INTRACELLULAR BICARBONATE AND pH OF SKELETAL MUSCLE IN CHRONIC RENAL FAILURE

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Summary

In 11 controls and 10 patients suffering from untreated uraemic acidosis intracellular bicarbonate and skeletal muscle pH (needle biopsy) were determined. In all patients a significant intracellular acidosis, not related to any extracellular indices was found. It is concluded that the chronic proton load is able to effect intracellular buffer composition; moreover the action of other factors such as derangements of cell metabolism and nutritional imbalance could be operating.

Introduction

Cell buffer mechanisms are believed to play a major role in the whole body defence against acid-base derangements. As previously demonstrated in uraemic acidosis the extracellular steady-stage is maintained at the expense of a progressive titration of cell buffers, particularly in bone. A significant participation from soft tissue has also been repeatedly emphasised but the reported literature is poor and inconclusive [1—4]. Skeletal muscle, about 40 per cent of total body cell mass, represents the main regulatory site of soft tissue buffering processes.

We have investigated the intracellular acid-base composition of muscle in chronic renal failure (CRF) patients, selected for their untreated metabolic acidosis by means of a direct measurement of intracellular bicarbonate and pH by muscle needle biopsy.

Materials and methods

Ten patients (5 males and 5 females) suffering from CRF (glomerular filtration rate range 4—30ml/min/1.73m²) and presenting an untreated metabolic acidosis were admitted to the present study. Eleven subjects with normal glomerular filtration rates and acid-base balance served as controls.

In all subjects arteriovenous blood samples were drawn for pH, pCO₂ and
HCO$_3^-$ determination. Venous blood samples were also used to determine plasma sodium, potassium and chloride.

In all subjects, whose consent was previously obtained, muscle needle biopsies from quadriceps femoris were performed after at least two hours of rest.

Muscle samples were then analysed in order to obtain muscle water (H$_2$O$_m$) and acid-labile carbon dioxide content (TCO$_2$).

Extracellular water (H$_2$O$_e$) was calculated from muscle chloride content according to Cotlove [5]: in CRF patients with glomerular filtration rates below 7ml/min/1.73m$^2$ a membrane potential value according to Cotton [6] was used.

H$_2$O$_m$ was determined by the difference between the fresh tissue samples and their dry weight.

Intracellular water (H$_2$O$_i$) was determined by the difference between H$_2$O$_m$ and H$_2$O$_e$.

Intracellular bicarbonate (HCO$_3^-$) was measured by TCO$_2$ method according to well-established techniques [7–8] and corrected for H$_2$O$_e$ and H$_2$O$_i$ values previously determined in different muscle samples.

In order to calculate intracellular pH (pH$_i$) the Henderson-Hasselbalch equation was used, assuming cellular pCO$_2$ values to be the same as venous CO$_2$.

Results

In Table I extracellular electrolyte and acid-base indices are listed. Data are reported for each patient, while controls data are expressed as mean ± SD. An extracellular metabolic acidosis was present in all CRF patients, as compared to controls, while no difference existed in electrolyte patterns. No correlation was found between acidaemia and glomerular filtration rates.

In Figure 1 the relation between the TCO$_2$ extracted from muscle samples and muscle weight is reported. It is possible to observe the linear relation found either in controls (●) and in CRF patients (■). The slopes of two lines were significantly different (p<0.001) demonstrating that in CRF patients a clear depletion of muscle TCO$_2$ was present.

In Table II muscle TCO$_2$, HCO$_3^-$ and pH$_i$ are reported: data are presented for each patient while control data are expressed as mean ± SD. A condition of intracellular acidosis was found in all patients.

All the above parameters were significantly different (p<0.001) to controls.

From the comparison of extra- and intracellular data (Tables I and II) no correlation was found between HCO$_3^-$, pH$_i$ and glomerular filtration rate nor to blood bicarbonate levels.

Statistical analysis

Data are expressed as mean ± SD. For the comparison of individual means in the two groups the Student’s ‘t’ test for unpaired data was used. The Student’s ‘t’ test was also used in the comparison of the slopes of the two lines reported in Figure 1.
<table>
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<th>Patients</th>
<th>Sex</th>
<th>Age (years)</th>
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<th>pH</th>
<th>PaCO₂ mmHg</th>
<th>HCO₂ mmol/L</th>
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Controls
n=11
30–75 years

| Mean     | 118 | 7.40 | 39 | 25 | 140 | 4.3 | 101 |
| SD       | 12  | 0.02 | 2  | 1  | 2   | 0.4 | 2   |

Student's 't' test: p<0.001 p<0.005 p<0.001 p<0.001 NS NS NS
Figure 1. The relation between TCO₂ extracted and related muscle weight in chronic renal failure patients and controls. For some subjects more than one value is presented.

