PART XI
NEPHROLOGY—METABOLIC ASPECTS
Chairmen: A Blumberg
           B Watschinger
Decreased Drug Conjugation as a Pathogenic Factor in the Aetiology of Analgesic Nephropathy

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Summary

Following paracetamol ingestion by patients with analgesic nephropathy, the proportion of drug excreted in urine in the non-conjugated form was greater than that excreted by normal subjects. The proportion was also increased with urinary infections and noted to diminish as the infections responded to antibiotics. Organisms isolated from these infections demonstrated in vitro deconjugating activity. It is suggested that decreased drug conjugation occurring as a result of several possible mechanisms is a significant factor responsible for increasing the toxicity of drugs causing analgesic nephropathy.

Introduction

There are several unexplained peculiarities about analgesic nephropathy. Although analgesic abuse is common, it is only a small proportion of the abusers that end up with signs and symptoms of the disease. The geographic distribution is uneven, the disease is more common in females, and the incidence is not particularly high in patients with rheumatoid arthritis who consume large amounts of analgesics. There is diversity of opinion as to which particular analgesics or combinations are responsible for the nephropathy. Despite all this, it is generally accepted that phenacetin is an important agent responsible for this disease. The major metabolite of phenacetin is N-acetyl-para-aminophenol (NAPA) which is identical to the analgesic ‘paracetamol’. This latter drug is also strongly implicated as an aetiological agent, either directly when used as an analgesic or indirectly as a toxic metabolite of phenacetin (Nanra & Kincaid-Smith, 1972). NAPA is known to
undergo conjugation with glucuronic acid and to a lesser extent with sulphuric acid in the liver. This biotransformation inactivates the compound, rendering it more polar and less lipid-soluble. However not all NAPA in the body is conjugated. A variable percentage is eliminated in the free form. It is this unconjugated moiety that has the greatest toxic potential. By being more lipid soluble it tends to diffuse back across the lipid membranes of the urinary epithelium and affect the surrounding tissues. The amount of NAPA which appears in the urine in a non-conjugated and toxic form could conceivably be increased by a number of mechanisms. It could occur as a result of inhibition, idiosyncratic deficiency or defect of hepatic microsomal conjugating enzymes; alternatively the preformed conjugates could be hydrolysed within the urinary tract by tissue lysosomal or bacterial enzymes. The purpose of our investigation was to compare the excretion of free and conjugated drugs in apparently healthy subjects with that in patients suffering from analgesic nephropathy, and to assess the ability of bacteria causing urinary infections in these patients to hydrolyse drug conjugates.

MATERIALS AND METHODS

The investigation was limited to determinations of free and conjugated forms of NAPA as these would be present in the urine following ingestion of either phenacetin or paracetamol containing formulations, which in our experience are the analgesics most likely to be abused.

NAPA Analysis

The free and total (i.e. free plus conjugated) NAPA in 5 ml aliquots of urine were separated by thin-layer chromatography before and after incubation with 50 µl beta-glucuronidase/aryl sulphatase (5.2/2.6 U per ml) using butanone as the eluent according to a method described elsewhere (Furman et al, 1976). The silica gel corresponding to the area of the NAPA streak was removed and added to 3 ml 95% ethanol. The mixture was stirred, filtered under suction and read spectrophotometrically at 248 nm (λ max of NAPA). The results were not expressed quantitatively but simply as the ratio of free:total NAPA thereby eliminating errors due to collection, dilution, urinary concentration, pH, rhythms and other sources of technical and biological variability.

Standard Test

On the day of test each subject or patient was required to empty his or her urinary bladder at 22.00 h. An aliquot of this urine was used as a blank or control sample. Following this a 1 g dose of paracetamol was taken orally. The following morning at 06.00 h the bladder was emptied again and this urine plus any overnight collection was pooled for NAPA analysis.
Study 1. Free:total NAPA Ratio in Urine of Normal Subjects and Patients with Analgesic Nephropathy

The standard test was performed on 50 healthy student volunteers who showed no abnormality on urinalysis. Thirty-two were males and 18 females. All except 4 subjects were aged 20–30 years, the others being 53, 52, 40 and 40 years old respectively.

Twenty patients with clinical, radiological and/or histological evidence of analgesic nephropathy were similarly tested. Most of these patients were suffering from advanced chronic renal failure. The sex, age and creatinine clearance value of these patients are listed in Table I. The ages ranged from 36 to 65 years, and the mean creatinine clearance was $6.5 \pm 3.3$ SD ml/min. Four patients who were receiving intermittent peritoneal dialysis were tested during interdialysis periods.

