Mechanisms of Antibiotic-Induced Nephrotoxicity

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ABSTRACT

Each of the major classes of clinically useful antibiotics can cause nephrotoxicity. Major differences exist among classes, and these are reviewed with regard to the frequency of occurrence of nephrotoxicity, the direct or indirect toxic effects of the drugs, their site(s) of action within the kidney, current views of their pathophysiological effects and observations which provide clues to the biochemical mechanisms underlying their adverse effects on kidney structure and function. Even for a single abnormal kidney function, such as non-oliguric reduction of glomerular filtration rate by gentamicin, the physiological and biochemical mechanisms may be complex. In no instance has the biochemical mechanism underlying antibiotic-induced nephrotoxicity been definitely established.

Introduction

Nephrotoxicity has long been recognized as a serious complication resulting from antibiotic administration. The vast array of antibiotic types and derivatives available to the clinician and the increased monitoring for early signs of renal impairment have reduced the risk of kidney damage compared to the early days of sulfonamide and neomycin therapy. However, the improved technical capacity to maintain the severely debilitated patient who is markedly more susceptible to a variety of opportunistic pathogens and the increasing incidence of microbial drug resistance often necessitate the use of antibiotics with relatively low therapeutic indices and significant nephrotoxic potential. In addition, some widely employed antibiotics with low potential for inducing renal disease will infrequently have significant detrimental effects on the kidneys of certain patients. These idiosyncratic drug responses may reflect intrinsic or environmentally-induced changes in renal function or drug metabolism. The intent of this paper is to review concisely current insights into the mechanisms underlying the most frequently encountered types of antibiotic-induced nephrotoxicity. The aminoglycosides, amphotericin, and the polymixin
are reviewed in detail based on a literature search complete through December, 1980. Additional aspects of this topic can be found in excellent reviews by Appel and Neu¹ and Sanders and Sanders.⁵⁶

**General Features of Antibiotic-Induced Nephrotoxicity**

Several general considerations help to provide an overview of the pathophysiological effects of antibiotics on the kidney. (1) Toxicity has been documented to occur upon administration of the major classes of therapeutically useful antibiotics. (2) The frequency of occurrence of antibiotic-induced nephrotoxicity varies greatly and depends upon the class of antibiotic, the properties of particular derivatives within one antibiotic class, and the physiological status of the patient. (3) Several anatomically and functionally distinct kidney sites are susceptible targets for the adverse effects of antibiotics. (4) Antibiotic-induced nephrotoxicity may be indirectly or directly mediated.

### TABLE I

**Commonly Employed Antibiotics Causing Nephrotoxicity**

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Chemical Structure</th>
<th>Microbial Target</th>
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<tr>
<td><strong>Amphotericin</strong></td>
<td>Large double bonded ring structure (polyene)-amino sugar conjugates</td>
<td>Membrane permeability (fungal)</td>
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<tr>
<td><strong>Polymyxins</strong></td>
<td>Small cationic polypeptides</td>
<td>Membrane permeability</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td>Three covalently bonded amino sugars</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td><strong>Sulfonamides</strong></td>
<td>Derivatives of p-aminobenzene-sulfonic acid</td>
<td>Folic acid synthesis</td>
</tr>
<tr>
<td><strong>Rifampicin</strong></td>
<td>Complex double bonded ring structure</td>
<td>Ribonucleic acid synthesis</td>
</tr>
<tr>
<td><strong>Penicillins</strong></td>
<td>Thiazolide (five membered) - S-lactam ring conjugates</td>
<td>Cell wall synthesis</td>
</tr>
<tr>
<td><strong>Cephalosporins</strong></td>
<td>Thiazolide (six membered) - S-lactam ring conjugates</td>
<td>Cell wall synthesis</td>
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As shown in table I, these antibiotics include a diverse group of chemically dissimilar compounds which exert their antimicrobial effects through site-specific action on several distinct biological targets. The structural and functional dissimilarities among these agents, e.g., polymyxin and penicillin, suggested the likelihood that differences would occur in their target sites within the kidney, in their biochemical mechanisms of action on renal tissue, and in their frequencies of inducing renal damages. The order of arrangement of the antibiotics listed in table I reflects in general their propensity for impairing renal function. Thus, most patients receiving amphotericin B have evidence of some degree of nephrotoxicity.⁷,¹³,⁶⁸ The high frequency (approximately 20 percent) of occurrence of renal damage associated with colistimethate (polymyxin E)³⁵ and other polymyxins⁵¹ limits their clinical application to situations in which other antibiotics cannot be employed. Nephrotoxicity associated with the aminoglycosides ranges from 2 to 25 percent of treated patients, depending upon the specific aminoglycoside administered, the criteria for defining toxicity, and the patient population.⁹,¹⁵,⁵⁹

