Antibiotic-Induced Nephrotoxicity

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INTRODUCTION

Antibiotics have long been, and remain, a major cause of drug-related renal toxicity, causing both acute renal failure and tubulointerstitial disease, depending on the drug. Most cases of acute renal failure are related to acute tubular necrosis, for which aminoglycosides have long been one of the leading causes. Tubulointerstitial disease is more commonly seen with β-lactams (hypersensitivity nephropathy), but can also be caused indirectly by aminoglycosides (tubulointerstitial injury).

Two complicating factors make the nephrotoxicity of antibiotics more common than expected. First, many of these drugs are given in combination, either between themselves or with other drugs. Consequently, the toxicity of one agent may be aggravated by the other one, as exemplified by vancomycin–aminoglycosides combinations, or the coadministration of aminoglycosides and nonsteroidal anti-inflammatory agents. Second, many antimicrobials are removed from the body essentially, or at least predominantly, through the renal route. The serum levels of these drugs will therefore increase as renal function becomes impaired, either as a consequence of the drug toxicity itself or because of concomitant renal damage caused by another drug or by another cause of nephrotoxic reaction. This applies both to drugs eliminated by glomerular
filtration and those that are removed from the body by tubular secretion if renal function is severely compromised (creatinine clearance of 20 ml/min or less).

These situations are common in severely ill patients for whom effective antimicrobial chemotherapy must be utilized. Thus, toxic levels of aminoglycosides and vancomycin are often observed in these patients unless close monitoring of both renal function and the drugs is performed. The same may occur with certain penicillins, fluoroquinolones, and tetracyclines, for which drug monitoring is not routinely performed.

These considerations explain many cases of antibiotic-related toxicities, and necessitate careful adjustment of doses, based on age, sex, body weight, and renal function. Clinicians must, however, be warned against inappropriately moving towards low doses of antimicrobials for fear of toxicity (a trend that was frequent in the late 1980s and early 1990s). It is indeed now well established that inefficient antimicrobial therapy leads to clinical failure as well as to risk of resistance (Craig, 2001), thereby exposing the patient to additional toxicities related to the persistence of the infection and the need to prolong the therapy. Efforts must be directed at selecting the most appropriate antibiotic and administering it at the maximal acceptable dose as defined by the peak serum concentration, the area under the 24 hour serum concentration, and the time during which the serum concentration will remain above the critical threshold (Amsden et al., 2000).

This chapter reviews the data available on the three classes of antimicrobial agents that have been most commonly associated with renal toxicities: aminoglycosides, β-lactams, and vancomycin. Other antibiotics will be only briefly touched upon because data are scanty and the mechanisms often less clear.

**AMINOGLYCOSIDES**

Aminoglycoside antibiotics are polar, cationic molecules that are all rapidly excreted by the kidney without significant metabolism. The first group comprises streptomycin and its closely related derivative dihydrostreptomycin, which were widely used from the late 1940s through the mid 1960s to treat Gram-negative infections and tuberculosis. Emergence of resistance and the desire to enlarge the spectrum towards “difficult to treat bacteria” such as *Pseudomonas aeruginosa* led to the development and introduction of kanamycins (represented mostly by kanamycin A
and tobramycin, and, in some countries, by dibekacin), sisomicin, and gentamicin (which is a mixture of three major components referred to as gentamicin C₁, C₁₄, and C₂ in approximately equimolar amounts). These were extensively used up to the mid 1980s. Further development of resistance to these naturally-occurring molecules eventually triggered the design of semisynthetic derivatives (amikacin [from kanamycin A], netilmicin [from sisomicin], isepamicin [gentamicin B], and arbekacin [from dibekacin]), which were made to resist bacterial inactivating enzymes responsible for this resistance (Mingeot-Leclercq et al., 1999).

Despite all these developments, ototoxicity and nephrotoxicity remained of concern to clinicians and resulted in a marked limitation in the use of these otherwise potent and life-saving antibiotics. Because it proved very difficult, if not impossible, to separate antibacterial efficacy and toxicity (Price, 1986), the pharmaceutical industry largely, if not entirely, abandoned this field of research in the late 1980s. In parallel, there has been an intense effort in laboratory and clinical research (more than 1,500 publications are referenced in public databases since 1969). Therefore, there is now a deep knowledge of the epidemiology, pathology, molecular mechanisms, and clinical significance of these toxicities, but no safe compounds. This explains why efforts at reducing aminoglycoside toxicity have met with such interest since the mid 1990s, and even today these remain the only means of protecting patients.

**Epidemiology**

The reported incidence of aminoglycoside nephrotoxicity varies from 0 to 50%, with most reports in the 5 to 25% range (Bertino, Jr. et al., 1993; Lane et al., 1977; Lerner et al., 1983). This variability results from differences in definition of nephrotoxicity, nature and frequency of the criteria used to assess renal function, and, perhaps most importantly, the clinical setting in which the drugs are used. The incidence in aged patients suffering from multisystem diseases and exposed to other potential nephrotoxicants ranges as high as 35 to 50%, whereas this figure may be close to zero in young healthy volunteers (Appel, 1990; Lane et al., 1977). In prospective randomized studies with definitions of nephrotoxicity that reflect a substantive decrement of glomerular filtration rate in seriously ill patients, the reported incidence of nephrotoxicity varies between 5 and 10% of patient courses (Lane et al., 1977;
Smith et al., 1977). In surveys of the etiology of acute renal failure in hospitalized patients, about half of the drug-induced cases of renal toxicity were attributable to aminoglycosides.

**Clinical Features**

Nephrotoxicity induced by aminoglycosides manifests clinically as non-oliguric renal failure, with a slow rise in serum creatinine concentration and a hypoosmolar urinary output developing after several days of treatment. The first signs of tubular dysfunctions or alterations are: release of brush border and lysosomal enzymes, decreased reabsorption of filtered proteins, wasting of potassium, magnesium, calcium, and glucose, and phospholipiduria. Progression to dialysis-dependent oliguric-anuric renal failure is unusual unless other risk factors are present (Appel, 1990). The renal failure is generally reversible. In a few patients there has been documented recovery of renal function despite continued administration of the aminoglycoside (Trollfors, 1983). Conversely, cases of fatal anuria have been reported. Occasionally, a Fanconi's syndrome (Casteels-Van Daele et al., 1980) or a Bartter's-like syndrome (Landau and Kher, 1997) has been observed.

**Risk Factors for Aminoglycoside Nephrotoxicity**

Based on clinical observations, there appear to be a variety of factors that predispose to the development of renal dysfunction with aminoglycoside therapy (Appel, 1990). It is important to distinguish between patient- and drug-related factors since this will guide the caregiver in determining the most appropriate course of action.

**Patient-Related Factors**

Age

The incidence of amikacin nephrotoxicity rises with advancing age, from 7% in patients under age of 30 years to 25% in patients over 75. The most likely mechanisms are twofold. First, the number of active nephrons decreases with age, leaving the patient with less reserve upon injury of a large proportion of nephrons. Second, because of the falling renal function, dosages may often be excessive if only based on insensitive renal function tests such as the measurement of serum urea nitrogen or serum creatinine concentrations, which rise
significantly only when a large proportion of active nephrons is damaged (Moore et al., 1984).

*Pre-existing Renal Diseases*

In patients with pre-existing renal disease, as estimated by serum creatinine concentration greater than 2 mg/dl, the study of Moore et al. (1984) found no increase in toxicity risk if the dose was carefully adjusted. However, pre-existing renal failure clearly exposes the patient to inadvertent overdosing. In addition, kidneys from patients with pre-existing renal disease may have decreased ability to recover from ischemic or toxic insults (Beauchamp et al., 1992b; Manian et al., 1990).

*Gender*

Female gender was identified as a risk factor in one study but not confirmed in others (Kahlmeter and Dahlager, 1984; Moore et al., 1984). Conversely, male gender was also reported in a retrospective analysis as a risk factor (Bertino et al., 1993). The matter is, therefore, unsettled. Animal studies are of little value in this context since most are performed with rats, and it is known that male laboratory rats tend to be spontaneously proteinuric, which in itself may be a risk factor.

*Volume Depletion/Hypotension*

Depletion of intravascular volume is an important risk factor for aminoglycoside-induced nephrotoxicity, whether induced by sodium depletion, hypoalbuminemia, or diuretics, even when systemic acid-base and electrolyte-volume status are maintained (Gamba et al., 1990). Hypokalemia and hypomagnesemia may be both predisposing risk factors or consequences of aminoglycoside-induced damage (Nanjie and Denegri, 1984; Zaloga et al., 1984).

