PART XXXIV

GUEST LECTURE

Chairmen:  A J Wing
            P Maldague
PREVENTION OF AMINOGLYCOSIDE NEPHROTOXICITY

M E De Broe

University of Antwerp, Belgium

Introduction

Since the introduction of streptomycin in the mid 1940s, the aminoglycosides have remained a mainstay of our antibiotic arsenal. Several generations of ‘new’ cephalosporins have not replaced the aminoglycosides in the treatment of serious infections, possibly because of the ‘gap’ in their antimicrobial spectrum. The narrow therapeutic-toxic (nephrotoxicity, ototoxicity and rarely neuromuscular paralysis) range of these powerful drugs is, however, still a major concern [1].

In this paper, recent insights into the renal handling and the mechanism of nephrotoxicity of these drugs will be reviewed. It should help the clinician to better define the profile of the patients at risk and the drug-related conditions which increase the risk of nephrotoxicity. This information should contribute to the design of measures for the prevention of this common cause of acute renal dysfunction [2].

In addition to the intrinsic nephrotoxicity of the aminoglycosides, certain patients have been found to be particularly vulnerable (Table I).

<table>
<thead>
<tr>
<th>TABLE I. Prevalence of nephrotoxicity of aminoglycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>General use</td>
</tr>
<tr>
<td>Bodey et al (1972) [3]</td>
</tr>
<tr>
<td>Random hospital population</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Critically ill patient</td>
</tr>
<tr>
<td>Plaut et al (1979) [7]</td>
</tr>
<tr>
<td>Young volunteers</td>
</tr>
<tr>
<td>Smith (1981) [8]</td>
</tr>
</tbody>
</table>
Renal handling of aminoglycosides

Aminoglycosides are highly charged polycationic hydrophilic drugs which cross biological membranes poorly or not at all. After intravenous or intramuscular injection these non-absorbable drugs distribute in the vascular and interstitial (extracellular) space. They are not metabolized and are eliminated unchanged almost entirely by the kidneys [1]. Aminoglycosides are filtered by the glomerulus at a rate almost equal to water. After entering the luminal fluid of the proximal renal tubule a small, toxicologically important portion of the total filtered drug is reabsorbed and stored in the proximal renal tubular cells (Figure 1).

The transport of these hydrophilic polycationic drugs into the cell involves interaction with acidic negatively charged phospholipid binding sites (phosphatidylinositol) at the level of the brush border membrane of the proximal tubule. This phospholipid receptor appears to be a common anionic binding site that is competitively shared by amino acids, cationic polypeptides, proteins and the aminoglycosides [9].

After charge-mediated binding, the drug is taken up into the cell in small invaginations of the cell membrane, a mechanism called ‘carrier mediated pinocytosis’. This has been demonstrated by electron microscopic autoradiography [9,10]. Within one hour, the drug on the luminal side of the proximal tubule cell is translocated into apical cytoplasmic vacuoles. These endocytic vesicles fuse with lysosomes, allowing the aminoglycosides to be stored unchanged in lysosomes. This storage has been clearly demonstrated by cell fractionation, autoradiography at ultrastructural level and more recently by immunofluorescence [11]. Water and other small molecules (below 200 dalton) can escape from the lysosomes through diffusion. Aminoglycosides, however, are not able to escape from this organelle because of their relatively high molecular weight (≥450 dalton), their cationic nature and their binding to the acidic phospholipids inside the lysosome (pH 4.5) [12,13]. Moreover, since pinocytosis is a continuing phenomenon these drugs tend to accumulate extensively inside the lysosomes.