The remaining classes of antibiotics listed in table I are much less frequently associated with untoward renal effects. From the search for improved therapeutic indices and more useful antimicrobial action spectra, many analogs of classic antibiotic types have become available. These derivatives often differ markedly in their renal effects. For example, the aminoglycosides, tobramycin and amikacin, are markedly less nephrotoxic than gentamicin in both animal models⁴⁰,⁴¹,⁴³ and in man.⁵⁹,⁶⁰,⁶¹ The relationship between empirically determined nephrotoxicity and chemical modifications of the parent antibiotic can help to identify particular portions of the antibiotic molecule which function in the induction of renal damage and may suggest possible
physiological mechanisms of action of these drugs.

In addition to the intrinsic toxicity of an antibiotic, its nephrotoxicity depends upon physiological influences and pharmacokinetic considerations, such as the maximum concentration achieved and the duration of significant levels of the antibiotic. Pre-existing renal dysfunction, the disease process rendering the individual susceptible to infection, changes in renal blood flow, electrolyte imbalance and pre- or concomitant administration of other nephrotoxic drugs, increases the likelihood of deleterious effects of antibiotics on the kidney. These factors and the problem of distinguishing between the patient’s underlying illness and drug-induced nephrotoxicity make difficult the determination of the incidence of antibiotic-induced renal disease in patients. Such factors further complicate the determination of the mechanisms responsible for the deleterious action of antibiotics on the kidney.

With the possible exception of the Loop of Henle, each region of the nephron can be damaged by one or more antibiotic classes. The proximal tubule, site of the quantitatively most significant reabsorptive functions, is particularly vulnerable. If generalized effects, such as phenomena related to hypersensitivity, are excluded, most antibiotics affect only one or a few targets. For example, the major site of action of the aminoglycosides is the proximal tubule,\(^2,16,32\) the site of potential toxic action of the currently used sulfonamides is the collecting duct. Site specificity of antibiotic-induced nephropathy implies several possibilities: (1) there exist cell types with distinctly susceptible targets for the deleterious effects of certain specific antibiotics; (2) the drug may achieve toxic concentrations in only certain kidney regions; and (3) toxic metabolites may be generated in only certain kidney cells. However, in addition to the apparently direct deleterious action of many antibiotics on renal functions, certain antibiotics may act indirectly.

In table II is indicated the association of each of the major classes of antibiotics with either immunity-mediated phenomena or direct toxic action. In general, the immunity-related nephrotoxic activities of antibiotics occur infrequently. Interstitial nephritis brought about by methicillin is a rare complication that appears to be associated with prolonged exposure to high levels of antibiotic.\(^3,10\) Fever, eosinophilia, and rash, indicators of an immune reaction, often precede or occur concomitantly with the penicillin-induced renal lesion.\(^3\) Penicillins, serving as haptens, produce allergic reactions through their ability to stimulate antibody production and to initiate hapten-antibody reactions. In patients, circulating antibodies to penicillins are not directed to the intact penicillin molecule but to its degradation products, e.g., the penicilloyl group, that can form covalent bonds to proteins.\(^40,50\)

Target organ specificity, as for example might be suggested to explain the occurrence of interstitial nephritis, could result from at least four biochemical factors. (1) The intrinsic activity of the metabolic pathway converting the antibiotic to the activated hapten may be restricted to a specific tissue and thereby elevate its local concentration. (2) Conjugation of the