*Liver Diseases*

Liver dysfunction was identified as a risk factor in a retrospective analysis of two large clinical trials and was then validated in two additional prospective trials (Lietman, 1988). This is particularly true for patients with biliary obstruction, cholangitis, or both, rather than for other causes of liver disease, such as alcoholic cirrhosis (Desai and Tsang, 1988). There is no simple explanation to this observation.
Sepsis

Because of the unique role of aminoglycosides in treating patients with difficult Gram-negative sepsis, the hemodynamic and metabolic perturbations of the sepsis syndrome were often associated with an increase in drug-induced nephrotoxicity. Acute or chronic endotoxemia amplifies the nephrotoxic potential and renal uptake of gentamicin in rats (Auclair et al., 1990; Ngeleka et al., 1990; Tardif et al., 1990). There is a lack of definitive studies in humans, although cases of renal failure that could be associated with the administration of batches of endotoxin-contaminated gentamicin have been observed. The mechanism can be complex, but is likely to result from increase in the release of oxygen intermediates in renal tubular cells mediated by endotoxins (or other bacterial toxins or virulence factors). The latter may then be additive to the membrane damage produced in the same cells by the aminoglycosides themselves (Joly et al., 1991).

Fever

Fever *per se* increases renal metabolic demands. When associated with shock, ischemia, and the development of foci of tissue necrosis, this enhances aminoglycoside nephrotoxicity by accelerating the course and severity of the toxic insult (Spiegel et al., 1990; Zager, 1988).

Drug-Related Factors

Duration of Aminoglycoside Therapy

A large array of clinical data supports the notion that duration of therapy is a critical factor for developing the clinical manifestation of aminoglycoside-induced nephrotoxicity. The mechanism is probably that the kidney does not use all of its nephrons at each time (leaving a subpopulation unaltered which can then be recruited once the main population becomes less functional), and proximal tubules are capable of regeneration. If regeneration is insufficient, renal function will be affected once a sufficiently large proportion of all available nephrons have been recruited and intoxicated, which typically may take about four to seven days.

Drug Choice

Much dispute has been heard in this area. In the rat model, tobramycin was clearly found to be less toxic than gentamicin, both in animals and in
humans (Gilbert et al., 1978). The respective positions of amikacin and
netilmicin (to limit the discussion to the most-commonly used
aminoglycosides) have been subject to many more controversies.
Commercial interests have largely fueled these, but genuine differences
in scientific and practical approaches have also been important. Thus,
high-dose studies, which are necessary in rats to cause overt renal
dysfunction, showed that netilmicin was considerably safer than
gentamicin. Likewise, amikacin was also declared much less toxic.
The problem may relate to the dosages and criteria used. Indeed, when
low, clinically relevant, doses are used, netilmicin appears as toxic
as gentamicin, whereas amikacin appears a mild or nontoxic drug.
(Lerner et al., 1983; Smith et al., 1977).

The question that has not been adequately answered is whether these
differences are sufficient to translate into differences in clinical toxicity, in
view of the abundance of risk factors discussed above that may make
any comparison quite hazardous. As an example, one clinical study
found netilmicin less toxic than tobramycin, but the level of toxicity of
tobramycin itself in that study was much lower than was usually
reported, suggesting that risks factors had been minimized in the study
population. With respect to amikacin, many studies used this antibiotic
as a second-line drug after failure with another aminoglycoside.
Amikacin, indeed, is active against many strains resistant to gentamicin
or tobramycin. In case of resistance to the former drugs, the change to
amikacin will often have taken place after three or four days of exposure
to the first drug. Amikacin-induced toxicity may have been largely
due to the first treatment and by the greater length of these successive
treatments. In studies where amikacin was used as first-line agent,
toxicities are usually very mild.

Frequent Dosing Intervals

Based on numerous clinical trials, the results of which have been
bundled into an impressive series of meta-analysis, a multiple daily
dosing of aminoglycosides clearly appears as a safe and efficacious
treatment method (Gilbert, 1997). This mode of treatment does not
prevent drug toxicity but may reduce the risk.

Concomitant Administration of Drugs

The toxicity of aminoglycosides can be enhanced by the coadministration
of other drugs and, conversely, other nephrotoxic drugs can amplify
the nephrotoxic potential of aminoglycosides. This has been clearly demonstrated for combinations of aminoglycosides and the glycopeptide antibiotic vancomycin (Kibbler et al., 1989; Wood et al., 1986) or, to a lesser extent, teicoplanin (Wilson, 1998), the antifungal amphotericin B (Gilbert, 2000; Kibbler et al., 1989), the anesthetic methoxyflurane (Barr et al., 1973), the immunosuppressant cyclosporine (Whiting et al., 1982), and the anticancer drug cisplatin (Jongejan et al., 1989; Salem et al., 1982).

**Handling of Aminoglycosides by the Kidney**

The first step towards understanding the pathophysiology of aminoglycoside nephrotoxicity was made in the 1970s by the demonstration of accumulation of aminoglycosides in the renal cortex (Fabre et al., 1976; Luft and Kleit, 1974). This finding was first documented in animals, but later repeatedly confirmed in the human kidney (De Broe et al., 1984, 1991; Edwards et al., 1976; Verpooten et al., 1989). Autoradiographic, micropuncture and immunocytochemical studies have shown that aminoglycosides are primarily taken up and concentrated by S1 and S2 proximal tubule cells (Molitoris et al., 1993; Pastoriza-Munoz et al., 1984; Silverblatt and Kuehn, 1979; Wedeen et al., 1983). The amount of aminoglycoside taken up by the renal cortex is only a small fraction of the total administered dose (about 2 to 5%) (Fabre et al., 1976), but it must be emphasized that aminoglycosides are not metabolized by mammalian cells. Any quantity of the drug that is retained by the kidney therefore remains chemically unmodified.

**Mechanism of Cortical Uptake**

Much attention was focused on the identification of pathway responsible for aminoglycoside uptake, and the mechanism proposed remained controversial for a long time. The drug can be taken up into the cell from both the luminal and basolateral membranes, although binding and uptake by brush border membrane predominates (Bennett, 1989). Nowadays, the consensus is that megalin, an endocytic receptor expressed on the apical surface of the proximal tubular epithelium, represents the major route of entry of aminoglycosides through the brush border of proximal tubular cells. This has been elegantly demonstrated with mice having genetic or functional megalin deficiency. These mice do not accumulate aminoglycosides in their proximal tubular cells
and are protected against aminoglycoside-induced nephrotoxicity (Schmitz et al., 2002).

**Intracellular Handling**

Following internalization, aminoglycoside antibiotics traffic via the endocytic system and accumulate primarily in lysosomes. Inside the lysosomes, the aminoglycosides accumulate in very large amounts (reaching concentrations that exceed 10 to 100 times the serum concentration). Information obtained both from cell cultures (LLC-PK1 cells) and *in vivo* (rat kidney) indicates that a small but quantifiable amount of the internalized gentamicin (5 to 10%) traffics directly and rapidly from the surface membrane to the Golgi apparatus in both (Molitoris, 1997; Sandoval et al., 1998, 2000; Sundin et al., 2001). This finding for gentamicin is consistent with movement along the endocytic pathway. It is also consistent with the previously known movement of Shiga toxin and ricin from the surface membrane to the Golgi apparatus (Johannes and Goud, 1998; Lord and Roberts, 1998; Sandvig and van Deurs, 1996). Because aminoglycosides are polar molecules, most of the drug taken up into proximal tubular cells (whether in lysosomes or in the Golgi apparatus) will remain for a considerable length of time. The half-life of the drug in kidney tissue may amount to several days (Fabre et al., 1976) as opposed to the short serum half-life (2 to 3 hr), mainly reflecting cell turnover with a small contribution of true exocytosis.

**Mechanisms of Toxicity**

A major difficulty and a point of many controversies has been, and still is, ascertainment of which changes of the numerous that have been described are truly responsible for toxicity. Several hypotheses have been suggested. An intriguing aspect of aminoglycoside nephrotoxicity is that very large amount of drugs (usually 10 times the therapeutic dose) must be administered to animals in order to cause clear-cut acute tubular necrosis and concomitant alteration of renal function (Gilbert, 2000; Parker, 1982). This is in sharp contrast to the clinical situation, in which a sizeable fraction of patients experience a loss in renal function upon treatment with clinically acceptable doses (Smith et al., 1980).

The question has therefore been raised as which subclinical features seen in animal studies are responsible for toxicity, and how these pertain to the further development of clinical toxicities (Tulkens, 1986).
However, the demonstration of a sequence of events, from a subclinical alteration to overt toxicity, is not necessarily a proof of a cause and effect relationship. It is indeed possible that only the more drastic changes, such as a decrease in glomerular filtration (Baylis et al., 1977) or extended necrosis (Parker, 1982) (both seen in animals only at high doses), really cause toxicity. Taking account of this caveat, the following sections review the various hypotheses that have received strong experimental support over the past 20 years.

