Serum Hippurate and its Excretion in Conservatively Treated and Dialysed Patients with Chronic Renal Failure

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Summary

54 healthy volunteers or patients with normal kidney and liver function, 17 patients with decreased kidney function and 12 dialysed patients were evaluated for their serum hippurate accumulation and kidney excretion. It was found that there was an inverse relationship between serum hippurate and the clearance of endogenous creatinine (C\text{Cr}) and a free relationship between fractional excretion of hippurate and C\text{Cr}. The excretory capacity in residual nephrons was increased. This was caused by the greater glomerular filtration load which increased up to 25 times and tubular secretion which increased 7 times in dialysed patients. The relative contribution of glomerular filtration to hippurate excretion rose from about 20% in controls to almost 50% in dialysed patients. True kidney adaptation was localized in the organic anion transport system of proximal tubules.

Key words
Serum hippurate – Hippurate excretion – Hippurate secretion – Glomerular filtration

Introduction

Metabolic acidosis with an increased anion gap is a typical sign of renal failure leading to increased protein breakdown in acidotic patients (Mitch et al. 1989). As a result, the mechanisms of organic anion accumulation and the impairment of their excretion in renal failure are intensively studied (Moller et al. 1983, Pritchard 1988, Ullrich and Rumrich 1988, Dzúrik et al. 1991). Hippurate is a representative organic anion occupying almost 25% of the organic anion transport system (OATS) capacity even at normal kidney function (Gulyassy et al. 1986, 1987). Its concentration in renal failure increases almost 100 times and it is a good marker of the accumulation of middle molecular substances (Vanholder et al. 1986, Schoots et al. 1987). It is even recommended to determine hippurate in standard nephrological diagnostics (Schoots et al. 1988).

It was found in our pilot study (Spustová et al. 1988) that serum hippurate concentration increased with the decreasing clearance of endogenous creatinine and the power correlation described best this relationship. This study was extended to
comprise dialysed patients and a greater number of subjects with normal kidney function as well as conservatively treated patients. The data obtained and evidence of kidney adaptation are presented in this paper.

**Patients and Methods**

*Patients:* 54 healthy volunteers or patients with normal kidney and liver function (C), 17 patients with decreased kidney function due to the conservatively treated renal failure (ND) and 12 patients on intermittent dialysis (D) were investigated.

*Procedure:* Two-hour urine collection in C, ND, six-hour urine collection in D patients and a sample of venous blood were obtained in the morning hours after overnight fasting. Blood was centrifuged and both serum and urine samples were kept at −20 °C before analysis.

*Analyses:* Hippurate was determined by reverse phase HPLC (Spustová 1989). Serum was purified on a Pre-Sep column (Laboratorní Přístroje, Prague), followed by HPLC using Separon SGX C18 column (Laboratorní Přístroje, Prague) with UV detection. Creatinine concentration was determined by the kinetic method using commercial kits (Lachema, Brno).

*Statistical analyses:* The null hypothesis of group differences was tested by the Student and Wilcoxon tests and the correlations and regression curves were calculated by the least square method for at least five types of relationships. The correlation with the highest r coefficient was accepted to be optimal (Tallarida and Murray 1981).