This sequence of events explains why, although only a very small fraction of filtered drug is taken up, there is progressive accumulation of aminoglycosides in the lysosomes of proximal tubular cells. This is supported by the finding that at a steady-state gentamicin serum concentration of 5μg/ml obtained by continuous infusion in a rat of 240g, the renal cortical concentration is 30μg/g after one hour [14]. Assuming the rat renal cortex is 0.8g, the total cortical gentamicin is 24μg under these conditions. The gentamicin load to the kidneys is 5μg/ml x 93 per cent non-protein bound x 2ml/min clearance x 60 min = 558μg. The fraction of the filtered load stored in the kidneys is therefore 24/558 (4.3%). Aminoglycosides accumulate in proximal tubular cells by lysosomal storage. Since proximal tubular cells occupy 60 per cent of the cortical mass, and lysosomes five per cent of proximal tubular cell, the lysosomes represent three per cent of the cortical mass [15]. The concentration of gentamicin in lysosomes should therefore reach a value of 30/0.03 = 1000μg/ml after one hour infusion, i.e. 2mM concentration, a value 200-fold higher than serum concentration.
Figure 1. Renal handling of aminoglycosides. After almost free filtration a small toxicologically important portion of the drug is reabsorbed by 'carrier mediated' (A) pinocytosis (B) and stored in lysosomes. This explains why these hydrophilic polycationic drugs 'cross' the membrane of the proximal tubular cell.
There is no conclusive evidence for a substantial uptake from antiluminal surface of proximal tubule cells, or for secretion of aminoglycosides. Most of the filtered drug is excreted in an unchanged form into the urine.

**Mechanism of nephrotoxicity**

Once trapped in the lysosomes of proximal tubular cells aminoglycosides inhibit lysosomal phospholipases and sphingomyelinase [16,17]. In parallel with enzyme inhibition, undigested phospholipids originating from the turnover of subcellular lipid membranes accumulate in lysosomes. The overall result is a phospholipidosis due to a non-specific accumulation of phospholipids, glycerophosphatidides and sphingomyelin, in myeloid bodies (Figure 2).

![Ultrastructural appearance of proximal tubular cell in aminoglycoside-treated patient](image)

**Figure 2. Ultrastructural appearance of proximal tubular cell in aminoglycoside-treated patient (4 days at therapeutic dose).** Lysosomes (large arrow) contain dense lamellar and concentric structures. Brush border, mitochondria (small arrow) and peroxisomes are unaltered. Upon higher magnification the structures in lysosomes show a periodic pattern. The bar in the left part represents 1μm, in the right part 0.1μm. Reprinted from De Broe et al, Kidney Int [18] with permission.

The evolution of this phospholipidosis (Figure 3) is at least in part dependent on the quantity of aminoglycosides stored in the lysosomes [19].

Once a threshold in cortical drug concentration is reached, the lysosomal phospholipidosis progresses and the lysosomes continue to swell even in the absence of any further drug administration. This may result in the loss of integrity of the restricting membranes and release of high amounts of aminoglycosides, lysosomal enzymes and phospholipids into the cytosol.
Figure 3. Evolution of aminoglycoside induced lysosomal phospholipidosis in proximal tubular cells

The clinically observed delay in onset of aminoglycoside nephrotoxicity [20] can easily be understood since it takes time for lysosomal phospholipidosis to result in intracellular release of the lysosomal contents and subsequent cell necrosis.

Binding of the aminoglycoside to sensitive targets such as mitochondria [21] or basolateral cell membranes may now occur with inhibition of sodium-potassium adenosine triphosphatase [22] and inhibition of a cytosolic phosphatidylinositol specific phospholipase C [23]. Overt cell necrosis and regeneration is readily apparent. As a consequence, clogging of certain tubular segments by necrotic cells and cell debris, increased intratubular pressure and decrease of glomerular filtration rate may ensue. Below the cortical threshold, however, there is regression of the drug-induced biochemical and morphological changes in the absence of any sign of cell necrosis or regeneration.

Aminoglycosides are polycationic drugs which bind to the negatively charged brush border. It is therefore not surprising that they compete with low molecular
Figure 4. Histopathological alterations of the kidney in a 67-year old female patient with aminoglycoside induced acute renal failure.

A. Epithelial desquamation and cell necrosis with obliteration of tubuli by necrotic masses. Vacuolated tubular cells, absence or thinning of brush border and tubular space limited only by a naked basal membrane at some places. Interstitial oedema and cellular infiltration is mild. Silver methenamine x 350.

B. Regenerating tubular cells (star) characterized by large vesicular nuclei basophilic cytoplasm and mitotic figure (arrow). Silver methenamine x 860
weight, freely-filtered and negatively charged β₂-microglobulin for binding sites on the brush border membrane. The reabsorption of β₂-microglobulin has been found to be competitively inhibited and its excretion enhanced by aminoglycosides [24]. Increase in the urinary excretion of tubular brush border enzymes such as alkaline phosphatase and alanine aminopeptidase are also early manifestations of the effect of aminoglycosides on the kidney. These 'early' indicators of renal effects are, however, too sensitive and non-specific to be of clinical value since every patient receiving aminoglycosides will exhibit these effects [25]. Tubular enzymuria cannot, therefore, be considered an indication of nephrotoxicity.