**Lysosomal Alterations**

When in lysosomes, aminoglycosides induce a marked phospholipidosis that has been demonstrated in cell culture models (Aubert-Tulkens et al., 1979), experimental animals (Feldman et al., 1982; Giuliani et al., 1984; Josepovitz et al., 1985; Knauss et al., 1983), and humans (De Broe et al., 1984). This phospholipidosis develops rapidly and involves all major phospholipids, with, however, a predominant increase in phosphatidylinositol on a relative basis (Feldman et al., 1982; Knauss et al., 1983). Accumulation of phospholipids within lysosomes is responsible for the formation of the so-called “myeloid bodies” that were described and linked to toxicity as early as in the mid 1970s (Kosek et al., 1974; Watanabe, 1978).

It has been proposed that phospholipidosis induced by aminoglycosides result primarily from impaired phospholipid degradation due to inhibition of lysosomal phospholipases A and C and sphingomyelinase (Aubert-Tulkens et al., 1979; Laurent et al., 1990), and that it is related to the binding of the cationic aminoglycosides to phospholipids at the acid pH prevailing in lysosomes. The link between the lysosomal phospholipidosis and further cell damage has, however, remained largely indirect so far (Laurent et al., 1990). Moreover, some aminoglycosides, such as netilmicin, induce a conspicuous phospholipidosis (Toubeau et al., 1986) without marked necrosis (Luft et al., 1976). This lack of a link between subclinical alterations and overt renal toxicity in animals may, however, be related to differences in dose-effect relationships between netilmicin and other aminoglycosides (Hottendorf et al., 1981).

**Mitochondrial Alterations**

Besides metabolic alterations in lysosomes, aminoglycosides also induce changes in mitochondria, namely a competitive interaction with
magnesium resulting in reduced mitochondrial respiration (Bendirdjian et al., 1982; Simmons et al., 1980; Weinberg and Humes, 1980), which has been considered as a cause of toxicity.

This hypothesis highlighted the lack of evidence showing that aminoglycosides could reach mitochondria before cell necrosis and postmortem redistribution of the drug stored in lysosomes. Yet, release of oxygen radical species triggered by aminoglycosides at the level of mitochondria has been proposed as a potentially important mechanism (Ueda et al., 1993; Walker and Shah, 1988).

Traffic of part of the cellular gentamicin to mitochondria in vivo has been observed (Sundin et al., 2001). This observation may have a particular significance since polyamines are known to be able to activate mitochondria and cause the release of cytochrome c, an important step leading to apoptosis (Mather and Rottenberg, 2001). Proteomic analysis following gentamicin administration has also indicated an energy production impairment and a mitochondrial dysfunction occurring in parallel with the onset of nephrotoxicity (Charlwood et al., 2002).

Inhibition of Protein Synthesis

Aminoglycosides act as antibiotics by inhibiting prokaryotic protein synthesis through binding to ribosomes, blocking peptide synthesis initiation, and causing mistranslation (Carter et al., 2000; Davies et al., 1965; Tai and Davis, 1979). Studies indicate that gentamicin administration in vivo reduces renal cortical endoplasmic reticulum protein synthesis ex vivo very rapidly (Bennett et al., 1988; Buss and Piatt, 1985). More recent data point to a major in vivo inhibitory effect of gentamicin on protein synthesis after only two days of antibiotic administration (Sundin et al., 2001). The mechanism by which this occurs is unknown, but could involve inhibition of nuclear transcription or alteration of endoplasmic reticulum- or Golgi-mediated posttranslational modifications.

An intriguing hypothesis raised by the study of the influence of gentamicin on the expression of specific proteins is that failure to translate high levels of mRNA into proportionally high levels of protein could attenuate the expression of stress response gene products, and thus diminish the possibility of recovery in gentamicin intoxication (Dominguez et al., 1996). Gentamicin has been found to significantly reduce Na\(^+\)/glucose cotransporter (SGLT1)-dependent glucose transport
and to downregulated mRNA and protein levels of the SGLT1 in pig proximal tubular LLC-PK₁ cells (Takamoto et al., 2003).

**Inhibition of Na⁺/K⁺-ATPase**

Aminoglycosides inhibit Na⁺/K⁺-ATPase activity in basolateral membranes (Cronin et al., 1982; Williams et al., 1984), and the binding of the drugs to these membranes has been correlated with toxicity (Williams et al., 1987). Inhibition is, however, seen only when aminoglycosides are presented to the cytoplasmic face of the membrane (Williams et al., 1984). It is not known whether this occurs in vivo at therapeutic doses.

**Apoptosis**

The observation that aminoglycosides induce apoptosis in vivo at therapeutically relevant doses (El Mouedden et al., 2000a) shed light on the mechanisms of the early stages of nephrotoxicity. Apoptosis, also called programmed cell death, was first described in 1972 (Kerr et al., 1972) from studies of tissue development kinetics and differentiation, but has now been demonstrated to be a key determinant in cell response to many environmental signals leading to cell death. It is characterized by specific features, such as cell shrinkage, increased cytoplasmic density, condensation of chromatin, and fragmentation of DNA. It can be triggered in the kidney by a very large array of toxic agents (Davis and Ryan, 1998).

In the context of aminoglycosides, rats show a clearly detectable apoptotic reaction in proximal tubules after only four days of treatment, which becomes conspicuous after 10 days (see Figure 16.1A). This reaction is dose-dependent and occurs in the absence of necrosis (El Mouedden et al., 2000a). Gentamicin-induced apoptosis can be also demonstrated using cultured renal cells (LLC-PK₁ - see Figure 16.1B, MDCK) and nonrenal cells (embryonic fibroblasts) (El Mouedden et al., 2000b). Current work suggest that lysosome destabilization may be a key triggering event in the onset of apoptosis in LLC-PK₁ cells upon incubation with gentamicin, setting the link between lysosomal accumulation of the drug and the ensuing toxic events. Other pathways, such as those involving mitochondria, may also be involved. It is also possible that both pathways are interrelated; the lysosomal destabilization eventually causes mitochondrial depolarization and opening of the
Figure 16.1  Morphological changes in rat renal cortex (A, C, D) upon treatment with gentamicin at low doses (10 mg/kg, 10 days) and in cultured LCC-PK1 renal cells (B) upon incubation with gentamicin under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated in A, B, and C (approximately 10 μg/g tissue) (El Mouedden et al., 2000b). (A) Typical image of apoptosis (shrinkage necrosis; open arrows) in a seemingly normal proximal tubule; (B) Typical image of nuclear fragmentation in a single cell; (C) Autoradiographic demonstration of thymidine incorporation in nuclei of proximal tubular cells (arrowheads); (D) Peritubular infiltration by endothelial and fibroblasts-like cells. (A, D) Hematoxylin-eosin/periodic acid Schiff staining; (B) 4',6-diamidino-2-phenylindole staining; (C) Animals injected with 3H-thymidine one hour before sacrifice and tissue sections processed for autoradiographic detection of radioactively-labeled structures followed by light hematoxylin/eosin counterstaining. (A, C, D: reproduced from Laurent et al. (1983) with permission; B, unpublished data from H. Servais.)

mitochondrial permeability transition pore, a known triggering event of not only apoptosis but also necrosis, depending upon the severity of this change (Halestrap et al., 2000; Lemasters et al., 1998).

Regeneration

The kidney has a large capacity to regenerate and thereby to compensate for tubular insults. This explains why necrosis and other related
processes may occur without being detected by functional deficits. The importance of this fact is best demonstrated in rats in which fairly high doses (10 times the human dose) can be given for periods as long as 40 days. After a first episode of acute renal failure, related to the synchronous necrosis of a large proportion of the proximal tubules, renal function returns to normal, as if the animals had become refractory to the toxic effects of the drug (Elliott et al., 1982a, 1982b). This "resistance" to gentamicin is a state of persistent tubular cell injury obscured functionally by preservation of the glomerular filtration rate and histologically by asynchrony of cell necrosis and regeneration (Houghton et al., 1986).

Tubular regeneration has been extensively studied by means of $^3$H-thymidine incorporation and other methods, and has been shown to occur very early on during treatment, even at low, clinically significant, doses (see Figure 16.1C) (Laurent et al., 1983, 1988; Toubeau et al., 1986). It may therefore be speculated that all patients exposed to aminoglycosides experience focal losses of tubular tissue (through apoptosis or necrosis), and that the quality and extent of regeneration determines whether or not active renal function is maintained during treatment.

After about 10 days of exposure to gentamicin in subtoxic doses, however, the kidney will show signs of mild peritubular inflammation and fibroblast proliferation (see Figure 16.1D). This will eventually lead to chronic tubulointerstitial nephritis with progressive renal failure. Cessation of treatment is associated with microcystic and inflammatory changes, suggesting that the renal response to tubular injury can be dissociated from the amount of toxin in the renal cortex (Elliott et al., 1982a; Houghton et al., 1988).

