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Review

Mechanisms of resistance to xenobiotics in human therapy

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Abstract. Xenobiotic resistance is the major cause of failure in human therapies. Because of their serious clinical and economical consequences, resistance phenomena have been intensively studied in the case of antibacterial, anticancer, antipaludic and anti-human immunodeficiency virus-1 therapies. Beside pharmacological factors that can impede the action of the drugs, several cellular mechanisms of resistance have been described. Surprisingly, these mechanisms are conserved among bacteria, eucaryotic cells, parasites and viruses. The efficiency of drugs can be circumvented by alteration of the drug cellular concentration (altered influx, enhanced efflux or sequestration), detoxification, alteration of the drug target, nonactivation or inactivation of the drug, or by enhanced DNA repair.

Key words. Antibiotic; cytotoxic drugs; antipaludic drugs; anti-HIV-1 drugs; MDR; drug detoxification; drug inactivation; DNA repair.

Introduction

The intensive use of a drug is followed by the emergence of cellular resistance to this compound. Such resistance has already been described in a wide range of organisms and microorganisms and is the major obstacle to successful chemotherapies. All kinds of chemotherapies are involved in xenobiotic resistance, more specifically antibacterial, anticancer, antipaludic and more recently anti-HIV(human immunodeficiency virus)-1 therapies, with serious clinical and economic consequences.

Resistance can result from (i) decreased cellular drug concentration resulting from an altered influx of the drug inside the cell, from an intensive drug efflux or from drug sequestration; (ii) increased cellular detoxification; (iii) qualitative and/or quantitative alteration of the drug target; (iv) failure to activate the prodrug to its active form; (v) enhanced drug inactivation; or (vi) enhanced DNA repair functions (fig. 1).

Many of these mechanisms may coexist in cells. Some innate mechanisms are naturally found in the cells before any treatment, while others can appear de novo during disease progression and treatment. The modulation of some of these resistance mechanisms has been described recently and shows promise for successful chemotherapy.

Decreased drug influx from extracellular medium to the cell

Since lethality is obtained for intracellular drug concentrations above a given threshold, alteration of the

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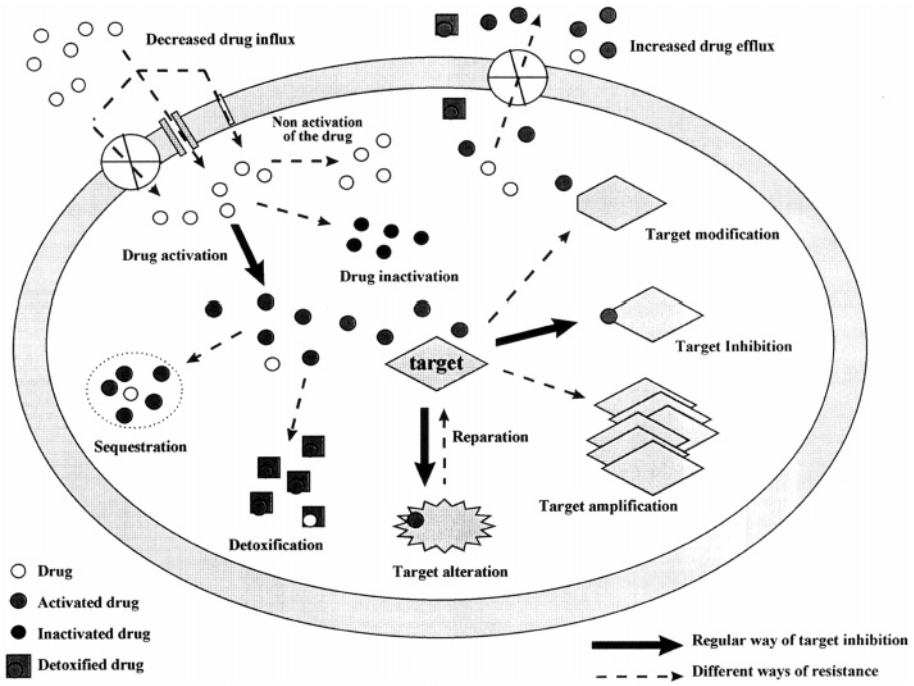

Figure 1. Cellular mechanisms of drug resistance.

drug influx inside the cell is one mechanism of resistance.

Usually, this results in a low level of resistance, but high levels can be acquired through a combination of decreased permeability and other mechanisms. Otherwise, it is most often linked to cross-resistance to structurally and functionally unrelated compounds.

