

Review
Antibiotic Safety Assessment

Bernard Rouveix

Service de Pharmacologie Clinique, CNRS UPRES A 8068, Hôpital Cochin, 27 rue du Fbg Saint Jacques, 75679 Paris Cedex 14, France

Abstract

Antibiotics usually have positive risk-benefit ratios, their adverse effects being generally mild and reversible on treatment cessation. However, severe adverse drug reactions (ADR), associated with significant mortality and morbidity have resulted in the withdrawal of several active antibiotics, including new fluoroquinolones. Adverse reactions to antibiotics are often poorly documented. The purpose of this article is to examine current tools for investigating and preventing antibiotic toxicity and to suggest future lines of investigation. Structure/ADR relationships have been investigated with various antibiotics (β -lactams, macrolides, quinolones, etc.) in an attempt to reduce the risk of adverse reactions. Some reactions can be linked to the drug's stereochemical composition. In the case of quinolones for instance, particularly ofloxacin and its derivatives, experimental data show that individual enantiomers have different toxicities. Another major factor that influences the risk of ADRs in a given population is metabolic variability, due to genetic differences in the relevant drug-metabolizing enzymes. Idiosyncratic antibiotic toxicity can be caused by a chemically reactive metabolite. Recent advances in molecular biology, and especially in individual genomic characterization (DNA chip technology, etc.), could in future be useful for identifying patients who are at a special risk of ADR. Finally, certain pharmacokinetic parameters (AUC, C_{max}, etc.) can be used to predict adverse effects.

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1. Introduction

There was a time when launching a new antibiotic was a relatively simple undertaking. For example, a few decades ago the aminoglycosides were heralded as miracle drugs giving excellent clinical results, better than anything previously seen; their marked toxicity (including deafness and nephrotoxicity) was initially seen by prescribers as an acceptable risk, and was subsequently avoided by clever dose adjustments. Such a situation is unimaginable today: indeed, if penicillin were to be discovered now, it would no doubt stumble at one or several development hurdles.

Contrary to popular belief, drug development is rarely halted because of animal or human toxicity. A survey of seven British pharmaceutical companies between 1964 and 1985 showed that the main reasons for halting antibiotic development were inefficacy (poor antibacterial activity) and 'bizarre' pharmacokinetic behaviour. Animal and human toxicity only halted the development of 11 and 10% of candidate antibiotics, respectively. The

figures for drugs other than antimicrobials were 17 and 16%, showing that toxicity observed during the development phase is viewed as less of a problem with antibiotics than with other drugs [1,2].

In the United States, adverse drug reactions (ADR) cause about 100 000 deaths each year, making them the 4th–6th most frequent cause of death [3,4]. More and more drugs are being withdrawn from the market because of serious adverse effects (Table 1). This applies to antibiotics too, and particularly to the fluoroquinolones: more than 10 000 different fluoroquinolones have been patented, but only 12 have been approved for clinical use.

In 1992, temafloxacin was withdrawn from the market, 6 months after it had been launched, because of a risk of haemolysis, liver and kidney failure and clotting disorders (the 'temafloxacin syndrome'). The incidence of this syndrome was very high, at one case per 3500 treatments. Three years later, sparfloxacin saw its indications restricted because of severe phototoxicity. Trovafloxacin, first released onto the market in December 1997, was withdrawn in June 1999 because of the risk of fatal cytolytic hepatitis; this time, however, the

E-mail address: bernard.rouveix@cch.ap-hop-paris.fr (B. Rouveix).