Both regeneration and assessment of fibrosis and other signs of tubulointerstitial inflammation have therefore been used as surrogate markers in many studies evaluating and comparing aminoglycosides at low doses (Hottendorf and Gordon, 1980; Tulkens, 1986). The whole process of regeneration and fibrosis induced by aminoglycosides has also been examined within the context of the release of growth factors (Leonard et al., 1994; Morin et al., 1992). While studies aiming at stimulating regeneration have been disappointing, it has now been recognized that macrophages, myofibroblasts, transforming growth factor β, endothelin, and angiotensin II may contribute to the development of renal fibrosis in gentamicin-treated rats (Geleilete et al., 2002).
Means of Protection

Reducing or protecting against aminoglycoside nephrotoxicity has attracted much effort and attention over the last decade, based either on purely clinical approaches, or on various experimental methods. The former will be discussed in detail because they are the only ones so far that have been shown to decrease aminoglycoside toxicity in patients.

Clinical Approaches

The Once-Daily Schedule

Up until the late 1980s, aminoglycosides were commonly recommended for administration in divided doses over a 24 hour period, and typically administered on 12 or 8 hour schedules (i.e., the total daily dose divided in two or three administrations at 12 or 8 hour intervals, respectively). Kinetic and toxicodynamic studies revealed, however, that this mode of administration resulted in enhanced drug uptake and toxicity as compared with a once-daily schedule (Bennett et al., 1979; Giuliano et al., 1986). In parallel, pharmacodynamic studies examining the antibacterial activity of aminoglycosides in vitro (Blaser et al., 1985), in experimental infections (Gerber et al., 1989; Leggett et al., 1990), and in patients (Moore et al., 1987) indicated that a large serum peak concentration to minimum inhibitory concentration ratio was a key determinant in their therapeutic efficacy.

Aminoglycosides are indeed typically concentration-dependent antibiotics and have a large post-antibiotic effect (Amsden et al., 2000). This led a series of investigators to test the once-daily schedule, first in limited clinical situations from the mid and late 1980s (Powell et al., 1983; ter Braak et al., 1990; Tulkens, 1991), then to a more wide usage in the mid 1990s (Nicolau et al., 1995; Prins et al., 1993), and eventually leading to more than 300 publications to date and several meta-analyses (Blaser and Konig, 1995; Ferriols-Lisart and Alos-Álminana, 1996; Munckhof et al., 1996). This schedule is now recommended in most cases for reasons of both potentially improved efficacy and potentially decreased toxicity (Gilbert, 1997, 2000).

The bottom line of all these efforts is that nephrotoxicity is usually delayed, but not suppressed, with the once-daily schedule. This is particularly well illustrated by the results presented in Figure 16.2, where it can be seen that both multiple-daily and once-a-day dosing
Figure 16.2 Appearance of nephrotoxic reaction in patients given netilmicin once-daily (OD) or on 12 hour or 8 hour multiple doses schedules (MD)(ter Braak et al., 1990). Nephrotoxicity was defined as an increase in serum creatinine concentration of more than 50% over baseline. Patients (141) were predominately elderly subjects with severe bacterial infections and received simultaneously 2 g ceftriaxone/day. Netilmicin treatment did not differ significantly in mean daily dose per kg body weight (average 6.6 mg/day) nor duration of therapy between the two treatment arms except for schedule. Compared with patients receiving conventional doses, patients treated with a once-a-day dose had higher serum peak netilmicin levels and lower trough levels. (Reproduced with minor modifications with permission.)

of netilmicin will eventually lead to a similar proportion of patients experiencing nephrotoxic reactions. Patients receiving the drug once daily, however, will be safer for two to three days more than those treated with the conventional schedule. This is understandable since the once-daily schedule will not abolish but will only reduce drug uptake. Therefore, treating patients for an extended period of time will eventually cause the cortical drug level to reach in both cases the critical threshold that results in tubular insult and ensuing functional damage. This is the basis for the current recommendations to limit aminoglycoside treatments to a maximum of seven days unless there is clear and defined medical reason to the contrary. It is indeed important to note that pharmacodynamic considerations predict that the once daily schedule will be at least as effective, and even perhaps more effective
than the divided dose schedule (Blaser et al., 1985, 1987; Amsden et al., 2000), so that a short course will be effective.

When all studies are considered at a fixed time point, the once-daily schedule appears almost never more, and often less, nephrotoxic than the divided-dose schedules (with a global risk factor calculated for eight studies and a total of 802 patients of 0.9, with a confidence interval of 0.63 to 1.31; Verpooten et al., 2003), while being as or more efficacious (Blaser and Konig, 1995) and offering obvious practical advantages, including less necessity of monitoring serum levels (see below). A survey made in the late 1990s among 500 acute care hospitals in the U.S. revealed that 74.7% of them use the once-a-day schedule or a close variant of it (Chuck et al., 2000).

**Individualized Pharmacokinetic Monitoring**

Individualized pharmacokinetic monitoring represents another approach that has been used by clinicians and clinical pharmacists in attempts to minimize aminoglycoside toxicity (Dahlgren et al., 1975; Pancorbo et al., 1982) while ensuring sufficient serum levels to obtain maximal therapeutic effects (Zaske et al., 1982). This has led to intense efforts at designing the most appropriate models and approaches to define “optimal” peak and trough levels, which were to be considered as gold standards and used for comparing drug toxicities (Smith et al., 1980). This, however, sometimes led to contradictory results (Bertino et al., 1993; Burton et al., 1991; Pancorbo et al., 1982), but did allow identification of patients at risk (Bertino et al., 1993; Mullins et al., 1987), reassessment of accepted “normal” therapeutic ranges (McCormack and Jewesson, 1992; Watling and Dasta, 1993), and led to minimized costs (Bertino et al., 1994).

When examined in large patient populations, individualized pharmacokinetic monitoring eventually demonstrated a significant negative risk factor of up to 0.42 vs. controls, together with substantial savings (Streetman et al., 2001). The outcomes, however, remained blurred by the misconception that a high peak was associated with toxicity, while a minimum trough level was necessary for activity. As has been shown, the converse is true, but can only be demonstrated if comparing different schedules of administration. Comparing schedules is essential because changing the dose but not the schedule modifies simultaneously, and to a similar direction, the $C_{\text{max}}$, the AUC and the $C_{\text{min}}$. These are indeed covariables with respect to the dose, making impossible to ascribe
toxicity or efficacy to one of them independently of the others by simply manipulating the dose.

The introduction of once-daily dosing largely modified the way monitoring was carried out, by concentrating more on the peak levels (for efficacy), while trough levels (often vanishingly low at 24 hours) tended not to be recorded (Cronberg, 1994). Currently, optimal true peak levels (i.e., extrapolated at time = 0) are set at around 20 mg/l for gentamicin, tobramycin, and netilmicin, and 60 mg/l for amikacin, with trough (24 hour) levels lower than 1 and 3 mg/l, respectively, for patients with normal renal function (Gilbert, 2000). These levels will be obtained in most patients, decreasing the necessity of systematic monitoring. However, surveillance may be useful in patients at risk. A nomogram using the eight-hour serum value, as an indicator of both the potential peak level (and thereby allowing adjustment of the dose) and the elimination constant (to detect impending renal failure), has been developed (Nicolau et al., 1995). It seems fairly popular in the U.S. as about one-third of the hospitals using the once-daily schedule have adopted it (Chuck et al., 2000).

This nomogram, as well as others, may not be optimal (Wallace et al., 2002). Application of the principles of individualized pharmacokinetic monitoring to the once-daily schedule (by adjusting the dose interval) may still ensure a further reduction of toxicity while allowing the use of larger doses to potentially increase efficacy (Bartal et al., 2003). Additional reduction of toxicity of the once-daily schedule by selecting the most appropriate time of the day for administration has been reported (Rougier et al., 2003) with early afternoon appearing as optimal. The practicability of this approach needs, however, to be critically assessed.

**Experimental Approaches**

**Reduction of Cortical Uptake**

Reduction of cortical uptake has been attempted by complexing aminoglycosides with polyanionic substances, such as dextran sulfate (Kikuchi et al., 1991) or inositol hexasulfate (Kojima et al., 1990b), or anionic β-lactams, such as piperacillin (Hayashi et al., 1988) or moxalactam [latamoxef] (Kojima et al., 1990a), fosfomycin (Fujita et al., 1983), or even simply with pyridoxal-5'-phosphate (one coenzyme form of vitamin B6; Smetana et al., 1992). Other attempts have been directed at: decreasing
the electrostatic attraction of aminoglycosides and their brush border binding sites by infusion of bicarbonate (Chiu et al., 1979) to raise urine pH and decrease the polycationic character of aminoglycosides (the pKa of the aminofunctions of the common aminoglycosides span from 5.5 though to approximately 8), or competing with aminoglycosides by means of calcium (Humes et al., 1984) or lysine (Malis et al., 1984). Lack of marked efficacy or intrinsic toxicity has, however, prevented all these approaches from being developed in the clinics.

Stimulation of exocytosis by fleroxacin (a fluoroquinolone antibiotic) has also been attempted (Beauchamp et al., 1997). Pasufoxacin, an experimental fluoroquinolone, also has shown a protective effect against arbekacin-induced nephrotoxicity, and that this was attributable to a suppression of uptake of arbekacin in cortical renal tubules (Kizawa et al., 2003). The concomitant use of an aminoglycoside and a fluoroquinolone to prevent toxicity runs, however, against the rules of the safe and restricted use of antibiotics.