Altered influx of cytotoxic drugs

Described for methotrexate [1], cisplatin [2], vincristine [3] and daunorubicine [4], an altered influx can be relatively specific for a class of compounds whose intracellular concentration depends on a specific cellular transporter. Thus, a quantitative or qualitative alteration of such a transporter has a direct effect on drug entry. A modification of the membrane composition or topology can also result in decreased permeability [4–6]. How such alterations happen is unknown. One possibility is membrane protein overexpression. This mechanism of resistance is not encountered frequently in eucaryotic cells and is most often associated with more usual and effective mechanisms. Moreover, it can often be reversed by the use of higher concentrations of the drug, which can then reach the cellular target by passive diffusion.

Altered import of the antimalarial drug chloroquine in *Plasmodium falciparum*

The uptake of the antimalarial drug chloroquine was recently shown to be associated with carrier-mediated transport [7]. In some chloroquine-resistant parasite isolates, this import mechanism has a lower transport activity and reduced affinity for chloroquine, suggesting that the modulation of chloroquine influx kinetics is responsible for resistance to this drug.

Antibiotic resistance mediated by cell wall impermeability

Because of structural differences between Gram-negative and Gram-positive bacteria cell walls (fig. 2), antibiotics use different mechanisms to enter these microorganisms. Peptidoglycan is the major constituant of Gram-positive bacterial cell wall (fig. 2A). This lattice structure does not limit the entry of molecules with molecular weights lower than 100 kDa. The only limiting factor of this passive diffusion is the hydrophobicity of the solute. Thus, drug resistance depends exclusively on the chemical structure of the drug and is not regulated by the cell itself.

In contrast, Gram-negative bacteria are covered by an additional membrane outside the cytoplasmic mem-

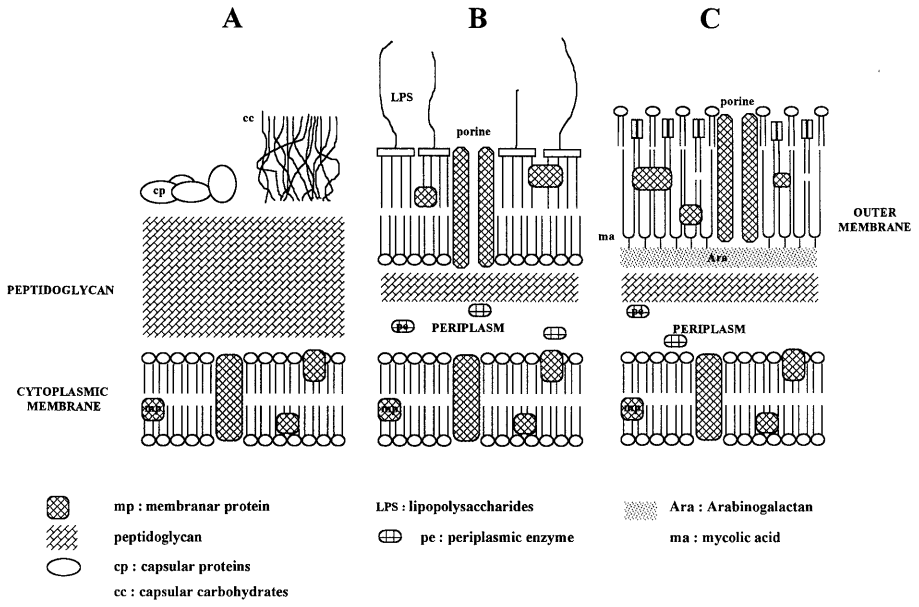

Figure 2. Structure of bacterial cell walls. (*A*) Gram-positive bacteria; (*B*) Gram-negative bacteria; (*C*) mycobacteria.