Table 1
Examples of drugs withdrawn from the market since 1990

Drug	Therapeutic class	Date authorized	Date withdrawn	Adverse effect
Grepafloxacin	Quinolone	11/97	10/99	QT prolongation
Astemizole	H ₁ antihistamine	12/88	6/99	Interactions
Trovafloxacin	Quinolone	11/97	6/99	Hepatotoxicity
Bromfenac	Analgesic	12/96	6/98	Hepatotoxicity
Mibefradil	Calcium antagonist	6/97	6/98	Interactions
Terfenadine	H ₁ antihistamine	3/85	2/98	QT prolongation
Dexfenfluramine	Appetite suppressant	4/96	9/97	Pulmonary hypertension, cardiac valve damage
Felbamate	Anticonvulsant	8/93	8/94	Bone marrow aplasia
Flosequinan	Vasodilator	12/92	7/93	Increased mortality
Temafloxacin	Quinolone	1/92	6/92	'temafloxacin syndrome'

risk was low, at about 0.006%. Finally, grepafloxacin, registered in November 1997, was withdrawn by the company in October 1999 because of cases of QT prolongation (seven deaths in Germany); the estimated frequency was below 0.0001% [5,6].

Thus, several new antibiotics have been withdrawn from the market in recent years because of rare or exceptional but life-threatening adverse effects. The number of patients enrolled in phase I to III clinical trials is far too small to detect such rare adverse effects prior to market release. Between one and five thousand subjects would have to be treated to observe at least one case of a rare adverse effect (frequency between 0.01 and 0.1%), while this number rises to between fifty thousand and half a million when the adverse effect is exceptional (frequency between 0.001 and 0.01%). It is, therefore, impossible to catalogue every potential adverse effect of a new antibiotic before it is released onto the market.

Drug companies must nonetheless precisely assess the risk-benefit ratios of their new candidate antibiotics, at both the individual and population levels, before going ahead with further development. And even when this is done correctly, the road to market remains fraught with pitfalls. In the absence of a clearly demonstrated therapeutic benefit relative to a reference drug, the 'principle of precaution' will be applied; if there is an objective risk, even one that is very low, development will be stopped, or the licensing terms will be modified, or the drug will be suspended or even withdrawn.

How then to define and prevent, as far as possible, the risk of poor tolerability? There are at least four approaches to this thorny question.

The first two concern adverse effects linked to the molecular structure of the antibiotic or to its racemic nature.

2. Structure–toxicity relationships

The relationship between antibiotic structure and activity is well established, contrary to the structure–

tolerability relationship. For example, the mechanism underlying immunoallergic reactions to β -lactam agents has been widely studied. These reactions are linked to the antigenic determinants carried by the β -lactam ring or by the side chains it bears. Most of these determinants are now known. They explain cross-reactions among penicillins, and between penicillins and cephalosporins. They also explain why aztreonam, which has no structural homology with other β -lactams, does not cause such reactions [7].

The risk of drug interactions with macrolides is partly dependent on their molecular structure. These interactions, controlled by hepatic cytochrome P450 isoenzymes, are no doubt influenced by the steric accessibility of the tertiary amine function, and the molecule's hydrophobia and pKa [8]. Knowing a macrolide's molecular structure would, therefore, allow one to anticipate, to a certain extent, the risk of drug interactions. It has also been suggested that macrolides with a 16-membered aglycone ring (josamycin, midecamycin, spiramycin, etc.) do not form inactive complexes with P450 isoenzymes because a second sugar is fixed to the chain bearing the tertiary amine, making the latter inaccessible. In contrast, the accessibility of the tertiary amine in macrolides with 14-membered lactone rings (troleandomycin, erythromycin, etc.) would explain the high frequency of interactions. This hypothesis is controversial, however, as clinical interactions have been observed with 16-membered-ring macrolides such as josamycin, with a substantial increase in ciclosporin serum concentrations. In addition, the better tolerability of 14-membered-ring macrolides, in which only the aglycone nucleus has been modified semisynthetically (e.g. clarithromycin, roxithromycin and dirithromycin), points to other mechanisms. Azithromycin, a 15-membered-ring macrolide, has a low risk of interactions and could thus provide useful information in this setting.