Prevention of Phospholipidosis

Polyaspartic acid has been shown to protect against both morphological and functional signs of aminoglycoside-induced nephrotoxicity in the rat (Beauchamp et al., 1990a; Gilbert et al., 1989). Further studies showed that this polyanionic peptide enters cells by endocytosis and reaches lysosomes where it forms ion-pair complexes with aminoglycosides, thereby preventing them from interacting with phospholipids (Kishore et al., 1990). Interestingly, this results in an increased cortical accumulation of gentamicin but does not alter the pharmacokinetic parameters relevant to the therapeutic effect of aminoglycosides (Whitten et al., 1996). Although potentially promising, the patent holder has barred by proprietary considerations and by lack of initiatives for the clinical development of polyaspartic acid. Daptomycin (a lipopeptide antibiotic active against Gram-positive organisms) also protects against lysosomal phospholipidosis (Beauchamp et al., 1990b) and nephrotoxicity (Wood et al., 1989) in experimental animals. Similarly to polyaspartic acid, daptomycin binds to phospholipid bilayers. However, the contribution of daptomycin to the membrane charge density and its effects on the lipid packing both combine to counteract the inhibition of phospholipase activity due to aminoglycosides (Gumani et al., 1995), and result in an activation of phospholipases when aminoglycosides are present (Carrier et al., 1998). Daptomycin has been recently approved
in the U.S. for clinical usage and evaluations of its potential to reduce aminoglycoside toxicity in patients at therapeutic doses is therefore awaited with interest.

Reduction of Cell Death and Increase of Cell Repair

This has been attempted with a number of compounds. Compounds have included, on the one hand, desferroxamine (Ben Ismail et al., 1994), methimazole (Elfarra et al., 1994), vitamin E plus selenium (Ademuyiwa et al., 1990), and lipoic acid (Sandhya et al., 1995), and on the other hand, ulinastatin (Nakakuki et al., 1996), fibroblast growth factor 2 (Leonard et al., 1994), and the heparin-binding epidermal growth factor (Sakai et al., 1997). Unfortunately, no clinical data are available.

Protection against Vascular and Glomerular Insults

Such protection can be obtained using calcium channel blockers (Lortholary et al., 1993), but the intrinsic pharmacological properties of these agents have prevented any clinical development. Trapidil, an antiplatelet and vasodilator agent, may also protect by antagonism of platelet-derived growth factor, vasodilation, inhibition of thrombocyte aggregation, and nitric oxide release (Buyukafsar et al., 2001).

Concluding Remarks on Aminoglycoside Nephrotoxicity

Although having attracted so much attention from laboratory and clinical researchers, the basic mechanisms of aminoglycoside nephrotoxicity, and especially the biochemical events leading to cell damage and glomerular dysfunction, still remain poorly understood. Yet, this has not prevented the generation of a vast amount of useful information concerning risk factors and the design of various means of protection. It now remains to be seen whether drug design can make use of this knowledge to obtain truly less-toxic compounds.

β-LACTAMS

β-Lactam antibiotics (penicillins, cephalosporins, carbapenems, and monobactams) are among the most important antimicrobials. As shown in Figure 16.3, all these drugs share the same pharmacophore, a five-membered ring (six-membered in the case of cephalosporins)
Figure 16.3 Structural formulae of the four main classes of \( \beta \)-lactam antibiotics used in clinics. All these antibiotics share the same pharmacophore consisting in a \( \beta \)-lactam ring (denoted \( \beta \) in the figure) fused with a five- or six-membered ring carrying a carboxylic acid function (shown by the vertical arrowheads). Monobactams, in which the pharmacophore consists in a \( \beta \)-lactam ring only, carry an hydrogenosulphate group (equivalent proton donor) function at the same distance from the \( \beta \)-lactam rings as the carboxylic acid function in the other \( \beta \)-lactams. This pharmacophore mimics and has the same configuration as the dipeptide D–Ala–D–Ala (from left to right: NX\(_2\)-CO-NX-CX\(_2\)-COOH, in which X are the corresponding substituents; for carbapenems, the first N atom is absent), which explains their antibacterial activity (Ghuysen et al., 1965). Mimicking a peptide has direct consequences on \( \beta \)-lactam handling by the kidney.

Carrying a free carboxyl function fused with a \( \beta \)-lactam ring (monobactams possess only the \( \beta \)-lactam ring and the carboxyl function is replaced by an hydrogenophosphate group acting as an equivalent proton donor). This common pharmacophore mimics the structure of a D–Ala–D–Ala dipeptide (Tipper and Strominger, 1965), which is the basis for the mode of action of this class of antibiotics (Ghuysen et al., 1981). As shall be indicated, this common structure also explains some aspects in the renal handling, and therefore the potential toxicity, of \( \beta \)-lactams. Differences among the very numerous derivatives relate primarily to the side chains, which govern critical properties
related to spectrum, resistance to $\beta$-lactamases, and, to some extent, pharmacokinetic properties (Petri, 2001).

Severe nephrotoxicity related to tubular necrosis was quickly recognized as a potential hazard of some $\beta$-lactams after the introduction of cephaloridine in the mid 1960s (Hinman and Wolinsky, 1967; Seneca, 1967). The fact that such toxicity had not been observed, at least to the same extent, with penicillins and other cephalosporins already in clinical usage at that time indicated that it was related to specific structural determinants distinct from the pharmacophore. This led to systematic screening of all new compounds under development for alterations of renal function. The result has been that, in contrast to aminoglycosides, antimicrobial activity and nephrotoxicity were quickly dissociated, at least in a blinded fashion. Most of the new $\beta$-lactams developed and commercialized over the past 20 years have, therefore, no or only very limited gross nephrotoxicity. Conversely, derivatives that were overtly nephrotoxic were quickly halted in their development based on simple screening studies. In parallel, the allergenic potentials of $\beta$-lactams were also taken into consideration, and compounds prone to induce allergic reactions (which are the main cause of the interstitial nephritis toxicity caused by $\beta$-lactams) were eliminated from the development programs. As a result, the molecules now in use are intrinsically nontoxic or only mildly toxic.

While this is obviously to the advantage of patients, it has largely cloaked the structure–relationship data (because of proprietary restrictions) and has limited the number of published mechanistic studies. Furthermore, little or no effort was spent in assessing means of protection as they were not considered essential in view of the availability of nontoxic derivatives. The study of $\beta$-lactams has nevertheless been rewarding in terms of a better understanding of renal physiology and of renal drug transport, on the one hand, and of basic mechanisms of cell toxicity on the other hand.

**Pathophysiology**

$\beta$-Lactam antibiotics are among the most important groups of drugs excreted by the kidney (Kamiya et al., 1983; Nightingale et al., 1975; Tune, 1997). This predictably results in increased exposure of susceptible targets within the kidney to potential toxic drugs. With penicillins, renal toxicity has varied, ranging from allergic angitis to interstitial nephritis, the latter of which is most commonly observed with
methicillin, and to tubular necrosis. Administration of massive doses of any penicillin, but most often carbenicillin and ticarcillin, may result in hypokalemia owing to the large amounts of nonreabsorbable anion presented to the distal renal tubules, which alters proton excretion and secondarily results in potassium loss.

**Interstitial Nephritis**

Interstitial nephritis is the most frequent pattern of drug-induced immunologically mediated renal injury (Kleinknecht et al., 1978). It occurs as an apparent hypersensitivity response to β-lactams and is usually ranked among the late immunological reactions to these drugs (Levine, 1966). Indeed, β-lactams behave like haptens, which may bind to serum or cellular proteins to be subsequently processed and presented by major histocompatibility complex molecules as hapten-modified peptides. The most common form of haptenization for penicillins is the penicilloyl configuration, which arises from the opening of the strained β-lactam ring, yielding an additional carboxylic function that allows the molecule to covalently bind to the lateral and terminal aminofunctions of proteins. Serum molecules thus facilitate haptenization. This reaction occurs with the prototype benzylpenicillin and virtually all semisynthetic penicillins, but other derivatives (called minor determinants) can be formed in small quantities and stimulate variable immune responses.

Since all β-lactams share the same basic structure, they are all disposed to give rise to haptenization. Variations in the side chains, and the corresponding differences in the chemical nature of the haptons, however, explain why clinical consequences are variable from one class of β-lactams to another (Pham and Baldo, 1996; Zhao et al., 2002). Cross-reactivity between penicillins and cephalosporins is accordingly far from complete (Romano et al., 2000) and is, even, rare (Novalbos et al., 2001). The potential relation of β-lactam allergy to other medication reactions involving structurally unrelated drugs needs to be further explored.