Study 2. Effects of Urinary Tract Infections on Urinary NAPA Ratios

The standard test was performed on 7 patients with active urinary infections before, and then repeated after 14 days of appropriate antibiotic therapy. In each case the diagnosis was based on the presence of more than $10^5$ organisms per ml of urine. Three of these patients suffered from analgesic nephropathy (GB, CH and ME of Table I), one patient had chronic pyelonephritis and 3 were young females (aged 18, 22 and 29 years) with no obvious clinical, biochemical or radiological evidence of a renal lesion other than the infection.

Study 3. In vitro Deconjugating Activity of Organisms Isolated from Urinary Infections

Seven strains of E.coli isolated from the patients with urinary infections were cultured in nutrient broth at $37^\circ$C until stable $(10^6$ bacteria/ml). Fifty $\mu$l of each culture was added to 5 ml urine from a normal volunteer in which the free:total NAPA ratio had been predetermined to be 0.07. After incubation in a waterbath for 16 hours the proportion of free NAPA was re-estimated.

RESULTS

Study 1

The mean ratio of free:total NAPA in the urines of the 50 healthy volunteers was $0.08 \pm 0.04$ SD. Only 13 subjects had ratios greater than 0.10, the highest being 0.21. In the analgesic nephropathy patients (Table I) the ratios were generally greater, the mean being $0.17 \pm 0.11$ SD. The difference between these patients and the normal subjects being highly significant (p < 0.001). In 3 patients (GB, CH and ME) the ratios listed in Table I are the lowest of a series
TABLE I. Free:Total Urinary NAPA Ratio in 20 Patients Suffering from Analgesic Nephropathy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Free:Total NAPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>F</td>
<td>47</td>
<td>10</td>
<td>0.47</td>
</tr>
<tr>
<td>WV</td>
<td>M</td>
<td>36</td>
<td>6</td>
<td>0.03</td>
</tr>
<tr>
<td>GC</td>
<td>F</td>
<td>47</td>
<td>12</td>
<td>0.21</td>
</tr>
<tr>
<td>VD</td>
<td>F</td>
<td>36</td>
<td>15</td>
<td>0.22</td>
</tr>
<tr>
<td>WW</td>
<td>M</td>
<td>49</td>
<td>7</td>
<td>0.13</td>
</tr>
<tr>
<td>MB</td>
<td>F</td>
<td>50</td>
<td>6</td>
<td>0.12</td>
</tr>
<tr>
<td>GB</td>
<td>F</td>
<td>52</td>
<td>6</td>
<td>0.32</td>
</tr>
<tr>
<td>CG</td>
<td>F</td>
<td>48</td>
<td>5</td>
<td>0.07</td>
</tr>
<tr>
<td>CH</td>
<td>F</td>
<td>44</td>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td>EP</td>
<td>F</td>
<td>64</td>
<td>5</td>
<td>0.20</td>
</tr>
<tr>
<td>PR</td>
<td>M</td>
<td>42</td>
<td>8</td>
<td>0.19</td>
</tr>
<tr>
<td>ME</td>
<td>M</td>
<td>56</td>
<td>3</td>
<td>0.19</td>
</tr>
<tr>
<td>MW</td>
<td>M</td>
<td>65</td>
<td>6</td>
<td>0.07</td>
</tr>
<tr>
<td>DM</td>
<td>F</td>
<td>53</td>
<td>7</td>
<td>0.05</td>
</tr>
<tr>
<td>JV</td>
<td>F</td>
<td>44</td>
<td>10</td>
<td>0.12</td>
</tr>
<tr>
<td>GM</td>
<td>F</td>
<td>54</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>AT</td>
<td>M</td>
<td>48</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td>PD</td>
<td>F</td>
<td>50</td>
<td>3</td>
<td>0.22</td>
</tr>
<tr>
<td>DN</td>
<td>F</td>
<td>43</td>
<td>8</td>
<td>0.10</td>
</tr>
<tr>
<td>FS</td>
<td>F</td>
<td>48</td>
<td>4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

of estimates performed. The higher ratio values for these patients are given in Table II having been noted when they had active urinary infections before antibiotic therapy.