The gastrointestinal adverse effects of macrolides have also been linked to their molecular structure. The most likely mechanism explaining these effects is the activation of motilin receptors. Fourteen-membered-

ring macrolides are far more prone to inducing gastrointestinal migrating complexes than 16- and, possibly, 15-membered-ring macrolides [9].

Many relationships have been established between the molecular structure, activity and tolerability of fluoroquinolones [10–13] (Fig. 1). For example, adding a halogen (F or Cl) to position 8 of the molecule increases its absorption and anti-anaerobe activity. A piperazine or pyrrolidine group, alkylated in C7, prolongs the half-life of elimination and increases activity against Gram-positive bacteria. While some adverse effects of fluoroquinolones, such as gastrointestinal intolerance or joint toxicity, are independent of their structure, many others seem to be structure-dependent. This is the case of CNS effects and interactions with theophylline and non steroidal anti-inflammatory drugs, which are strongly influenced by substitutions of the pyrrolidine nucleus and by a piperazinyl ring in C7 (which conditions the degree of binding to GABA receptors), in gatifloxacin, sparfloxacin and levofloxacin, for example. It is likely that new fluoroquinolones (e.g. gatifloxacin and gemifloxacin) will not have neurological effects such as seizure-promoting activity. Phototoxicity is also influenced by the nature of the substituent at position 8, halogens leading to photoreactivity (e.g. sparfloxacin and lomefloxacin), while compounds bearing a hydrogen or a methoxy group are better tolerated (moxifloxacin, gatifloxacin). The trifluorated quinolones (temafloxacin, tosufloxacin, fleroxacin and trovafloxacin) carry a high risk of severe idiosyncratic and immunoallergic adverse effects, the most serious being the ‘temafloxacin syndrome’ and trovafloxacin hepatotoxicity. Some fluoroquinolones are very poorly tolerated. In a clinical trial of fleroxacin involving 79 patients, adverse effects occurred in 84% of cases, and were severe in 48%. In Japan, tosufloxacin has been linked to eosinophilic pneumonitis. Trovafloxacin can cause hepatotoxicity with eosinophil infiltration.

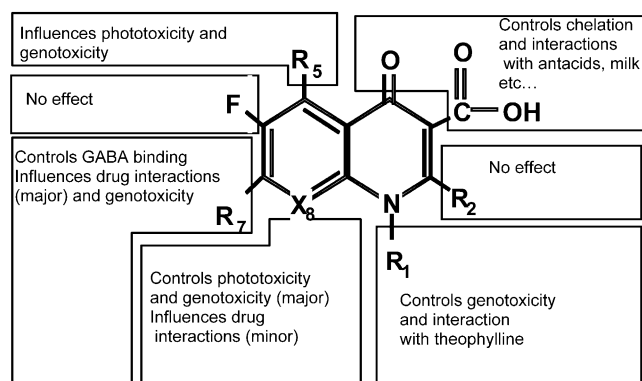


Fig. 1. Relationship between the structure and toxicity of fluoroquinolones (after [10]).

These examples show that structure–toxicity relationships, that are barely studied prior to marketing can provide important information on the likely toxicity of a new antibiotic.

3. Stereoisomers and toxicity

About one-quarter of drugs are composed of a racemic mixture of two isomers that often have different pharmacodynamics, pharmacokinetics and toxicity. The racemate can be problematic, especially when the adverse effects of the combination are due to the least active enantiomer. Isolating and developing a single isomer can be useful if it optimizes the risk-benefit ratio, reduces the risk of drug interactions and simplifies the pharmacokinetics. There are many examples of drugs produced as the racemate, enantiomer or non racemic mixture [14–16].