**Table 1**

**Hippurate serum accumulation and urinary excretion**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Non dialysed patients</th>
<th>Dialysed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>83±2</td>
<td>261±24**</td>
<td>954±68**</td>
</tr>
<tr>
<td>Creatinine clearance (ml/s)</td>
<td>1.75±0.05</td>
<td>0.54±0.08**</td>
<td>0.15±0.01**</td>
</tr>
<tr>
<td>Serum hippurate (μmol/l)</td>
<td>3.6±0.4</td>
<td>24.3±5.0**</td>
<td>167.5±39.0**</td>
</tr>
<tr>
<td>Hippurate filtration (nmol/s)</td>
<td>6.7±0.7</td>
<td>11.0±2.3*</td>
<td>12.8±2.0**</td>
</tr>
<tr>
<td>Hippurate filtration/ml C&lt;sub&gt;Cr&lt;/sub&gt;</td>
<td>3.6±0.4</td>
<td>31.0±9.6**</td>
<td>90.0±15.0**</td>
</tr>
<tr>
<td>Hippurate secretion (nmol/s)</td>
<td>25.2±2.8</td>
<td>18.8±2.7</td>
<td>14.9±2.1</td>
</tr>
<tr>
<td>Hippurate secretion/ml C&lt;sub&gt;Cr&lt;/sub&gt;</td>
<td>14.2±1.6</td>
<td>46.0±9.1**</td>
<td>100.0±12.0**</td>
</tr>
<tr>
<td>Hippurate excretion (nmol/s)</td>
<td>31.9±3.2</td>
<td>29.8±4.3</td>
<td>27.7±2.9</td>
</tr>
<tr>
<td>Hippurate excretion/ml C&lt;sub&gt;Cr&lt;/sub&gt;</td>
<td>17.8±1.8</td>
<td>77.0±18.0**</td>
<td>190.0±19.0**</td>
</tr>
<tr>
<td>Fractional hippurate excretion</td>
<td>5.5±0.4</td>
<td>3.3±0.4*</td>
<td>2.4±0.26**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. * p<0.05, ** p<0.001 (versus control subjects).
Results

Creatinine: The three groups differed markedly both in serum creatinine concentration and clearance of endogenous creatinine (Tab. 1).

Serum hippurate concentrations were very low in the control subjects. Markedly higher values were found in ND and mainly in dialysed patients (Tab. 1) with an extensive variability caused by the intentional inclusion of patients with various kidney function impairment. An inverse power correlation between serum hippurate and $C_{Cr}$ fitted best (Fig. 1) with the variability of serum hippurate in patients with kidney disease and illustrated low serum hippurate even at markedly decreased $C_{Cr}$. However, serum hippurate rose steeply after the exhaustion of excretory capacity reserves.

![Fig 1](image_url)

The relationship between serum hippurate concentration ($S_{Hip}$) and clearance of endogenous creatinine ($C_{Cr}$). C - healthy volunteers or patients with normal kidney function, ND - patients with decreased renal function due to conservatively treated renal failure, D - patients treated by haemodialysis.
Urinary hippurate: No change of urinary hippurate excretion was detected in patients with kidney disease (Tab. 1), which indicated normal hippurate production and excretion at the new steady state with an increased serum hippurate even in dialysed patients.

Fractional hippurate excretion ($FE_{Hip}$) 5.0 in control group pointed to its very effective secretion by OATS in proximal tubular cells (Tab. 1). However, a part of control subjects excreted hippurate with fractional excretion greater than 5.0 (Fig. 2) which indicated that the kidney participates in hippurate synthesis. The $FE_{Hip}$ was decreased both in ND and dialysed patients. The free power correlation between the $FE_{Hip}$ and $C_{Cr}$ made the participation of additional factors influencing the hippurate excretion very probable.

![Graph showing the relationship between fractional excretion hippurate ($FE_{Hip}$) and clearance of endogenous creatinine ($C_{Cr}$). For abbreviations see Fig. 1.](image-url)
MECHANISMS OF HIPPURATE EXCRETION. At least four mechanisms participate in hippurate excretion. a) Glomerular filtration: About one fifth of hippurate is excreted by this mechanism at normal kidney function. The relative amount depends on FEHip. b) Hippurate secretion by OATS in proximal tubules: This is the decisive mechanism increasing FEHip up to 5.0 due to the extreme affinity of OATS to glycine conjugates. Hippurate is the main endogenous organic anion transported by OATS and it occupies almost a quarter of the OATS capacity already during normal kidney function (Gulyassy et al. 1986, 1987). The remaining three quarters represent a reserve for the transport of other organic anions and for the decreased kidney function. This reserve enables the kidney to keep low serum hippurate up to the marked reduction of Ccr. c) Back diffusion of hippurate in the distal nephron represents probably a non-ionic diffusion (Braun et al. 1963). Its significance is small, as illustrated by the high FEHip in all three groups of evaluated subjects. d) Hippurate synthesis in the kidney: The increase of FEHip above 5.0 is a sign of hippurate synthesis in the kidney. The human kidney resembles rat, rabbit and dog kidneys in this respect (Irjala 1972) synthetizing hippurate. The limiting significance of benzoate and glycine (Irjala 1972, Spustová et al. 1987) and the positive feedback mechanism by hippurate (Spustová et al. 1987) have been proven. Renal hippurate synthesis was significant in some subjects of the control group.