<table>
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<td>Antibiotic - Induced Nephrotoxicity Arises By Two Different Mechanisms</td>
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<th>Indirect Action (Immunity-Medicated)</th>
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<tr>
<td>Penicillins (especially methicillin)</td>
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<td>Cephalosporins (especially cephalothin)</td>
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<td>Sulfonamides</td>
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<td>Rifampicin</td>
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hapten to a particular tissue-specific protein may generate antibodies which react with the protein directly. This may lead to localized autoimmune disease. (3) One tissue may possess the ability to concentrate the antibiotic to levels sufficient for the generation of significant quantities of the hapten. (4) The specific capacity of a tissue to bind antigen-antibody complexes formed elsewhere may predispose it to immunologically-mediated damage. It is unlikely that an interstitial concentrating mechanism contributes to the occurrence of interstitial nephritis induced by penicillins because immune-mediated nephrotoxicity occurs with penicillins that differ from one another markedly with regard to protein binding and serum half life. The kidney is one of the target organs in drug-induced serum sickness, the glomerulus being an especially frequent site of non-specific localization of antigen-antibody complexes. Although this phenomenon would seem to be a logical consequence in many penicillin recipients who possess antibodies to the drug, the absence of the characteristic glomerular lesion seen in other immune complex disease and the extreme rarity of penicillin-induced glomerulitis suggest that immune mechanisms account for penicillin-induced nephrotoxicity. Certain cephalosporins also act indirectly on the kidney. Some instances of cephalothin nephropathy have a strong resemblance to penicillin-induced nephropathy. Nephrotoxicity associated with cephalothin is frequently characterized by signs of hypersensitivity including rash, eosinophilia, and interstitial nephritis. In neither penicillin-induced nor cephalosporin-induced renal lesions is the exact mechanism of immunemediation established.

With this general background, the direct toxic actions of antibiotics as they pertain to the kidney will be discussed.

Mechanisms of Nephrotoxic Antibiotics

Consideration of the spectrum of chemical structures, the known mechanisms of antimicrobial actions (table I), and the diverse sites of action of the nephrotoxic antibiotics suggests that a variety of distinctly different mechanisms must account for their direct toxic action on renal structure and function. Although much progress has been made in identifying the anatomical sites which are the targets for renal damage by antibiotics, there currently exists only a partial description of the physiological events that are associated with the derangement of renal functions by antibiotics. In no case has the biochemical mechanism underlying specific renal physiological alterations been established. For certain antibiotics, however, descriptions of their action are sufficiently detailed that one may begin to predict their probable mechanism(s) of action. The known nephrotoxic actions of several antibiotic classes are summarized in the following sections.

AMPHOTERICIN B

Nephrotoxicity is the most serious side effect of amphotericin B, a polyene macrolide antifungal agent. Most patients receiving the drug demonstrate some degree of dose-dependent nephrotoxicity. Urinalysis reveals hematuria, pyuria, presence of tubular cells, and cylindruria but little proteinuria. Histologic changes in blood vessels and glomeruli occur infrequently and are not specific for the drug. In animals, amphotericin causes intratubular calcium deposits accompanied by necrosis of the proximal and distal convoluted tubules and thickening of the tubular basement membranes. Much of the effect of amphotericin on the kidney involves its action on the distal tubule. The drug causes a distal tubular acidosis charac-
terized by net acid excretion and reduced acidification of urine in response to an acid load. Both inulin and p-aminohippuric acid clearance decrease during acute administration of amphotericin. Decreased glomerular filtration rate and ischemic damage appear to be secondary to drug-mediated renal vasoconstriction. These effects are probably the result of the action of amphotericin on the distal tubule. It has been established that an increased delivery of chloride ion to the distal tubule can lead to a profound decrease in the glomerular filtration rate of the nephron. This suppression of glomerular function by the distal tubule is called tubulo-glomerular feedback. Both this tubulo-glomerular feedback mechanism and the acute ischemic change caused by amphotericin are attenuated by preloading animals with sodium or by pre-treating them with furosemide. These findings are consistent with the hypothesis that the acute amphotericin-mediated response includes an increased chloride permeability of the distal tubule followed by activation of tubulo-glomerular feedback.

How can this site-specific action be reconciled with the known biochemical activities of amphotericin? Polyene macrolide antibiotic toxicity toward eukaryotic cells in generally attributed to membrane permeability changes resulting from polyene macrolide-sterol interactions. Amphotericin is slowly excreted and has a half life of about 24 hours. It is almost completely protein bound; impaired renal function does not significantly alter the serum concentration. Thus, generalized cellular toxicity resulting from membrane alterations might be expected in the kidney. However, under certain circumstances it is possible to dissociate toxicity and permeability changes. In some in vitro experimental systems, cell toxicity has been demonstrated at polyene macrolide concentrations that do not elicit changes in membrane permeability. Alternatively, immediate membrane damage may occur in the absence of cell lethality. It appears, then, that the acute effect of amphotericin B on the distal tubule is a reflection of a rapid permeability change to chloride ions. With extended therapy, this may be accompanied by a more generalized cellular toxicity which results from continued exposure to the drug or from exposure to an increased concentration of this drug.