The basic characteristic of the immunoallergic acute interstitial nephritis is the presence of edema and focal or diffuse cellular infiltrates in the renal interstitium, particularly in the corticomedullary region, the subcapsular cortex, and around the glomerulus. In β-lactam-induced acute interstitial nephritis, the presence of eosinophils is frequently observed. The majority of the infiltrate cells are mononuclear,
fundamentally T-lymphocytes, although there are also monocyte-macrophages that may appear as epithelial cells.

The clinical syndrome is one of fever, macular rash, eosinophilia, proteinuria, eosinophiluria, and hematuria (Appel and Neu, 1977). Functionally, the reaction to β-lactam-induced interstitial nephritis is one of nonoliguric renal failure with a decrease in creatinine clearance and a rise in serum urea nitrogen and serum creatinine concentrations, which can progress to anuria and renal failure (Baldwin et al., 1968; Olsen and Asklund, 1976; Woodroffe et al., 1975). The incidence of acute interstitial nephritis induced by β-lactams is three times more frequent in males than in females. It can appear at any age but is most usual in young adults. The incidence and severity of nephrotoxicity associated with β-lactams are potentiated by aminoglycoside antibiotics (Dejace and Klastersky, 1987; Yver et al., 1976), renal ischemia (Browning et al., 1983), and endotoxemia (Tune et al., 1988; Tune and Hsu, 1985). Differential diagnosis from other causes of acute renal failure may be difficult, but coincident evidence of an acute allergic reaction to the β-lactam in use may help, as may the detection of eosinophils in the urine. Definitive diagnosis usually requires renal biopsy, and is important because a change in antibiotic therapy will usually result in rapid improvement in renal function (Linton et al., 1980).

**Acute Tubular Necrosis**

For the reasons discussed above, acute tubular necrosis is currently rare with β-lactams. It is encountered only with high-dose therapies (Atkinson et al., 1966), with the following rank order (as defined by studies in animals or humans): cephaloglycin, cephaloridine >> cefaclor, cefotiam, cefazoline, cephalothin >>> cephalixin, cefoperazone, cefotaxime and cetazidime (Boyd et al., 1973; Cojocel et al., 1988; Hottendorf et al., 1987). No tubulotoxic reaction has been reported for with monobactams, but it must be emphasized that only one molecule in this class (aztreonam) is in wide clinical use and that most of the toxicology data on compounds that have not been developed remains unpublished.

Carbapenems present a particular and specific situation. Imipenem, which was the first carbapenem introduced in clinical usage, is quickly hydrolyzed by a dehydropeptidase found in the brush border of proximal tubular cells. This not only causes a loss of drug (and a lack of efficacy in case of urinary tract infection) but also the release of
degradation products that are nephrotoxic in certain animal species (Moellering et al., 1989). Imipenem is, therefore, always administered in combination with cilastatin. The latter inhibits the dehydropeptidase and allows for both a sustained concentration of active antibiotic in urine and a sparing of the toxic effects on proximal tubular cells. Meropenem, and the other carbapenems, which are not susceptible to this degradation by renal dehydropeptidase, are intrinsically devoid of such toxicity (Topham et al., 1989).

Two factors account for the development of renal tubular damage caused by β-lactams, namely the accumulation of the drug in the proximal tubular cells via active transport mechanisms, and the ability of the drug to trigger cytotoxic events.

**Active Transport**

As recently reviewed by Inui et al. (2000), β-lactam antibiotics are not only filtered but also actively transported and secreted by the kidney at the level of the proximal tubular cells. An overview of the transporters involved is presented in Figure 16.4. The role of organic anionic transporters (OATs) in the renal handling of cephalosporins and carbapenems and the ensuing tubular toxicity was primarily based upon experiments using inhibitors. First, probenecid (Craig, 1997; Tune, 1972; Tune et al., 1974), p-aminohippurate (Tune, 1975), piperacillin (Hayashi et al., 1988), N-acyl amino acids (Hiouchi et al., 1993), and other organic anions (Craig, 1997) were found to reduce the uptake and the nephrotoxicity of cephaloridine in experimental animals. Second, betamipron (N-benzoyl-3-propionic acid), an inhibitor of OATs, was shown to inhibit the uptake of panipenem and imipenem into proximal cells and to prevent their nephrotoxicities (Hiouchi et al., 1994). This demonstrated that uptake by proximal tubule cells from the blood via a renal OAT at the basolateral membrane was apparently a necessary step for toxicity. At present, it is known that OAT1 and OAT3 are the major anion exchangers in rats (Cha et al., 2001; Jariyawat et al., 1999; Kusuhara et al., 1999; Sekine et al., 1997) and in humans (Sekine et al., 1997, 2000; Sweet et al., 2000; Tojo et al., 1999). OAT2, also localized at the basolateral membrane, may interact with cephalosporins, but with a lower affinity (Enomoto et al., 2002; Sekine et al., 1998).

Transport of β-lactams across the luminal membrane to the urine is primarily mediated by OAT4 (Babu et al., 2002a; Cha et al., 2000). This transport is slower for cephaloridine, which results in the accumulation
Figure 16.4 The main transport systems of β-lactam antibiotics in the kidney and the role in tubular toxicity. The most important transporters are members of the organic anion transporter (OAT) family (part of the major facilitator superfamily) that act as ion uniports or ion/H⁺ symports. Influx into cells occurs primarily through the OAT1 and OAT3 transporters located at basolateral membrane, which are inhibited by probenecid, p-aminohippurate, and other organic anions. OAT2 has only a low affinity for β-lactams. Once in cells, β-lactams are secreted into the urine through the activity of OAT4. Imbalance between influx and efflux may result in intracellular drug accumulation and triggering of cell toxicity. The peptide transporter 2 (PEPT2) and the organic cation transporter 5 (OCTN2) are responsible for luminal reabsorption of aminopenicillins and cephalosporins carrying quaternary nitrogen, respectively. They may contribute to the intracellular retention of these cephalosporins. The inorganic phosphate/Na⁺ transporter (Npt-1) is involved in the clearance of faropenem. The role of other transporters, such as the P-glycoprotein (MDR1), is suspected but not proven. (Based on data reviewed in Inui et al., 2000, and Van Bambeke et al., 2003.)

of high intracellular drug concentrations and selective damage of these cells in the proximal tubule (Takeda et al., 2002; Tune, 1997).

Other important transporters include the H⁺-coupled peptide symporters PEPT2 and the organic cation transporters OCTN2. PEPT2 will transport β-lactams in a inward fashion, from tubular fluid into the tubular epithelial cells, recognizing primarily aminopenicillins, such as cephalexin, but not aminopenicillins (Daniel and Adibi, 1993; Ganapathy et al., 1995). Although of the same subfamily as the intestinal PEPT1 transporter, the renal PEPT2 is quite distinct with respect to recognition of β-lactam antibiotics; anionic compounds such as cefixime or ceftibuten have a much greater affinity for PEPT1 than for PEPT2,
whereas the opposite is observed for zwitterionic \( \beta \)-lactams (Ganapathy et al., 1995, 1997). OCTN2, an organic cation/carnitine transporter, responsible for Na\(^+\)-coupled transport of carnitine in the kidney and other tissues, has been shown to transport cephaloridine, cefepime, and in general cephalosporins carrying a quaternary nitrogen in their side chain (as does carnitine), from the luminal medium into cells. Thus, OCTN2 will contribute to further increase the intracellular concentration of cephaloridine, and will cause inhibition of carnitine reabsorption in the kidney.

Interestingly, many of the \( \beta \)-lactam antibiotics that are not recognized by OCTN2 are good substrates for PEPT2 and vice versa. The mouse inorganic phosphate transporter Npt-1, which operates in the hepatic sinusoidal membrane to transport benzylpenicillin and mevalonic acid, is probably involved in the renal secretion of faropenem (an oral carbapenem) and is inhibited by anionic \( \beta \)-lactams, such as benzylpenicillin, ampicillin, cephalaxin, and cefazolin, and other anionic drugs, such as indomethacin and furosemide (Uchino et al., 2000). The involvement of other transporters, such as the P-glycoprotein (MDR1, PGP), is suspected but not proven (Susanto and Benet, 2002).

**Cytotoxicity**

While site-specific transport and accumulation are a first and necessary step, these are not sufficient to cause proximal tubular nephrotoxicity. Several parameters seem involved, such as:

- the presence of the pyridinium ring (cephaloridine) and of the substituted 3-methylthioretatrazole (cefotiam) (Cojocel et al., 1988)
- the rate and extent of desacetylation of cephalosporins (Hottendorf et al., 1987; Williams et al., 1988)
- an activation of protein kinase C with an enhancement of superoxide anion generation (Kohda and Gemba, 2001)
- the activation of adenine receptors (Minami et al., 1994)
- the interference with carnitine-dependent fatty acid oxidation in mitochondria or reabsorption of carnitine in the kidney (Tune and Hsu, 1994, 1995).