Fig. 3
The relationship between hippurate filtration/ml Ccr and clearance of endogenous creatinine (Ccr). For abbreviations see Fig. 1.

Discussion
DETERMINATION OF GLOMERULAR FILTRATION RATE. The glomerular filtration rate is a clue variable for the evaluation of individual excretory mechanisms. Its determination by $C_{Cr}$ could be a source of error because it indicates falsely high values namely in renal failure and after a protein meal (Posthuma et al. 1990). To prevent this objection, urine was collected for 2–6 hours after overnight fasting and not for 24 hours. But even if $C_{Cr}$ still indicated higher values, it would decrease the proportion of tubular hippurate secretion and in fact the increase of hippurate secretion would be even higher.

KIDNEY ADAPTATION TO RENAL FAILURE. The impaired kidney is unable to increase $FE_{Hip}$ (Tab. 1). As a result, serum hippurate increases until its load in residual nephrons substitutes the decreased kidney function. This is apparent especially if the excretion is calculated per functional unit, i.e. per ml $C_{Cr}$ ($y = 25.2x^{-0.992}; r = -0.842; P<0.001$). The excretory capacity of residual nephrons increases by two mechanisms. The elevated serum hippurate increases the filtration load of the residual nephrons (Fig. 3) and the necessary amount of hippurate is disposable even for tubular secretion which shows a relationship similar to that illustrated for glomerular filtration ($y = 17.43x^{-0.844}; r = -0.764; P<0.001$). The increase of OATS activity is an adaptive process (Cheeseman 1990) dependent on dicarboxylates (Pritchard 1988, Ullrich and Rumrich 1988), on some amino acids, oxygen supply and $Na^+,K^+$-ATPase activity (Džúrik et al. 1991).

![Fig. 4](Image)

The contribution of hippurate filtration and secretion to hippurate excretion in controls (C), conservatively treated (ND) and dialysed (D) patients. Data (except those in brackets) are expressed per ml $C_{Cr}$.
To make the relationship more illustrative, the results of the evaluated groups are presented in Fig. 4. The glomerular load in residual nephrons increased about 9 times in ND and even 25 times in dialysed patients in comparison with the control group. The secretion of hippurate by proximal tubules increased 3 times in ND and 7 times in dialysed patients whereas the excretion of hippurate rose 4 times in ND and 10 times in dialysed patients. The amount of hippurate excreted by glomerular filtration corresponded to 21% in controls, 40% in ND and 47% of the excreted amount of hippurate in dialysed patients. Thus, the most pronounced increase concerned the filtered load.

It appears that marked adaptations develop in residual nephrons during the progression of kidney diseases. Many of them have been known for a long time, such as the increased FE$Na$, FE$K$ and FE$p$. These changes are understandable because they are caused by decreased reabsorption, i.e. decreased energy expenditure. Moreover, the adaptation even maintains ammonia production (Hoffsten and Klahr 1983) as a consequence of increased glutamine supply to the kidney. In fact, the glutamine breakdown is a source of 2-oxoglutarate and ATP production. The presented results serve as evidence about the adaptive increase of secretion, which is an unexpected finding which points to the complex nature of adaptive processes in residual nephrons.

The great variability of FE$Hip$ (Fig. 2), even in ND and dialysed patients makes the possibility of increasing hippurate secretion very probable. In fact, already on the basis of the pilot data (Spustová et al. 1988), an in vitro study was performed to look for substrates able to enhance the activity of the system transporting organic anions. Several dicarboxylates, their oxo- and amino acids were found to stimulate the organic anion transport system (Dzúrik et al. 1991). This should be confirmed in further in vivo studies.

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References