One of the very few examples of the use of isomers in the field of antibiotics is levofloxacin, the left-handed enantiomer of the racemic ofloxacin (Table 2). It is twice as active as ofloxacin and up to 128 times more active than the right-handed isomer D-ofloxacin. Some pre-clinical studies suggested that levofloxacin would be less toxic than D-ofloxacin. In the experimental seizure test (electroshock or pentylenetetrazol, strychnine or caffeine administration), the risk of seizures and death was increased by ofloxacin, D-ofloxacin or levofloxacin administration, but D-ofloxacin appeared to be more toxic than levofloxacin. Similarly, in in vitro models, levofloxacin is less inhibitory than D-ofloxacin for GABA binding to its receptors. Phototoxicity has been assessed in mice: levofloxacin appears to be relatively safe compared with ofloxacin (Aventis internal report).

Thus, this line of investigation appears promising but is rarely followed. For example, temafloxacin, which was withdrawn from the market, is a racemate whose two enantiomers have the same antibacterial activity. It would be useful to know whether the toxicity of this antibiotic is due to only one isomer.

Table 2
Activity and toxicity of ofloxacin isomers

	Levofloxacin S- (–)enantiomer	D-Ofloxacin R- (+)enantiomer
Antibacterial activity	+++	+
Toxicity		
Seizures	+	+++
Phototoxicity	+	++
Clastogenic activity	+	++
Mammalian opoisomerase II inhibition	+	+++

These first two approaches are most useful in the preclinical phase, while the latter two are more appropriate to clinical studies.

4. Identification of the toxic metabolite and of patients at risk

Unforeseeable idiosyncratic reactions, which are unrelated to the dose, have been an area of intensive research in recent years. These reactions include anaphylaxis, blood dyscrasias, hepatotoxicity and severe cutaneous reactions. They are generally serious and still too often fatal. They cannot currently be anticipated during the preclinical phase of antibiotic development, in the absence of relevant animal models and a good understanding of their mechanism in humans. These reactions recently led to the withdrawal of certain fluoroquinolones. The underlying mechanism seems to be immunological and to involve pharmacogenetic molecular reactions. Recent advances in immunology and in molecular and cellular biology should soon lead to the development of new techniques for investigating and preventing idiosyncratic reactions.

Other types of adverse effect are due to the synthesis of a chemically reactive metabolite (Fig. 2). These metabolites include epoxides, quinones, acyl halides and hydroxylamines. Many *in vitro* studies and some *in vivo* studies have shown that drugs can become toxic after biotransformation by cytochrome P450 isoenzymes. The variable metabolism of these drugs is linked to genetic polymorphism of CYP450 isoenzyme activity. Knowledge of this polymorphism is improving, and it is

likely soon to influence the choice and development of candidate antibiotics [17–20].

Too few metabolites responsible for idiosyncratic adverse reactions are identified and investigated. Some are well known, such as the hydroxylamine metabolite of chloramphenicol (responsible for bone marrow aplasia in one in 20 000 treated patients), and quinoneimine, the toxic metabolite of amodiaquine. Use of this latter antimalarial saw a revival during the emergence of chloroquine resistance, but has since fallen because of the risk of life-threatening neutropenia and hepatotoxicity. This toxicity was linked to the formation of an unstable metabolite, quinoneimine, in the liver and white cells. When quinoneimine is not activated by conjugation with glutathione, it acts as a hapten and couples to a protein; the resulting neo-antigen can cause hypersensitivity reactions and elicit specific antibodies. High titres of antibodies directed against quinoneimine have been found in the blood of patients who developed myelotoxicity or hepatotoxicity after amodiaquine intake [21]. By modifying the molecular structure of the parent drug it should be possible to prevent the formation of this toxic reactive metabolite.