Discussion

The main objective of the present study was measurement of muscle cell bicarbonate and pH in uraemic acidosis. Previous studies on intracellular compartments gave conflicting results and were not able to clarify the exact role of skeletal muscle buffering in this condition. A direct evaluation of skeletal muscle HCO₃⁻, by means of TCO₂ method, seems to offer a suitable approach to such problems.

In our patients (Table I), no correlation existed between acidaemia and glomerular filtration rate, suggesting that in this condition other factors than
TABLE II. Acid-base parameters of skeletal muscle in chronic renal failure patients and controls are listed

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age (years)</th>
<th>TCO₂ nmol/mg</th>
<th>HCO₃⁻ mmol/L/H₂O_j</th>
<th>pH_j</th>
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Mean          8.3  7.3  6.88  
SD            1.1  1.6  0.09

Controls  

n=11  
30–75 years

Mean  

12.3  11.7  7.05
SD   

1.0  1.1  0.05

Student’s ‘t’ test  

p<0.001  p<0.001  p<0.001

nephron reduction are operating (exogenous and dietary factors, catabolism, body potassium stores, acid excretion/glomerular filtration rate, PTH levels). In all patients muscle analysis clearly showed a condition of intracellular acidosis as demonstrated by a significant decrease of TCO₂, HCO₃⁻ and pH_j: muscle intracellular buffers seem to be operating against the chronic proton load. On the other hand the intracellular acid-base parameters were not related either to glomerular filtration rate, acidaemia or any other of the extracellular indices considered.

This is not surprising if we think that intracellular buffering is a complex process not merely related to extra → intra H⁺ flux (or intra→extra HCO₃⁻ flux). In fact pH_j regulation is based upon inter-relationships between physico-chemical (mainly cell proteins) and dynamic buffers, being the latter related to cell metabolic activity (metabolic pathways to produce or consume protons) [9–10].

Intracellular buffering efficiency can then be impaired not only by the severity or chronicity of the acidotic state, but also by other factors (not considered in this study) closely linked to metabolic and nutritional balance.

Thus to explain the intracellular acidosis found in our patients we can advance
two hypotheses. Firstly a recruitment of cell metabolic buffers in muscle cells closely related to both glycolitic and oxidative cycle with the consequent metabolic derangement representing a "trade off" for the maintenance of a relatively steady extracellular acid-base status. Secondly the peculiar metabolic and nutritional imbalance of uraemia could have reduced cell buffers availability, thus resulting in an impaired tolerance to proton load.

The final common pathway of the intracellular acidosis found in our patients is undoubtedly an energy metabolism derangement: this could be the source of the muscular symptoms frequently encountered in CRF patients.

References


Open Discussion

NICHOLLS (Exeter) If you continue to give captopril beyond seven days, a steady state will be reached when there is no further sodium loss. If you then give indomethacin is this steady state maintained, or does sodium retention then occur?

DAL CANALE I do believe that it must reach a steady state when extracellular fluid volume does not influence proximal tubular reabsorption. Maybe only proximal tubular reabsorption because there is decreased plasma aldosterone. I think, but I have no proof, that the steady state can change again when you give indomethacin.

BANKS (Bristol) What happened to blood pressure and was the effect inhibited by indomethacin?

DAL CANALE Captopril significantly decreased blood pressure in our hypertensive patients. The decrease was still significant when indomethacin was added but the increase was less.

GOODWIN (Chairman) I wondered whether this effect on prostaglandin metabolism is peculiar to captopril or whether it is shared by other converting enzyme inhibitors such as enalapril?
DAL CANALE  Yes, we have information in dogs about this and some have similar effects.

GOODWIN  Does enalapril have this effect?

DAL CANALE  I do not know.

BANKS  Were your patients in sodium balance? Others have shown natriuresis on 100mg captopril and maybe your failure to do so resulted from not knowing the sodium intake. Did you weigh the patients?

DAL CANALE  Yes, our patients were in constant salt balance before starting the study, taking 100mEq of sodium daily, the balance being maintained for at least five days prior to the study. The decrease in body weight was about 1kg which approximately corresponds with the loss of 150mEq sodium and actually we had a measured loss of 180mEq.