino acids were higher than those of plasma with an extreme erythrocyte/plasma ratio of aspartate (50:1) and glutamate (11:1) [4]. Thus, it was necessary to consider the transport function of erythrocytes in the study of the blood transport of any substance.

**MM substances in erythrocytes**

There are some previous data on the presence of MM fractions in the erythrocytes. Chapman et al [6] described that most of the subfractions of fraction 7 (i.e. fraction 2 in the presented nomenclature) were present in the erythrocytes and their concentrations were much higher than in plasma. These data extend the previously published data to the additional fractions, i.e. 1—7 and it is remarkable that the highest erythrocyte/plasma ratio was found in fractions containing various MM inhibitors [7]. Moreover these results fit well with the low release of MM fractions from the erythrocytes. No definite assumptions could be made on the nature of binding and the localisation of MM substances in the erythrocytes, and it is our belief that no general decision will be possible even in future. It will be necessary to define it for individual MM substances. In any case the relatively slow release of IGN from the erythrocytes could reflect in apparently ineffective removal of MM substances from plasma with inappropriate conclusions on the elimination of MM substances.

**References**

7. Džúrik R. *Proc EDTA 1980; 17:* 577
Open Discussion

FAGUER (Paris) Our group at Hôpital Necker have also looked for middle molecules in erythrocytes after haemolysis. We used a slightly different method from you with Sephadex G15 gel permeation chromatography followed by anion exchange chromatography with DEAE A25. We have effectively found middle molecules in the haemolysate of healthy subjects and of uraemic patients. As regards middle molecule b4-2 that we are studying, it does not seem to be present in significant amounts in the erythrocytes.

DRÜEKE (Paris) Would you suggest that the accumulation of your middle molecule substances in the erythrocytes could interfere with the erythrocyte survival in those patients?

DZÚRIK Maybe, but I have no definite evidence.

VANTELON (Pontoise) Is there any relation between IGN and the ALAD inhibitors studied by Leber?

DZÚRIK This is an inhibitor which is certainly present in plasma of uraemic subjects, and according to its mobility on a column of Sephadex G15, it is highly probable that it is accumulated in erythrocytes. This could interfere eventually with haemoglobin synthesis, but if the erythrocytes are already present in the peripheral circulation this haemoglobin synthesis is of no great physiological significance. But it may be that the accumulation of this inhibitor on the receptors, if they are present in the premature erythrocyte, could interfere with haemoglobin synthesis; that means the maturation and production of erythrocytes in uraemic subjects. Whether it has any significance in healthy subjects is very difficult to say. It is very dangerous to extrapolate and the study must be done with each middle molecule inhibitor to be sure that it is valid for that inhibitor.

BERGSTROM (Stockholm) Dr Dzúrik, I am still a little worried about the identity of what you find in plasma and the erythrocytes. I think until we have a better method than yours to measure the activity, we cannot be sure of that. Could it be possible that by your treatment of the normal and uraemic erythrocytes you could change some substance in the erythrocytes in a way that will be toxic to glucose utilisation?

DZÚRIK I think this possibility could be excluded with a very high probability because the treatment first of all removes biological activity, and a second reason is that if it were to be changed, in all probability it would be lost during the purification procedure. Of course the definite answer would be not only biological identification but also immunochemical identification and these studies are in progress at the present time in our laboratory.