Study 2

All seven patients with urinary infections had urinary free:total NAPA ratios

TABLE II. Urinary Free:Total NAPA ratio in Patients with Urinary Infections Estimated Before and After Appropriate Antibiotic Therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Organism isolated</th>
<th>Antibiotic therapy</th>
<th>Free:Total NAPA Before therapy</th>
<th>Free:Total NAPA After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>F</td>
<td>52</td>
<td>E. coli</td>
<td>ampicillin</td>
<td>0.80</td>
<td>0.32</td>
</tr>
<tr>
<td>CH</td>
<td>F</td>
<td>44</td>
<td>E. coli</td>
<td>gentamicin</td>
<td>0.12</td>
<td>0.07</td>
</tr>
<tr>
<td>ME</td>
<td>M</td>
<td>56</td>
<td>E. coli</td>
<td>gentamicin</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>JK</td>
<td>F</td>
<td>26</td>
<td>E. coli</td>
<td>amoxicillin</td>
<td>0.33</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Chronic Pyelonephritis

Uncomplicated Urinary Infection

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Organism isolated</th>
<th>Antibiotic therapy</th>
<th>Free:Total NAPA Before therapy</th>
<th>Free:Total NAPA After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>F</td>
<td>29</td>
<td>E. coli</td>
<td>ampicillin</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>RB</td>
<td>F</td>
<td>18</td>
<td>E. coli</td>
<td>ampicillin</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>BG</td>
<td>F</td>
<td>22</td>
<td>E. coli</td>
<td>amoxicillin</td>
<td>0.11</td>
<td>0.08</td>
</tr>
</tbody>
</table>
that were greater prior to, than they were after antibiotic therapy (Table II). The ratios decreased in varying degree with clinical improvement. The series was too small to note any significant difference between the response in the chronic renal failure patients and those with normal renal function and infection. The analgesic nephropathy patient GB in whom as much as 80% of the initial urinary NAPA was present in a free form had marked pyuria and a clinical diagnosis of gram-negative septicaemia was made at the time when the standard test was first applied.

Study 3

All seven cultures of E. coli isolated from the patients in Table II exhibited deconjugating activity on being incubated with the test urine. The percentage of urinary NAPA conjugate that was hydrolysed ranged from 57 to 96%.

DISCUSSION

Many bacteria produce beta-glucuronidase and other deconjugating enzymes in the media of in vitro cultures (Barber et al, 1951; Dahlen & Linde, 1973; Jacox, 1951). Also, increased beta-glucuronidase activity has repeatedly been observed to occur in urine during the course of urinary infections (Bank & Barline, 1965; Schapiro et al, 1968; Vinacur et al, 1974). Consequently it was not unexpected to find that organisms responsible for urinary infections in our patients had both in vivo and in vitro deconjugating activity. These urinary deconjugases are generally considered to originate in lysosomes of renal tubular cells, and several authors have regarded such activity as indicating active pyelonephritis, as opposed to infection of the lower urinary tract (Bank & Barline, 1965; Schapiro et al, 1968). However increased beta-glucuronidase activity also occurs in the urine with a number of non-infective renal and bladder diseases (Kallet & Lapco, 1967; Schapiro et al, 1968), and correlation of enzyme activity with infection of the upper urinary tract alone cannot always be substantiated (Ronald, 1971).

Despite the positive relationship in our patients of increased proportions of deconjugated drug in the urine with urinary infections, it is not possible from these findings alone to attribute all of this increase to bacterial enzyme activity. Primary hepatic conjugation may have been impaired, and the role of enzymes from leucocytes or renal tubular cell lysosomes are not separated. Whatever the various mechanisms may be, our patients do seem to secrete an increased proportion of their analgesics as non-conjugates.

The efficacy of oestrogens and cytostatic agents acting on bladder carcinoma has been shown to increase with urinary deconjugation of the drugs concerned and similarly the toxicity of carcinogens responsible for neoplasms of the bladder (Connolly et al, 1973). In this regard the increased incidence of renal-pelvic carcinoma in phenacetin abusers with nephropathy (Høbye & Nielsen, 1971) migh
also be related to diffusion from urine of non-conjugated chemical carcinogens. In a recent histopathological study of analgesic nephropathy, Hesse et al (1976) observed characteristic pigmented and microvascular changes to extend around the lower end of the ureter in the bladder. This distribution of pathological changes gives further support to the theory that toxic effects may occur with diffusion from urine through the epithelium of the urinary pathway.

It seems obvious that the main aetiological factor of analgesic nephropathy must be the mass of analgesics ingested. However, we would like to suggest that patients who excrete increased amounts of these drugs and their metabolites in the non-conjugated form are more likely to develop the nephropathy. Urinary infections are generally regarded as being complications of analgesic nephropathy. Consideration might be given to the possibility that infections precede the nephropathy and are a continuing aggravating factor throughout the course of the disease. It is impossible to know what the conjugating capacities of our patients were before they developed analgesic nephropathy. We can only speculate that in some of them non-conjugation and deconjugation of drugs increased their vulnerability to toxic effects of analgesics.