**The Polymyxins**

The polymyxins—polymyxin B, colistin, and colistimethate—are basic, cyclic polypeptide antibiotics with a broad range of activity against Gram-negative bacteria. All are associated with significant nephrotoxicity typically characterized by decreased creatinine clearance, proteinuria and cellular casts in the urine. In fatal cases, histological studies have established proximal tubular damage and acute tubular necrosis. Physiological evidence for the action of polymyxins on the proximal convoluted tubule has been obtained from in vitro studies employing hippurate uptake by rabbit renal cortical slices. (Hippurate transport is a biochemical marker of proximal tubule function.) In man, the degree of inhibition of this organic acid transport system by the polymyxins is well correlated with their relative ability to induce nephrotoxicity. While the origin of the relatively specific effects of polymyxins on the proximal tubule may in some manner relate directly to the proximal tubule organic base transport system, this hypothesis remains to be verified.

It seems reasonable to assume that the kidney is susceptible to the surfactant action of the polymyxins through which their antibacterial action is mediated.
The interaction of these compounds with the phospholipid component of bacterial cell membranes, especially phosphatidyethanolamine groups, leads to changes in membrane permeability and cell death.\textsuperscript{23} Polymyxins bind to the cellular components of the kidney,\textsuperscript{37} and polymyxin B accumulates intracellularly to high levels in the kidney.\textsuperscript{34} The drug is retained for an extended period in an unmetabolized form. With regard to the chemical structure of the polymyxins, the degree of tissue binding, nephrotoxicity, inhibitory action on proximal tubular hoppurate transport, and antibacterial activity are directly related to the number of free amino groups in this antibiotic.\textsuperscript{46,48,52,64} It has been suggested that a balance between protonated and unprotonated amino groups in the molecule is important in both antibacterial and nephrotoxic effects since there is a pH optimum for hoppurate transport inhibition and phospholipid binding by polymyxins.\textsuperscript{23,46} From these observations it can be conjectured that proximal tubular damage by polymyxins may result from their relatively selective accumulation in cells of the proximal tubule where their concentration becomes sufficient to produce directly significant membrane damage.

THE AMINOGLYCOSIDES

Nephrotoxicity and ototoxicity are major factors limiting the clinical utility of the aminoglycosides. Included among the group of clinically useful aminoglycosides are neomycin, gentamicin, kanamycin, tobramycin, netilmicin, amikacin, and streptomycin. The preceding list is given in order of decreasing nephrotoxicity and is based upon a composite of data derived from experiments in animal models\textsuperscript{19,20,22,32,42,44} and clinical observations.\textsuperscript{9,59,69,61} Neomycin, sometimes employed orally to reduce the gut flora, is not administered intravenously because of its high frequency of nephrotoxic complications. The problems of extrapolating to man conclusions based on observations in animal models are numerous, but the agreement is surprisingly good for those aminoglycosides rigorously evaluated in man (e.g., comparing nephrotoxicity of gentamicin and tobramycin).\textsuperscript{44,60}

Clinical observations and animal experiments demonstrate that aminoglycosides can induce both structural and functional kidney damage.\textsuperscript{5,26,32,36} Typical findings include proteinuria, enzynuria (derived from proximal tubule lysosomal enzymes), hematuria, cylindruria, elevated blood urea nitrogen, reduced glomerular filtration rate and impaired urinary concentrating ability.\textsuperscript{1,2,16} These abnormalities may or may not be associated with oliguria, although the disease may progress to oliguric renal failure should aminoglycoside administration be continued. Proximal tubule damage progressing to acute tubular necrosis typifies the histological picture associated with aminoglycoside-induced renal damage.\textsuperscript{14,32,36} The glomeruli and blood vessels are not histologically altered. Myeloid body formation is a prominent feature of aminoglycoside nephrotoxicity and occurs most prominently in lysosomes of proximal tubule epithelial cells.\textsuperscript{20,32,36}

Aminoglycosides are not metabolized to a significant extent, and the major route of excretion is by glomerular filtration.\textsuperscript{1,2,32} In patients with severely impaired renal function, the serum half life of these drugs increases to 25 to 40 times that of the two hours\textsuperscript{58,38,41} seen in normal individuals. One feature of aminoglycoside antibiotics which distinguishes them from most other antibiotics is their accumulation in the renal cortex\textsuperscript{42,43,44,67} (consistent with the tubular action of aminoglycosides since the tubules are located in the renal cortex). The ratio of cortex-bound to serum aminoglycoside concentration ranges from 5 to 20, depending on experimental conditions.
Other classes of antibiotics, e.g., penicillins or cephalosporins, have cortical to serum ratios ranging from one to three. The capacity of the kidney to accumulate aminoglycosides is large, and repeated injections result in progressively increased cortical levels.