It is more useful to define aminoglycoside nephrotoxicity in terms of a reduction in glomerular filtration rate as reflected by a rise in serum creatinine. The serum creatinine is an adequate, inexpensive and convenient parameter for monitoring aminoglycoside nephrotoxicity.

The damage to the human kidney induced by aminoglycosides is most often mild, delayed in onset, reversible and only exceptionally leads to non-oliguric acute renal failure. The degree of reversibility of renal dysfunction associated with aminoglycosides is remarkable and can be attributed to the capacity of the proximal renal tubule to regenerate itself (Figure 4).

**Risk factors for aminoglycoside nephrotoxicity**

Some of the risk factors for aminoglycoside nephrotoxicity have been identified because of insights gained into their renal handling and mechanism of toxicity [26–28]. Others are derived from studies in experimental animals, case reports (mostly retrospective) and prospective, clinical trials. Although studies in experimental animals should be confirmed in clinical trials, they are useful in the recognition and control of potential risk factors. These factors can be divided into those related to the drug and its administration and those related to the clinical condition of the patient (Table II).

<table>
<thead>
<tr>
<th>Drug related</th>
<th>Patient related</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dose</td>
<td>1. Age</td>
</tr>
<tr>
<td>Duration</td>
<td></td>
</tr>
<tr>
<td>Dose regimen</td>
<td></td>
</tr>
<tr>
<td>2. Prior aminoglycoside treatment</td>
<td>2. Prior renal insufficiency</td>
</tr>
<tr>
<td></td>
<td>Hepatic insufficiency</td>
</tr>
<tr>
<td>3. Choice of the drug</td>
<td>3. 'Critically ill patient'</td>
</tr>
<tr>
<td></td>
<td>Sodium-volume depletion</td>
</tr>
<tr>
<td>4. Associated drugs</td>
<td>4. Other causes</td>
</tr>
<tr>
<td>Diuretics</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td></td>
</tr>
<tr>
<td>Cis-platinum</td>
<td></td>
</tr>
<tr>
<td>Amphotericin</td>
<td></td>
</tr>
</tbody>
</table>
It is obvious that those risk factors which can be prevented or modified must be identified as soon as possible and exploited by the clinician.

Drug related risk factors

Dose, duration, dose regimen Since it is clearly established that the first step in nephrotoxicity of aminoglycosides is the uptake of the drug into the proximal tubular cell, it is not surprising that dose and duration of drug administration are risk factors. Uptake of aminoglycoside into the proximal tubular cell is a low affinity, high capacity [9] and, for some, a non-linear saturable process [14]. The duration of exposure of the brush border to these drugs is, therefore, the critical first step in the nephrotoxic process. This explains why despite maintenance of therapeutic serum drug concentrations prolonged administration can result in nephrotoxicity. Although a safe duration of therapy has not been clearly identified, a treatment with aminoglycosides should not exceed two weeks.

Dose regimen may be an important determinant of aminoglycoside nephrotoxicity. Several groups [29–31] have shown that the severity of experimental nephrotoxicity of gentamicin is greater when the total daily dose is divided or given by continuous infusion rather than given as a single bolus.

The rationale for this important observation may be found in the recent comparative studies of renal cortical uptake-storage kinetics of different aminoglycosides [14]. Indeed, steady state elevations of serum gentamicin and netilmicin were associated with non-linear increase in renal cortical levels, strongly suggesting saturable uptake (Figure 5). Cortical uptake of tobramycin, however, was linearly related to serum values.

Amikacin showed a mixed kinetic pattern for cortical accumulation; a linear pattern at high serum concentrations, saturation kinetics at low serum concentration.

In other words, for gentamicin and netilmicin renal cortical uptake is less ‘efficient’ at high serum concentrations than the uptake observed at low serum concentrations.

Since renal cortical uptake of gentamicin and netilmicin is not linearly related to serum concentrations, a pronounced effect of dosage regimen on cortical accumulation can be expected. Indeed, the fraction of drug taken up by proximal tubule cells is much higher when the same amount of drug is given by continuous infusion than by intermittent or single injection(s) [32].