brane and the peptidoglycan layer (fig. 2B). This outer membrane contains lipopolysaccharides and phospholipids, and acts as a permeability barrier that excludes many antibiotics [8]. Nevertheless, Gram-negative bacteria do exchange many products with their environment. Antibiotics can enter the cells by two means. The hydrophobic antibiotics are able to cross directly the lipid bilayer, while other compounds cross the membrane through protein channels. The porins assemble into large water-filled channels that allow the non-specific and spontaneous diffusion of small (less than 600 Da), rather hydrophilic solutes such as nutrients, metabolites, waste products or hydrophilic antibiotics including β-lactams, some quinolones and chloramphenicol [9, 10]. OmpF (outer membrane protein F) is the major porin in a wide range of bacteria. The level of expression of this protein fluctuates in response to environmental signals. Thus, negative regulation of the *ompF* gene, which results in a decreased number of membrane channels, is a common way that Gram-negative bacteria develop resistance to hydrophilic antibiotics [11–13]. In a variety of resistant bacteria, it was shown that the decrease of *ompF* expression is associated with elevated expression of *micF*. *micF* appears to destabilize *ompF* messenger RNA (mRNA) by producing an RNA transcript whose 3′ end is complementary to the 5′ end of *ompF* mRNA. When *micF* is present in multicopy, *ompF* mRNA levels and the amount of OmpF porin are greatly reduced [14–18]. This alteration of porin expression only affects therapies with antibiotics using these membrane spanning channels to enter the bacteria. Thus, hydrophobic molecule entry is not disturbed by this membrane-specific modification. However, development of an unusual lipid bilayer, whose fluidity is well regulated, results in low permeability of the outer membrane to these molecules [19]. Moreover, the relatively nonporous outer membrane of some bacterial species, such as mycobacteria, whose cell wall includes mycolic acid as a major con-

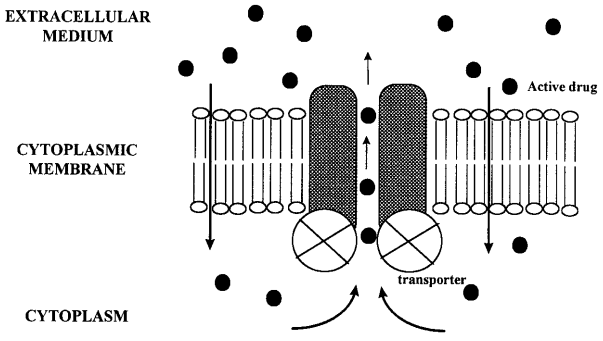

Figure 3. Schematic representation of xenobiotic resistance mediated by cellular efflux of drugs. The active drug enters the cell by passive diffusion and is actively exported through the cytoplasmic membrane by a transporter.

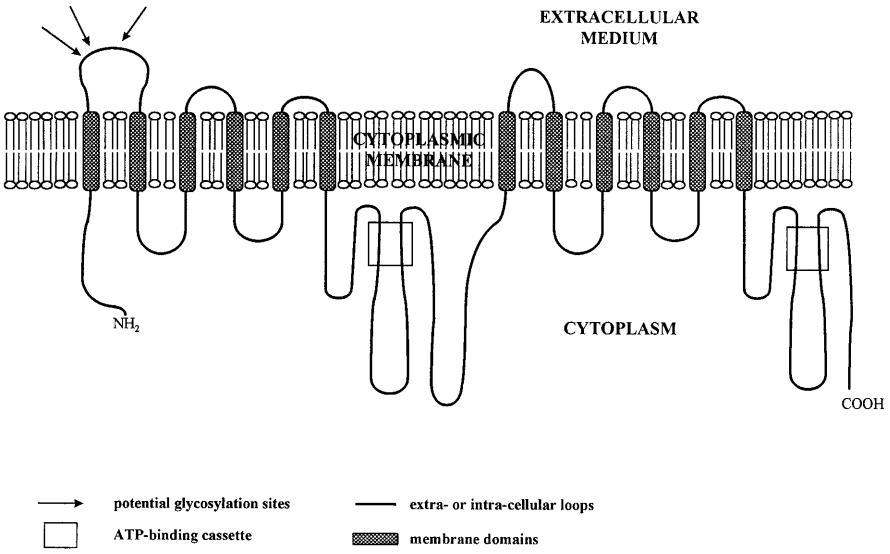

Figure 4. A model of transmembrane MDR phosphoglycoprotein.

stituent (fig. 2C), or *Pseudomonas*, accounts for their intrinsic resistance to many antibiotics.

Membrane alterations leading to decreased permeability do not have a major impact on the success of chemotherapy and are most often associated with more efficient resistance mechanisms [12, 20].

Reduced drug accumulation due to increased cellular efflux

A drug that initially enters the cell can be subsequently transported back into the extracellular environment. In many cells, this enhanced efflux is responsible for xenobiotic resistance. As with decreased drug influx, increased efflux results in low concentrations of drug inside the cell. This mechanism of resistance can be specific for one drug or for a class of therapeutic agents but most often leads to high-level cross-resistance to various unrelated drugs. Drug efflux is associated with the activity of specific proteins or protein complexes within the cytoplasmic membrane (fig. 3). Such transport proteins have been described in many cases of cancer and malaria chemotherapies in eucaryotic cells, but also in antibiotic-resistant procaryotic cells. These efflux systems are characterized by high functional and structural homology, and since the exit process requires energy, they have been referred to as pumps.