Another example is intolerance of cotrimoxazole (trimethoprim-sulphamethoxazole), which is about 50 times more frequent in HIV-infected patients than in other immunodepressed patients (5%) and in subjects with normal immunity (< 3%). The mechanisms underlying this intolerance are not fully known, but they involve activated CD8 T lymphocytes (that are sometimes cytotoxic for keratinocytes) and the reactive metabolite of sulphamethoxazole. These reactive metabolites (mainly hydroxylamines) are produced in larger amounts by HIV-infected patients whose acetylation

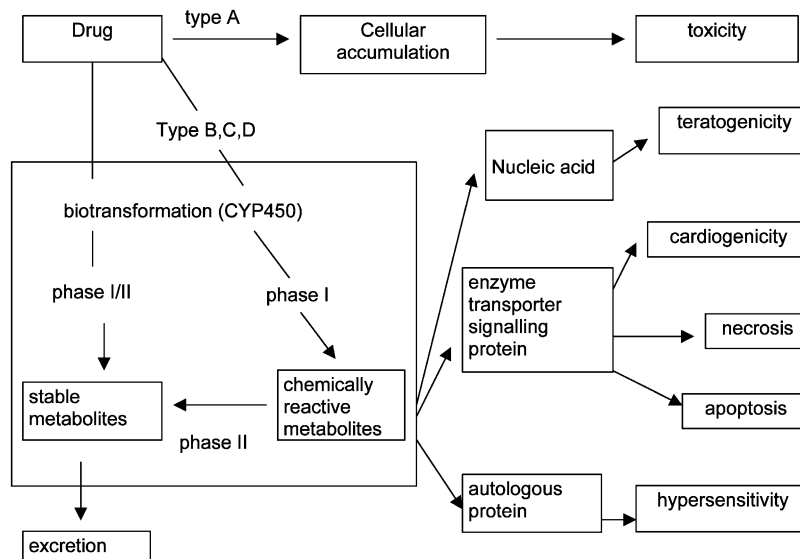


Fig. 2. Role of antibiotic metabolism in the mechanism of idiosyncratic adverse effects (phase 1: oxidation, phase 2: conjugation).

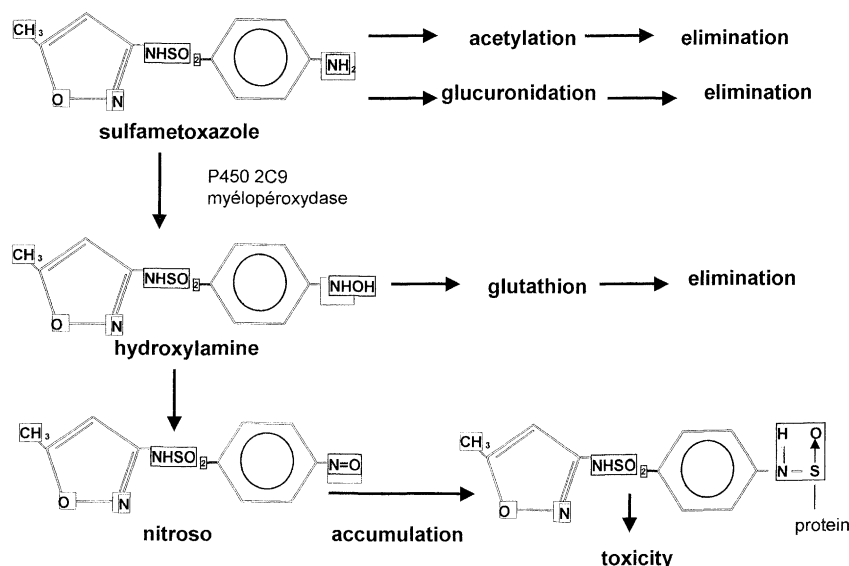


Fig. 3. Relationship between the metabolism and toxicity of sulphamethoxazole.

profile is slow, which deviates acetylation towards the metabolic pathway dependent on cytochrome P450 and myeloperoxidase. If detoxification of the metabolite derived from this pathway is defective (glutathion-S-transferase deficiency), there is a risk of toxicity (Fig. 3). These abnormalities appear to be linked to the genetic polymorphism of the relevant enzymes. Sulphamethoxazole and its reactive metabolite can not only behave as haptens but can also modulate the immune response and participate in non allergic toxic mechanisms. The blood of such patients contains T lymphocytes specific for sulphamethoxazole, and CD8+T lymphocytes in skin rash biopsy specimens [22,23].