There is considerable evidence for non-linear uptake of gentamicin in the human kidney. Indeed, gentamicin given in a dose of 4.5mg/kg/day over one day as a continuous infusion results in a several fold higher cortical concentration than the same dose given as three bolus injections (De Broe et al, unpublished observations). Since recent studies have shown comparable antibiotic efficacy with spaced bolus injections [33,34] a thrice daily or twice daily dosing schedule will decrease the risk of nephrotoxicity for gentamicin in humans without interfering with its therapeutic effect. How far these results in humans are applicable to other members of the aminoglycoside group remains to be determined.
Prior aminoglycoside treatment According to Smith [26] there is currently no convincing evidence that prior aminoglycoside exposure is a risk factor for subsequent nephrotoxicity although some clinical studies suggested the contrary [35,36]. Storage of the aminoglycosides in lysosomes of proximal tubule cells and their very slow release from this compartment is now clearly established [19,20,37]. This storage and subsequent lysosomal phospholipidosis plays a central role in the development of nephrotoxicity. One can, therefore, reasonably understand that a kidney previously (one or two months) exposed to aminoglycosides which still has the stigmata of phospholipidosis, will show an ‘accelerated’ form of phospholipidosis and prompt nephrotoxicity.

Choice of the drug An impressive number of comparative studies [28] of aminoglycosides nephrotoxicity in humans are available.

It is difficult to draw firm conclusions from these studies because of the multiple clinical variables and because most of the studies failed to achieve critical methodological standards for comparative drug trials [38].

It is clear, however, that all aminoglycosides are nephrotoxic for the human
kidney and that the same mechanism is involved. Differences in renal cortical uptake and in lysosomal lipidosis, exist among members of the aminoglycoside family.

In a prospective controlled randomized comparative short-term study in humans using clinical doses, we could not distinguish gentamicin, tobramycin or netilmicin on the basis of tissue accumulation, lysosomal overloading or effect on lysosomal phospholipase A1. Amikacin induced significantly lower lysosomal overloading and no significant loss of phospholipase A1 activity compared to controls [18].

Associated drugs A number of studies in experimental animals have shown an increase in nephrotoxicity when loop diuretics were given with aminoglycosides compared to that observed with aminoglycosides alone [26,39]. This enhanced aminoglycoside nephrotoxicity observed with potent diuretics is most unlikely to be due to direct drug interactions. Indeed, extracellular fluid volume repletion minimizes or prevents the increased nephrotoxicity of aminoglycoside and loop diuretics. This suggests that the potent natriuretic action of these drugs is the cause of the increased nephrotoxicity [39,40]. The mechanism by which sodium and water depletion enhance nephrotoxicity is, however, unclear. Contraction of the extracellular compartment with increase in the serum aminoglycoside level, increased net drug reabsorption at the proximal tubule level, activation of hormonal systems modulating renal vasoconstriction and the effect of the potent loop diuretics on calcium and potassium have to be considered as possible pathophysiological mechanisms. In any case, extracellular fluid volume and sodium depletion must be avoided during the course of aminoglycoside treatment.

The concomitant administration of cephalothin and aminoglycosides clearly represents an additional risk factor due to intrinsic additive toxicity [26]. Amphotericin B, methoxyflurane, Cyclosporin A and cis-platinum, have each been associated with an increased risk of aminoglycoside nephrotoxicity [21]. Although many of these drugs can be nephrotoxic by themselves, convincing clinical evidence to support synergistic toxicity is not available.

Patient related risk factors

Age There are at least two reasons why advanced age has been suggested as a risk factor for aminoglycoside nephrotoxicity. Age goes along with a decrease in renal function and an important decline of the regenerative response to cell injury necrosis. Indeed, there is a reduction in glomerular filtration rate as a result of progressive vascular changes and glomerular sclerosis. Parallel with the diminution in renal function, there is a decrease in muscle mass and creatinine production. Hence the diminished glomerular filtration rate in the elderly is not necessarily reflected by a rise in serum creatinine [41]. Since the relationship between serum creatinine and creatinine clearance is not linear, an identical renal insult will result in a much larger increase in serum creatinine in older patients with reduced renal function, compared to the younger patients. Measurements
of endogenous creatinine clearances before and during aminoglycoside treatment in the elderly are therefore mandatory. The equation of Cockcroft and Gault [42] and the nomogram of Siersbaeck-Nielsen [43] take into account age, weight and sex and are useful methods for calculating the creatinine clearance ($C_Cr$) from the serum creatinine value ($Cr_s$):

$$C_{Cr} \text{ ml/min} = \frac{(140 - \text{age}) \times \text{weight}}{\text{Cr}_s \times 72}$$

Age in years, weight in kilograms, serum creatinine in milligram per decilitre. In females the same formula x 0.85 can be used.