In dogs, at stable therapeutic serum levels, the aminoglycosides can be sequentially ordered with regard to their levels of cortical accumulation. From highest to lowest these are—neomycin > gentamicin > kanamycin > tobramycin > streptomycin—an order which bears a remarkable similarity to their relative nephrotoxic potencies. Streptomycin is not accumulated to a significant extent and is the least toxic. Results generally similar to those in the dog are found in the rat model. One exception, netilmicin, an aminoglycoside with low nephrotoxicity, accumulates to an extent similar to highly toxic gentamicin. The toxic effects of all aminoglycosides are known to be both dose- and duration-dependent. In addition to being concentrated in the renal cortex, these antibiotics also are retained there in chemically unmodified form for an extended period of time. In the rat, for example, the half-time of gentamycin in serum is 0.5 hours, whereas in the renal cortex the half-time is 109 hours. A single dose may persist for days and, after cessation of drug administration following repeated doses, aminoglycoside may be detectable in urine for weeks. These results may explain delayed nephrotoxicity and development of nephrotoxicity in patients receiving a second course of therapy within a few weeks of previous drug therapy.

There is no definitely known mechanism explaining how these antibiotics cause their deleterious effect on cells of the proximal tubule. The aminoglycosides exert their effect on bacteria by binding to specific protein components of the small (30S) ribosomal subunit. This binding results in inhibition of protein synthesis and mis-translation during polypeptide chain elongation. Although these effects usually have not been demonstrated to occur with eukaryotic cytoplasmic ribosomes, there are reports that in eukaryotes inhibition of protein synthesis and messenger RNA misreading can occur in vitro. The proximal tubule specificity observed in vivo may result from the unusually high levels of aminoglycoside achieved and intrinsic or environmentally induced factors. Such factors appear to make certain tubular cells sensitive to the drug, perhaps at the level of the protein synthesis. Other mechanisms may, however, be involved. For example, neomycin has major effects upon polyphosphoinositide metabolism in guinea pig kidney both in vivo and in vitro. In view of the probable function of polyphosphoinositides in membrane permeability and ion transport, alteration of their metabolism could have profound effects on the cell. Finally, there may be unexpected effects of aminoglycosides on particular metabolic functions, for example, the inhibition of the Embden-Meyerhof pathway by kanamycin has been shown in the guinea pig.

A less generally appreciated second target for aminoglycosides is the glomerulus. Reduction of glomerular filtration rate often occurs after aminoglycoside administration and is frequently of the non-oliguric type. Baylis has investigated the mechanisms responsible for the impairment of glomerular filtration by gentamicin in the rat. Several factors alone or in combination could cause a fall in glomerular filtration rate. (1) There is a major and directly proportional dependence on the renal plasma flow rate. (2) Reduction of the hydrostatic pressure difference across the capillary wall has an important effect. (3) An increase in plasma protein concentration leading to elevated plasma oncotic pressure will decrease filtration rate. (4) Reduction in capillary
water permeability and/or effective surface area in the glomerulus will reduce the glomerular filtration rate. At low to moderate concentrations of gentamicin, micropuncture studies establish the significant alteration of the last pair of parameters. At high concentrations of the antibiotic, the reduction in glomerular filtration rate is multifactorial.\textsuperscript{5,6} The detailed molecular mechanisms accounting for any of these physiological changes by gentamicin are completely unknown.

Conclusions

Among nephrotoxic agents, the most common offenders are drugs prescribed for the treatment of clinical conditions not directly concerned with the kidney. In this category, antibiotics are major inducers of kidney disease. While the incidence of nephrotoxicity mediated by allergic responses is uncommon, high frequencies of renal impairment occur with the administration of the major antibiotic used to combat systemic fungal infections, amphotericin B, and the aminoglycosides used to combat broadly antibiotic resistant organisms such as \textit{Pseudomonas aeruginosa}. A detailed picture is beginning to be established of the pathological alterations which arise in the kidney upon antibiotic administration. A few of the circumstances which may potentiate antibiotic-induced nephrotoxicity are known, but it is unclear why in the same kidney some nephrons are damaged while others are not when presumably exposed to similar local concentrations of the drug. In no case has the underlying biochemical mechanism been definitively established for antibiotic-induced nephrotoxicity. Further clarification of the underlying mechanisms of toxicity could have major impact upon the design of new antibiotic analogs with reduced nephrotoxic properties and for the establishment of therapeutic regimens which ameliorate the nephrotoxic effects of antibiotics without impairing their antimicrobial activity.

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