Multidrug resistance (MDR) to cancer chemotherapy

The effectiveness of numerous cytotoxic drugs used in cancer chemotherapy is reduced by the overexpresion of a permeability glycoprotein (Pgp), which confers multidrug resistance (MDR). This Pgp is a 170-kDa mammalian membrane adenosine triphosphatase (ATPase) belonging to the large superfamily of integral membrane transporters, the ATP binding cassette (ABC) superfamily, or traffic ATPases [21]. Pgp has a very broad specificity for various unrelated substrates including *Vinca* alcaloids, colchicine, anthracyclines, epipodophillotoxins, taxoids and mitramycine. From localization studies and from previous analysis of Pgp-deficient mice, it was proposed that one putative function of Pgp could be the transport of endogenous substances (toxins, metabolites, waste, hormones etc), a hypothesis consistent with the role of Pgp as a transport protein. More recently, analysis of MDR knock-out mice, without any physiological abnormalities, revealed that the pharmacological function of Pgp is protection against cytotoxic drugs [22].

Mechanisms of MDR have been intensively studied during the last 10 years, and are described in many reviews (including [23–27]). Though the biochemical, pharmacological and genetic aspects of MDR are well characterized, its mechanisms are not yet fully understood.

The MDR protein (Pgp) consists of 1280 amino acids and has a tandem primary structure with two halves that exhibit striking sequences similarities. Computer modelling [28] showed that both halves are structurally similar and consist in an N-terminal hydrophobic membrane-spanning region and a C-terminal cytosolic region which binds nucleotides (fig. 4). Thus it was proposed that the drugs pass through a hydrophobic pore formed

by the transmembrane domain of one or more Pgp molecules. This efflux requires an energy-dependent conformational change in the protein for which the cytosolic ATP hydrolysis provides the energy.

The human *MDR1* gene was isolated from chromosome 7. Its complementary DNA (cDNA), and later the gene itself, have been sequenced. This gene codes for Pgp, and its expression is directly linked to the MDR phenotype [29]. Thus, in many tumour cells [30, 31] and in eucaryotic cells transfected with *MDR1* cDNA [32], a direct correlation exists between Pgp expression levels and level of resistance. *MDR1* regulation seems very complex and may occur at many levels, including DNA transcription and post-translation. In many human cancers, increased levels of a 4.5-kb mRNA do not result from genetic amplification of the *MDR1* amplicon [33] but rather from increased transcription of the gene *MDR1*. Its amplification, with five other genes, seems to be a late event in the MDR phenotype that occurs only in very high level resistant cells. Other mechanisms of transcription regulation may play a role in initiation of resistance [34].

Moreover, point mutations or post-translational modifications, such as site-specific phosphorylation of Pgp by protein kinase C (PKCα) [35, 36], or the associated expression of other proteins could regulate the level of activity and the substrate specificity of the efflux protein and contribute to its phenotypic diversity. Recent studies using protein kinase activators or inhibitors suggest that phosphorylation of Pgp modulates its biological activity. However, it is difficult to clearly establish the role of Pgp phosphorylation in regulating its multidrug transporter activity, and some conflicting results have been published [37–40]. Several studies have indicated that glycosylation is not crucial for Pgp function but may contribute to its targeting the cell surface [37].

Recently, MRP (multiresistance protein), another ABC 190-kDa transporter with 15% amino acid identity with MDR Pgp, was isolated from drug-resistant cell lines. This protein functions as an ATP-dependent pump for a large array of cytotoxic drugs as well as a variety of detoxification products like glutathione conjugates [23, 41–45]. The broad specificity of MRP overlaps but is distinct from that of Pgp [46]. As for Pgp, the mechanisms of MRP overexpression are not really understood. Eijdems et al. [47] showed that transcriptional activation of MRP genes is most often responsible for overexpression, while gene amplification determines higher levels of drug resistance.