At-risk antibiotics must not be administered to patients with predisposing factors such as a high level of the relevant P450 isoenzyme or a low concentration of glutathione, which may be useful for detoxification; these disposing factors include fasting, malnutrition, alcohol abuse and concomitant administration of drugs.

The development of DNA chips for simultaneous analysis of the expression of several thousand genes should make it possible to analyze the global transcriptional response to a given drug and to determine the metabolic pathway associated with toxic effects [24–27].

5. Toxicokinetics and toxicodynamics

Pharmacokinetics and pharmacodynamics are two key approaches in antibiotic assessment. In contrast, toxicokinetics and toxicodynamics are under-exploited and are hardly mentioned in international conferences of harmonization [28]. The goal of toxicokinetics is to avoid adverse drug effects through a precise assessment

of concentration–toxicity relationships. In the case of antibiotics, the onset of adverse effects may be linked to pharmacokinetic parameters such as the area under the curve (AUC), the peak serum concentration (C_{max}) or the mean residence time (MRT). The first two parameters are now used to predict drug efficacy. The toxicological approach is rarely used in clinical antibiotic therapy. One of the best examples is the modulation of aminoglycoside nephrotoxicity and ototoxicity by adjusting the dose and rate of administration [29]. Some clinical studies have shown that once-a-day aminoglycoside administration is less toxic for the kidneys than twice- or thrice-daily dosing, but other studies failed to confirm these initial results or showed no difference. However, most relevant studies suffered from an open-label methodology and brief treatment periods. Several recently published meta-analyses [30–32] concluded that the risk was either diminished or unchanged by once-a-day dosing. More recently, a relationship between weak renal toxicity and once-a-day administration was confirmed in a prospective randomized double-blind trial involving 123 patients, of whom 83 were treated for at least 72 h. In the group treated twice a day, 15.4% (6/39) of assessable patients developed nephrotoxicity, compared with none of the 35 patients in the group treated once a day. The AUC emerged as a good predictive marker of this toxicity [33].

6. Conclusion

Some rare but potentially life-threatening adverse effects lead to the abandonment or withdrawal of otherwise promising antibiotics, such as some recent

fluoroquinolones. These adverse effects are usually unpredictable because of their idiosyncratic nature, due to innate individual hypersensitivity. To be able to evaluate and prevent these iatrogenic risks, we need to understand the underlying mechanisms and to identify individual contributory factors.

This type of toxicity remains difficult to prevent. Among the most important outstanding questions is whether the molecular structure of an antibiotic is responsible for its toxicity and if its metabolism is genetically modulated (role of type 2 acetylation, cytochrome P450 2D6 or 2C19, glutathione 5-transferase, etc.). Traditional studies based on animal models and cell culture have successfully identified the toxic metabolite, both in standard conditions and after stimulation of their production through enzyme induction or by creating a detoxification deficiency (glutathione depletion, inhibition of epoxide hydrolases, etc.).

Finally, prevention implies the need to assess individual susceptibility. Acquired factors (multiple drug therapy, drug interactions, old age, malnutrition, alcoholism, etc.) and genetic factors (phenotyping and genotyping for antibiotics with genetic polymorphism) should be looked for.

When an antibiotic reaches the clinical development phase, the attributability of any rare but serious adverse effects must be thoroughly investigated. When a problem is discovered shortly before or after marketing, as was the case for trovafloxacin hepatotoxicity and grepafloxacin cardiotoxicity, for example, specific clinical studies are required to determine the degree of risk. The process can be assisted by strategies and decision trees drawn up by expert groups [34]. This can lead to a change in the summary of product characteristics, or, after a re-assessment of the risk-benefit ratio, to market withdrawal by the health authorities.

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