Unfortunately, many of these studies have been made using only a limited number of \( \beta \)-lactams, so that it is not possible to determine in-depth structure–activity relationships and generalizations are difficult
to draw. It is also possible that the mechanisms are genuinely multiple and depend on the chemical nature of each of the drugs studied, i.e., their side chains. Further studies combining detailed examination of the distribution, expression levels and activity of β-lactam transporters along the nephron, together with a molecular knowledge of the cytotoxic effects of each antibiotic accumulated by cells, will be needed for useful prediction of the nephrotoxicity of new compounds (Jariyawat et al., 1999).

Concluding Remarks on β-lactams

β-Lactams have shown at least two modes of renal toxicities, namely interstitial nephritis and tubular necrosis. While the former toxicity is intrinsically linked to their allergenic properties and cannot really be predicted, it remains rare and associated with only a few compounds in clinical practice. Differential diagnostic with other causes of interstitial nephritis is, however, desirable to avoid continuing exposure, or reexposure to the same drug. Tubular necrosis has been a problem with cephaloridine and a few other β-lactams when administered at high doses. The safety margin introduced by the systematic screening of new molecules should be sufficient for this toxicity to remain exceptional in clinical practice. It remains, however, a problem to be seriously considered in the development of all new β-lactams since it appears to result from a combination of common factors to this whole pharmacological class together with specific and idiosyncratic properties of each drug.

VANCOMYCIN

Vancomycin is a glycopeptide antibiotic of large molecular weight. This causes the drug not to be absorbed from the gastrointestinal tract, and to be unable to penetrate Gram-negative bacteria. Originally introduced in the clinics in the late 1950s as an agent active in case of staphylococcal and streptococcal infections, vancomycin has been notorious for early toxicity related to impurities and to histamine release (causing the so-called “red-man syndrome”). These adverse effects have been markedly reduced through better purification procedures and by giving the drug as a slow infusion over at least one hour. With these precautions, vancomycin is considered as safe, causing only relatively mild and self-limiting general toxicity (Elting et al., 1998;
Sorrell and Collignon, 1985), with the main side effects remaining phlebitis at the site of injection as well as nephrotoxicity (see below) and ototoxicity. The latter remains the most problematic because it can be irreversible (Feketi, 2000).

For many years, clinical usage of vancomycin has remained limited because of the availability of other antibiotics, including of \( \beta \)-lactamase-resistant penicillins and cephalosporins, \( \beta \)-lactamase inhibitors such as clavulanic acid (in Europe) or sulbactam (in the U.S. and some other countries), capable of counteracting the rise of \( \beta \)-lactamase producing staphylococci. Yet, the pandemic of nosocomial methicillin-resistant \textit{Staphylococcus aureus} infections which started in the mid 1970s (and the fact that these strains were resistant not only to all \( \beta \)-lactams, including those resistant to \( \beta \)-lactamase, but often also to aminoglycosides, macrolides, lincosamines, and fluoroquinolones; Livermore, 2000), heralded the comeback of vancomycin for systemic use in the mid-1980s. The only real alternative has been teicoplanin, which can be administered less frequently and is claimed to be less nephrotoxic. However, uncertainties about the necessary dosages of teicoplanin in severe infections have resulted in a lack of approval in the U.S. and many other countries outside Europe. The introduction of quinupristin/dalfopristin and linezolid in the late 1990s has not really changed this situation as both drugs have potentially worrying side effects and are facing emergence of resistance.

Vancomycin elimination is predominantly by glomerular filtration and clearance amounts to 90% of inulin clearance and 80% of creatinine clearance (due to protein binding) with only a minor portion of the drug being eliminated by nonrenal mechanisms (Krogstad et al., 1980). Clearance of vancomycin and creatinine are highly correlated among patients, enabling construction of nomograms for vancomycin dosage adjustment based on creatinine clearance (Moeller, Jr. et al., 1981) and body weight (Brown and Mauro, 1988).

There have been many disputes about the intrinsic nephrotoxic character of vancomycin and the role of impurities in this context. The incidence of nephrotoxicity associated with vancomycin appears indeed low (5 to 15%) when the drug is used alone (Goetz and Sayers, 1993; Rybak et al., 1990). In a recent study in China based on 84 patients suffering from Gram-positive infections, the prevalence of nephrotoxicity ranged from 11% to 14%, depending on which criteria were used. There was no significant difference in terms of nephrotoxicity prevalence whichever criteria of nephrotoxicity were applied. Nephrotoxicity could
be reversed, either during or after treatment, in 22 to 44% of patients. The development of nephrotoxicity was associated with lower respiratory tract infections and poor bacteriological response.

There is ample clinical evidence that vancomycin can enhance the nephrotoxic potential of aminoglycosides. Risk factors include the length of treatment with vancomycin longer than 21 days and vancomycin trough serum concentrations greater than 10 mg/l (Rybak et al., 1990). In a study comparing continuous and intermittent infusion of vancomycin, a significant rise in serum creatinine concentration was only observed in patients receiving other antibiotics, including aminoglycosides (Wysocki et al., 2001). Conversely, a prospective study evaluating the effect of aminoglycoside dosing regimens on rates of observed nephrotoxicity found by multivariate logistic regression analysis that concomitant use of vancomycin was a significant predictor of nephrotoxicity (Rybak et al., 1999). The same conclusion was reached from a study with oncologic patients (Elting et al., 1998). In this context, there are data that suggest that individualized pharmacokinetic monitoring results in less aminoglycoside-associated nephrotoxicity and fewer associated costs in patients exposed to vancomycin (Streetman et al., 2001). Pediatric patients are, however, at lower risk (Nahata, 1987), perhaps in relation to their better regenerating capabilities (Laurent et al., 1988).

Experimental studies support the concept that vancomycin and aminoglycosides potentiate their respective nephrotoxicities. Rats treated concomitantly with tobramycin and vancomycin show, indeed, more extensive tubular necrosis than those treated with either of these drugs alone, and also demonstrated more intense regeneration (Wood et al., 1986). This heralds early insult to cortical tissue (Laurent et al., 1988). A similar conclusion pointing to a more severe toxicity of the gentamicin-vancomycin combination compared to each of these drugs alone was reached in a study using enzynuria (alanyl aminopeptidase, γ-glutamyltransferase, N-acetyl-β-hexosaminidase) as criterion of tubular damage in vancomycin–gentamicin combinations (Fauconneau et al., 1992, 1997). Intriguingly enough, renal cortical levels of vancomycin were lowered in rats receiving both vancomycin and gentamicin as compared with rats receiving vancomycin alone. One possible explanation is the fact that increased tubular necrosis will result in cell shedding and ensuing decrease of cortical levels of the drug.

Unfortunately, very little is known about the uptake and handling of vancomycin in kidney. One study concluded that mediated transport for vancomycin occurred across the basolateral membrane, but not
across the brush border membrane. It implied that the nephrotoxicity of vancomycin is due to entry from this pole with absence of mediated egress at the brush border membrane (Sokol et al., 1989). The transporters have, however, not been identified. Moreover, the subcellular localization of vancomycin in kidney is largely unknown. One study examined the subcellular localization of tobramycin and vancomycin in the renal cortices by immunogold labeling. Tobramycin was detected over the lysosomes of proximal tubular cells as expected, together with vancomycin (Beauchamp et al., 1992a). The significance of this colocalization in molecular and cytopathological terms has, however, not been further investigated.

Another potentiation of toxicity that may have clinical significance is that of endotoxin. While endotoxin did not cause additional nephrotoxicity in rats treated with vancomycin alone, it caused marked toxicity (as assessed by the increase in blood urea nitrogen concentration, decrease in creatinine clearance, and rise in renal cortical DNA synthesis) in animals treated with vancomycin and gentamicin. This occurred to a more severe extent than in animals receiving gentamicin alone. It was concluded that endotoxin amplified the nephrotoxic potential of gentamicin alone as well as that of gentamicin plus vancomycin (Ngeleka et al., 1990).

The usefulness of vancomycin serum concentrations, the determination of a therapeutic range of values, and their correlation to antibacterial efficacy and drug toxicity in the clinical setting are controversial. Old reports of dose-related toxicities were, indeed, confronted with the problems of impurities. Actually, only the antibacterial efficacy of vancomycin and its correlation with reported therapeutic ranges may justify obtaining a vancomycin trough concentration in certain groups of patients (Freeman et al., 1993). Evaluation by decision analysis over a range of assumptions, varying probabilities, and costs reveals that pharmacokinetic monitoring and vancomycin dosage adjustment to prevent nephrotoxicity are not cost-effective for all patients. However, such dosage adjustments demonstrate cost-effectiveness for patients receiving concomitant nephrotoxicants, intensive care patients, and probably oncology patients (Darko et al., 2003). Serum level determination may also be helpful in patients with increased volume of distribution, in patients with decreased renal function, in children or neonates, and in elderly patients (Cunha, 1995).