Reversion of the MDR phenotype was described for the first time by Tsuruo in 1981. The simultaneous use of a calcium channel blocker, verapamil, with a cytotoxic drug restores cellular sensitivity to the cytotoxic agent [48, 49]. Several screening studies have identified over 100 molecules with this reverser property [25]. Phase I and II studies of these compounds in association with many cytotoxic agents pointed out their toxicity when used alone or in combination. Thus, verapamil and cyclosporin A, which display good reversion qualities, are very poorly tolerated [50, 51]. As the function of Pgp is probably the transport of a wide range of potentially toxic molecules, it can be assumed that more efficient modulators of MDR will produce a broad spectrum of toxicities. Moreover, it has recently been shown that the response to verapamil is short-lived and that tumour cells ultimately become resistant to chemosensitizers through a non-Pgp-mediated mechanism [52].

Because of the cardiac toxicity of verapamil, potentially less toxic molecules such as (*R*)-verapamil and dexverapamil have been developed in order to inhibit drug efflux. Several other promising MDR modulators [53–58] are still under investigation.

The structural diversity of these compounds suggests that there may be several mechanisms of MDR modulation and thus provides a rationale for the potential combination of reversing drugs. Various modulation mechanisms have been predicted, whereas the presumed major mechanism of reversion is competitive inhibition of drug efflux. In this case, modulators are substrates of Pgp, binding to the protein and being pumped out as cytotoxic drugs [59, 60]. On the other hand, it has been shown that treatment with MDR modulators can modify the level of Pgp expression in human cancer cells. But results in this field are controversial, and seem to be dependent on Pgp antagonists and cell lines. Thus, verapamil treatment was shown to decrease *MDR1* gene expression in two leukaemic multiresistant cell lines through modulation of its promotor activity [61], while verapamil, nefidipine and cyclosporin A induce increased levels of *MDR1* mRNA and of Pgp in a human colon carcinoma cell line [62]. Thus, a unique mechanism of chemosensitization cannot be attributed to all modulators [53, 63].

Some of these MDR reversers were shown also to be effective in modulation of the MRP phenotype [64]. Moreover [65], it was shown that a specific reduction in MDR [66] and MRP [65] expression can be achieved with antisense oligonucleotides complementary to the coding region of MDR and MRP mRNA, respectively. Consequently, this class of molecules provides an attractive and potentially highly specific treatment for resistance to conventional chemotherapy.

Multidrug resistance to antimalarial chemotherapy, the pfMDR

Human malaria caused by the intracellular parasite *Plasmodium falciparum* remains a serious disease in much of the tropical and subtropical world. Due to its specifi-

city, stability and safety, chloroquine has been one of the most successful and widely used antimalarial drugs. The biological activity of chloroquine is directed against the intraerythrocytic stage of *Plasmodium* infection. It has been proposed that the drug inhibits the detoxification process of haemoglobin digestion products that are generated at this stage of infection, the accumulation of these toxic products being ultimately responsible for parasite cell death. The rapid emergence of resistant parasites has markedly reduced the usefulness of chloroquine in antimalarial chemotherapy [67].

Resistance is always correlated with reduced accumulation of the drug in infected erythrocytes. A significant homology is found between this resistance mechanism and MDR of tumour cells. Compared with sensitive cells, chloroquine efflux is 40–50 times higher in resistant cells, while the entrance rates are the same. This efflux is linked to the expression of a 160-kDa phosphoglycoprotein, pgh 1, highly homologous to MDR Pgp [68]. Cowman et al. [69] showed that it is expressed in digestive vacuoles during the erythrocytic phase when chloroquine plays its antiparasite role. Its function could be to transport haemoglobin products.

Moreover, the *pfmdr*-1 gene isolated from chromosome 5 of *P. falciparum* and encoding pgh-1 is 54% homologous to *MDR-1* [70]. As for the MDR phenotype, the mechanism of pgh 1 overexpression is not really understood. Amplification of *pfmdr-1* is not detected in all cases of resistance [71] but can be detected in sensitive cells [72]. Mutated alleles of the *pfmdr-1* gene have been isolated and sequenced. They differ from the wild type by one to four mutations which could be responsible for changes in specificity of the transport protein [69, 73]. Another similarity with MDR is that verapamil has been reported to reverse chloroquine resistance of the human malarial parasites through a marked increase of chloroquine accumulation in infected erythrocytes [74].

Ltpgp A, a very similar phosphoglycoprotein, has been described in some parasites of the *Leishmania* family. The overexpression of this protein is responsible for resistance to a broad range of compounds [75, 76].

Bacterial resistance by an active antibiotic efflux

As for cancer and parasite chemotherapies, energy-dependent efflux systems represent a major mechanism of resistance to antibiotics. Efflux is associated with the transport of structurally diverse compounds and accounts for a variety of resistance phenotypes. This mechanism of bacterial resistance has been thoroughly described by Jenkinson [77].