Figure 6. Nomogram for rapid evaluation of endogenous-creatinine clearance. With a ruler, join weight to age. Keep ruler at crossing-point of line marked R. Then move the right-hand side of the ruler to the appropriate serum creatinine value and read the patient's clearance from the left side of the nomogram (from [43] with permission)
The derived endogenous creatinine clearance value allows calculation of an appropriate daily dose. A convenient method for that purpose is to multiply the usual dose when renal function is normal by the ratio of the patient's creatinine clearance to the normal creatinine clearance.

Aminoglycoside nephrotoxicity is non-oliguric and characterized by a remarkable degree of reversibility. Indeed, in experimental animals the potential for proximal renal tubular cell regeneration during high dose aminoglycoside treatment is enormous [19].

It is possible that the regenerative capacity of the ageing kidney is limited. An age associated decrease in turnover, growth and repair rates has been demonstrated in the human skin [44]. In this context it is worth mentioning that very young rats show a remarkable resistance to aminoglycoside nephrotoxicity [45].

Prior renal insufficiency Patients with pre-existing renal disease do not appear to be at a high risk for the induction of further renal impairment when treated with aminoglycosides. These are the conclusions of two well designed prospective studies [46,47], which appear to contradict impressions gained from poorly controlled retrospective studies and isolated case reports.

The widespread use of adjustment of the administration of aminoglycosides by reducing the dose or by extending the interval between dosing may have contributed to this apparent lack of risk in renal failure patients.

In the experimental animal we found, however, a rational basis for the absence of increased risk in renal failure. Continuous six-hour infusions of gentamicin in normal and remnant kidney renal failure rats demonstrated reduced uptake of drug in the hyperfiltering remnant kidney (Verpooten et al, unpublished observations). Thus, the hyperfiltration of residual nephrons may offer some protection against the development of phospholipidosis and subsequent nephrotoxicity.

In a prospective randomized clinical study, Moore et al [48] showed that liver disease was associated with the development of nephrotoxicity. Hepatic insufficiency leading to intra-renal vasoconstriction, reduced renal blood flow, and stimulation of the renin-angiotensin system are proposed as pathophysiological mechanisms.

Critically ill patients It is not surprising that the critically ill patient with sepsis, hypotension, hypoxaemia, sodium and volume depletion (see under Associated drugs heading), acidosis and decreased baseline glomerular filtration is at high risk for the development of a nephrotoxic reaction to aminoglycosides.

Several reports have defined other risk factors in experimental animals and measures by which it was possible to reduce the nephrotoxicity of aminoglycosides. Calcium-loading [49], phosphate depletion [50], streptozotocin-induced diabetes mellitus [51] and inhibition of angiotensin II [52] each provide some protection from the toxic effects of aminoglycosides. On the contrary, potassium or magnesium deficiency [27], acidosis [53] and male sex [27] have been associated with enhanced susceptibility to nephrotoxicity. The clinical relevance of these intriguing observations remains to be determined.
Monitoring of aminoglycoside therapy

There are two rationales for the monitoring of serum aminoglycoside levels: a therapeutic and a toxicological one. Serum drug monitoring early and during the aminoglycoside therapy of a critically ill patient should be performed to ensure the presence of adequate therapeutic drug concentration. Due to the over-emphasized importance of nephrotoxicity during aminoglycoside treatment underdosing rather than overdosing became a common prescribing error. Moreover aminoglycoside nephrotoxicity is usually mild and almost always reversible. Since the kinetics of creatinine and aminoglycosides are similar it is possible to rely on the simple, inexpensive and reliable serum creatinine determinations combined with a limited number of serum aminoglycoside levels for monitoring aminoglycoside nephrotoxicity.