As mentioned earlier, teicoplanin, a glycopeptide antibiotic similar to vancomycin except for its much longer half-life (due to an hydrophobic
side chain), was claimed to be devoid of nephrotoxicity. This proved correct when tested in volunteers at 6 mg/kg/day (Pierre et al., 1988), and was confirmed by clinical trials using this dose or lower ones (Wilson, 1998; Wood, 1996, 2000). However, high doses and prolonged treatment have been associated with toxicity, which appeared to consist of interstitial nephritis (Frye et al., 1992). More importantly, clinical failures were seen in the early 1990s in patients treated with the 6 mg/kg doses for severe infections, such as endocarditis (Gilbert et al., 1991), and concerns were also raised about the possible selection of resistance in *Staphylococcus aureus* and in coagulase-negative staphylococci. Unfortunately, none of the previously published animal studies had reported systematically on dose-effect relationships for teicoplanin toxicity in comparison with vancomycin (which is typically dosed in humans at 30 mg/kg/day). The introduction of teicoplanin in Japan, however, triggered such additional studies (Yoshiyama et al., 2000). These showed that the nephrotoxicity of teicoplanin was only one-fourth that of vancomycin in rats. Extrapolating these data to humans would suggest that teicoplanin, given at the high doses that may be necessary, could be as nephrotoxic as vancomycin.

Because of the renewed interest in glycopeptides, and of the mounting resistance against vancomycin, new glycopeptides have been obtained and are presently under development. Among them, oritavancin is active against vancomycin-resistant enterococci and therefore attracts much interest. The only safety data published so far, however, concern the phase I studies, in which oritavancin was well-tolerated (Barrett, 2001). Another derivative is dalbavancin, which is characterized by a very long half-life, making once-weekly administration possible. Preclinical studies in rats and dogs show that dalbavancin is well tolerated upon intravenous bolus administrations at doses several times higher than those expected to be used in humans. Phase I or phase II clinical trials have not reported major side effects in the range of concentrations where dalbavancin is effective (Seltzer et al., 2003). No dosage adjustments are necessary in case of mild renal insufficiency (Dowell et al., 2003).

Three new antibiotics of other pharmacocentral classes (quinupristin/dalfopristin, linoxolid, and daptomycin) have recently become available as treatment options for infections caused by drug-resistant Gram-positive cocci. Although apparently devoid of nephrotoxicity, these drugs have other adverse effects and are costly. Nevertheless, quinupristin/dalfopristin and linoxolid may be useful in selected patients
who cannot tolerate vancomycin, or in the case of infection with resistant organisms. Studies with daptomycin, which protects against aminoglycoside nephrotoxicity in animals, are awaited with interest.

Few studies have examined protective means against nephrotoxicity induced by glycopeptide antibiotics. Yet, fosfomycin (an antibiotic with limited usage) was found to decrease not only gentamicin but also vancomycin and teicoplanin toxicities (Yoshiyama et al., 2001). The usefulness and practicability of such antibiotic associations solely for a toxicological reason is, however, subject to criticism in view of the risk of emergence of resistance.

Concluding Remarks for Vancomycin

The nephrotoxicity of vancomycin and other glycopeptide antibiotics seems relatively minor, but is not clearly understood in molecular terms. The reasons are mainly a lack of basic studies with vancomycin, which is bearing its age, and the uncertainties concerning dosages and comparative, dose-dependence studies for teicoplanin. New glycopeptides are still in development and little public data is available. Despite those difficulties, it is fair to say that glycopeptide nephrotoxicity should not be of major concern to clinicians, except in the case of co-administration of aminoglycosides.

OTHER ANTIBIOTICS

Data on nephrotoxicity of other antibiotics are scanty and most available data consist of case reports with few, if any, systematic experimental studies. The field is also made difficult to review as data concerning established antibiotics are often old (or, for mechanistic studies, poor), while those related to new compounds are often not in the public domain. The following antibiotics may, however, be briefly discussed.

Colistin is a cationic polypeptide antibiotic from the polymyxin family. These antibiotics interact with membrane phospholipids, causing destruction of the bacterial membranes by detergent-like mechanisms and, thereby, by increasing cellular permeability. Colistin, introduced in 1962, was abandoned in the early 1970s because of initial reports of severe toxicities (Ito et al., 1969). However, the difficulties created by the emergence of multidrug resistant Pseudomonas aeruginosa in cystic fibrosis patients have forced clinicians to resume its usage. Few data, however, are available, even though some researchers
consider that the frequency of nephrotoxicity may be substantially less than previously believed (Beringer, 2001). Colistin is currently usually administered by aerosol, which tends to minimize its potential nephrotoxic effects.

The nephrotoxicity of quinolones has been linked to the development of crystalluria in experimental animals. However, crystalluria is unlikely to occur in humans at the clinical doses and renal damage has not been noted for ciprofloxacin. Premarketing clinical trials in humans ($n = 5,308$) of temafloxacin (which eventually was withdrawn for reason of uremic hemolytic anemia; Blum et al., 1994) reported no crystalluria nor clinically significant nephrotoxicity (Krasula and Pernet, 1991). Only a few reports of acute renal failure due to oral fluoroquinolone therapy have appeared, with signs of acute interstitial nephritis (Famularo and De Simone, 2002; Ramalakshmi et al., 2003). All patients had nonoliguric renal failure, which was completely reversed after discontinuation of the fluoroquinolone. Eventually, a Medline search was conducted over the period 1985 to 1999 on ciprofloxacin, norfloxacin, levofloxacin, ofloxacin, trovafloxacin, enoxacin, sparfloxacin, grepafloxacin, gatifloxacin, clinafloxacin, and moxifloxacin to ascertain the incidence and features of fluoroquinolone nephrotoxicity. The search failed to reveal more than case reports and temporally related events, with hard-to-estimate incidences and the possibility of multifactorial aspects (Lomaestro, 2000). Interestingly, fleroxacin and pefloxacin (an experimental fluoroquinolone) have been shown to reduce the nephrotoxic potential of gentamicin.

Nephrotoxicity was recognized as a risk factor of the combination of sulfamethoxazole and trimethoprim (cotrimoxazole) since its introduction on the European market in the late 1970s (Richmond et al., 1979). It is potentially related to tubulooobstructive effect caused by precipitation of sulfamethoxazole, as first observed after treatment with sulfonamides in the 1940s. It is particularly concerning in transplant patients (Ringden et al., 1984) as cotrimoxazole toxicity synergistic with that of cyclosporine (Pak et al., 1988). Surprisingly, however, animal studies showed a protective effect of cotrimoxazole on gentamicin-induced nephrotoxicity in rats (Izzettil et al., 1994). Further basic studies are probably needed in this context.

Tetracyclines may aggravate uremia in patients with underlying renal diseases, in relation to their capacity to impair eucaryotic as well as procaryotic synthesis. In addition, cases of nonoliguric renal failures were reported as early as in the mid 1960s (Lew and French, 1966).
Ascorbic acid, isoascorbic acid, and mannitol were found to be protective (Polec et al., 1971), which suggests that osmotic diuresis was contributing to eliminate a toxic agent, however the mechanism of this toxicity has remained largely unexplored.

More-recent studies showed that the OAT1 and OAT4 transporters mediated the efflux of tetracycline, whereas human (h) OAT2 and hOAT3 did not (Babu et al., 2002b). These results have been interpreted as suggesting that OAT1 mediates the basolateral uptake and efflux of tetracycline, whereas hOAT4 is responsible for the reabsorption as well as the efflux of tetracycline at the apical side of the proximal tubule. However, the link to tetracycline-induced nephrotoxicity in the human kidney remains to be established. A Fanconi's syndrome, associated with nausea, vomiting and polyuria, has also been repeatedly observed in patients receiving degraded tetracyclines since its original description in the mid 1960s (Brodehl et al., 1968) and may result from a direct insult to the proximal renal tubules.

Rifampin has been linked with renal insufficiency associated with heavy glomerular proteinuria. Acute interstitial nephritis together with effacement of glomerular epithelial cell foot processes, electron-dense deposits in mesangial matrix and subendothelial and paramesangial sites seemed to be the cause of the disorder, the clinical signs of which were reversible upon discontinuation of therapy (Neugarten et al., 1983). Other cases of acute tubular nephritis have been described (Gallieni et al., 1999). Toxicity was shown to involve tubular cells, as demonstrated by potassium wasting, acidifying defect, high fractional excretion of uric acid, and glucosuria (Cheng and Kahn, 1984). Progressive increases in enzumuria with no changes in glomerular filtration rate were also observed, and were additive to that caused by streptomycin (Kumar et al., 1992).

**CONCLUSIONS**

Nephrotoxicity remains of concern for many antibiotics, but progress in the understanding of the basic underlying mechanism coupled with the optimization of the clinical use of existing agents and the effective selection of less toxic derivatives has considerably reduced the incidence of this adverse effect. It is very rewarding to see how cooperation between chemistry, biology, and clinical sciences has been so effective in this context. Efforts should, however, be maintained because nephrotoxic reactions significantly contribute to endangering patients.
They also represent an important medical and economic burden in treatment of infectious diseases.

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