References

Bank, N and Barline, SH (1965) *New England Journal of Medicine*, 272, 70
Barber, M, Brookbank, BWL and Kuper, SWA (1951) *Journal of Pathology and Bacteriology*, 63, 57
Dahlen, G and Linde, A (1973) *Applied Microbiology*, 26, 863
Jacox, RF (1953) *Journal of Bacteriology*, 65, 700
Kallet, HA and Lapco, L (1967) *Journal of Urology*, 97, 352
Ronald, AR (1971) *Applied Microbiology*, 26, 863
Schapiro, A, Paul, W and Gonick, H (1968) *Journal of Urology*, 100, 146

Open Discussion

BLUMBERG (Aarau) You did not study patients with other renal diseases in renal failure, such as chronic glomerulonephritis, etc. Is it possible that chronic renal failure per se might impair the conjugation rate and this effect is not specific for analgesic nephropathy? We have just learned in the Workshop upstairs that glucuronidation may be impaired in chronic renal failure.
FURMAN As far as β-glucuronidase in the urine in chronic renal failure, which is not quite the same as the story for microsomal enzymes, we did find in some patients increased glucuronidase activity with active glomerulonephritis in the early stage. We also found this with rejection and it has also been found by others with carcinoma of the bladder. Concerning microsomal enzymes, as you say you have heard from another communication that enzyme activity tends to be decreased in chronic renal failure. This would not give you increased glucuronidase activity in the urine, as we are finding here with these patients; it would be the other way round.

TANGE (Melbourne) We are not unsympathetic to the idea that glucuronide conjugation might minimise the toxicity of these compounds. However, we have examined in animals the toxicity of various metabolites of phenacetin. Severe renal damage can be produced by a number of metabolites of phenacetin except paracetamol, which in these circumstances produces liver damage. The kidney damage in these animals is associated with a metabolic excretion in which the largest proportion of metabolites are excreted in the conjugated form, not only with sulphate but with glucuronide. Now the second point that I think the speaker might be interested in is that when we examined the effect of experimental nephrotoxins in the Gunn rat, which does not conjugate the glucuronide, we find that nephrotoxicity is increased. It is increased not only for phenacetin metabolites, but also for various salicylates in which glucuronide conjugation is not so important for excretion. In these animals both renal cortical damage and renal papillary necrosis may be observed. However, when we look at the heterozygous Gunn rat in which glucuronide conjugation is relatively normal, we also find this propensity for increased renal damage. And therefore I think we should be careful about concluding that failure of glucuronide conjugation is an important contribution to analgesic nephropathy.

FURMAN Thank you, I appreciate those remarks. It is always difficult to compare the results of acute or relatively acute animal experiments with the chronic changes we get in human beings. They may be analogous in a way, but they are not the same. I do not think we quite know yet what causes analgesic nephropathy but I accept these strictures and one only puts this as a possible added factor; this is only a theory.

TANGE Let me say just one more thing. Patients with analgesic nephropathy develop renal papillary necrosis, not all, but a large proportion. This is death of tissue in mass. Now I ask you to tell me of any other pathological example in which there is death of tissue in mass which is not an acute event. Even though in analgesic nephropathy this occurs at some time during a long natural history. If you have death of tissue in mass you are dealing with an acute event.

FURMAN Ah yes, but that does not always come early in the disease, and there are many other cases of analgesic nephropathy that do not have papillary necrosis. They just have a slowly progressive interstitial nephritis, pigmentary changes and the perivascular cuffing and have undoubted analgesic nephropathy. Papillary necrosis by its very presentation is fundamentally an acute event, but the vascular changes are very slow and progressive.

CATTELL (London) Dr Furman, as I understand it, what you have demonstrated
is a change in the ratio of the excretion of the free and the conjugated form of
the drug. Now is that truly an increase in the excretion of the free compound
or merely a reduction in the excretion of the conjugated form, possibly as a
consequence of the damage the kidney has sustained?

FURMAN It is very difficult to be sure. There is a little bit of each. There is a
very big element that seems to be related to infections and that really is not
related to the hepatic path or the amount of non-conjugation. I think this is
mainly deconjugation, but again I think that there is an element of both.

CATTELL Have you for example measured the clearance ratio between the
free drug and creatinine and compared the clearance of the conjugated form
against creatinine? Do the clearance ratios change as between normals and
patients with established analgesic nephropathy?

FURMAN This is a very difficult thing to do from a technical point of view
because it is very very difficult to find any of the drug that is conjugated in the
serum. The conjugated portion is excreted so rapidly and the methods for detec-
tion are relatively crude. I have not got a micro method that would be able to
measure this. I do not know if anyone has. This is a technical problem.