In patients at risk and in patients with pre-existing renal failure, however, measuring of glomerular filtration rate and determination of serum trough levels is mandatory (see Table III).

<table>
<thead>
<tr>
<th>TABLE III. Serum aminoglycoside monitoring in patients at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>DETERMINE GLOMERULAR FILTRATION RATE (GFR)</td>
</tr>
<tr>
<td>USING NOMOGRAM OR FORMULA</td>
</tr>
<tr>
<td>GIVE USUAL LOADING DOSE</td>
</tr>
<tr>
<td>MAINTENANCE DOSE ACCORDING TO GFR</td>
</tr>
<tr>
<td>AFTER 3–4 DOSES DETERMINE TROUGH LEVELS</td>
</tr>
<tr>
<td>SUB-THERAPEUTIC LEVELS</td>
</tr>
<tr>
<td>TOXIC</td>
</tr>
<tr>
<td>THERAPEUTIC CONCENTRATION</td>
</tr>
<tr>
<td>MONITORING GFR</td>
</tr>
<tr>
<td>10–12 DAYS = STOP</td>
</tr>
</tbody>
</table>

Conclusion

As soon as treatment with an aminoglycoside begins the proximal tubule cell starts accumulation of the drug and development of lysosomal phospholipidosis. Insight in renal handling, mechanism of nephrotoxicity and identification of risk factors, however, enable the clinician to reduce drastically the prevalence and degree of renal damage by aminoglycosides (Table IV).

All aminoglycosides are nephrotoxic, especially in patients who have to be treated with these drugs. On the other hand nephrotoxicity is usually mild, reversible and susceptible to secondary prevention. Excessive fear of an over-emphasized side effect will result in under-treatment of the critically ill patient.
TABLE IV. Guidelines for the prevention of aminoglycoside nephrotoxicity

- PATIENT
  IDENTIFY RELATED RISK FACTORS
- DRUG

USE SINGLE, TWICE DAILY DOSE REGIMEN
NEVER USE CONTINUOUS INFUSION

LIMIT TREATMENT COURSE TO 10 DAYS
RESPECT INTERVAL BETWEEN TREATMENT COURSES
MEASURE GLOMERULAR FILTRATION RATE OUT OF SERUM CREATININE BEFORE AND DURING THERAPY (Formula, Nomogram)

Acknowledgments

Support for research in this paper came from the following sources: Belgian National Fund for Scientific Research grants 3.0069.82 and by the Scientific Research Planning of the Belgian Government contract 82-87/47.

My thanks to RA Giuliano, G Paulus, F Roels, P Tulkens, GA Verpooten for the numerous fruitful discussions and to RP Weeden for critical advice on this manuscript.

The secretarial work of E Snelders is gratefully acknowledged.

References

5 Fridmond-Möller N, Maigaard S, Madsen PO. Infection 1980; 8: 283
7 Plaut ME, Schentag TJ, Jusco WJ. Lancet 1979; ii: 526
8 Smith CR. 12th International Congress of Chemotherapy, Florence, Italy. 1981: 174
9 Just M, Habermann E. Naunyn Schmiedebergs Arch Pharmacol 1977; 300: 57
10 Silverblatt FJ, Kuehn C. Kidney Int 1979; 15: 335
12 Ohkuma S, Poole B. Proc Nat Acad Sci USA 1978; 75: 3327
15 Pfaller W. Structure, Function Correlation on Rat Kidney. Berlin: Springer-Verlag. 1982: 1
Cronin RE. Clin Nephrol 1979; 11: 251
Lipsky JJ, Lietman PS. J Pharmacol Exp Ther 1981; 220: 287
Whelton A. J Clin Pharmacol 1985; 25: 45
Bennet WM, Plamp CE, Gilbert ON et al. J Infect Dis 1979; 140: 576
Reiner NE, Bloxham DD, Thompson WL. J Antimicrob Chemother 1978; (Suppl A) 4: 85
Lane AZ, Wright GE, Blair DC. Am J Med 1977; 62: 911
Lietman PS, Smith CR. Rev Infect Dis 1983; 5 (Suppl 2): 284
Epstein M. Fed Proc 1979; 38: 168
Cockcroft DW, Gault MH. Nephron 1976; 16: 31

973