The first antimicrobial drug efflux pump discovered conferred tetracycline resistance. This was followed by the description of several energy-dependent bacterial transport systems [78]. These pumps are usually about 400 amino acid residues long. Structural analyses predict 12–14 membrane-spanning α-helical segments that form a hydrophobic pore in the cytoplasmic membrane. In Gram-positive bacteria, the antibiotics are pumped out into the extracellular medium, while in Gram-negative bacteria the drug can accumulate in the periplasm. One way to overcome the outer-membrane barrier was suggested by the presence of auxiliary proteins that may bridge the cytoplasmic membrane transporter and outer-membrane channels (fig. 5) [8, 78, 79].

Active efflux of tetracycline is well documented both in Gram-positive and Gram-negative bacteria [80, 81]. Eight highly homologous genes have been isolated and sequenced: *tet (A)*, *tet (B)*, *tet (C)*, *tet (D)*, *tet (E)*, *tet (K)*, *tet (L)* and *tet (P)*. They show a high degree of homology with other bacterial transporters such as glucid transporters. Some of these genes are regulated at the transcription level by the repressor *tet R*, which is not active in the presence of tetracyclines [82]. The Tn10 *Tet (A)* gene is certainly the most widespread tetracycline efflux system among bacterial species. The Tet A pump is specific for tetracyclines and works as a proton antiporter. Amino acid substitutions in membrane-spanning regions of Tet A are responsible for the alteration of substrate specificity, showing that these residues may play a role in tetracycline recognition [83, 84]

The protein Nor A is involved in resistance to hydrophilic fluoroquinolones as well as lipophilic compounds. This protein, highly homologous to the

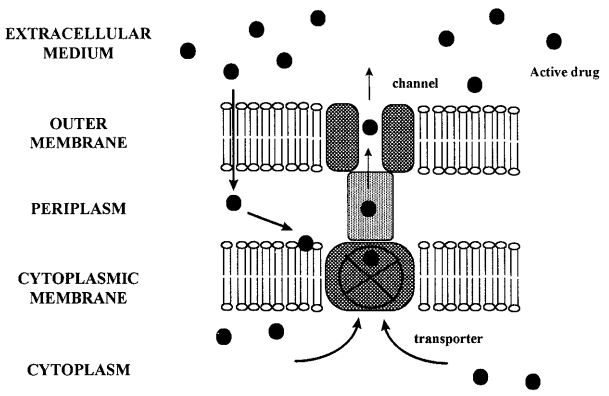

Figure 5. Proposed mechanism of drug efflux in Gram-negative bacteria [17]. The transporter is connected to an accessory protein which is itself associated with an outer-membrane channel protein. The active drug which enters the cell through porin channels or by passive diffusion can be pumped out by the transporter directly from the membrane bilayer or from the cytoplasm.

tetracycline pumps, contains 12 membrane-spanning domains and confers a high level of resistance [18, 85]. In the same way, erythromycin resistance can be linked to the expression of an ABC transporter, MrsA [86]; a chloramphenicol transporter has also been described [87].

Drug sequestration

Drug sequestration inside the cell can also affect drug concentration at its site of action. This resistance mechanism is uncommon and has been described in only a few cases of cancer chemotherapy. Alterations of doxorubicin distribution have been reported [88]. The drug can be sequestrated into small cellular vesicles whose number is related to the resistance level. These vesicles are able to trap other cytotoxic drugs such as daunorubicine, mithoxanthrone [89] and vinblastine [90, 91]. This sequestration phenomenom could participate in exocytosis and could be linked to degradation processes leading to elimination of an inactive drug.

Cellular detoxification

The fundamental role of glutathione has been ascertained in many tumour cells resistant to various cytotoxic compounds, including alkylating agents and drugs whose lethal activity is linked to electrophilic (quinones, cations) or radical species. Glutathione (GSH) is an intracellular cysteine-containing tripeptide (γ glutamyl-cysteinyl-glycine) present in high concentrations in mammalian cells. An increased conjugation with GSH is a major mechanism in the development of drug resistance [92]. The GSH thiol group performs a nucleophilic attack on the target toxicant and forms a stable conjugate which can be metabolized or/and expelled from the cell.

Glutathione-S-transferases (GSTs) are a major group of detoxifying enzymes. Since these enzymes catalyse conjugation with GSH, their level of expression is a major factor determining cellular sensitivity. Four isoenzymes are involved in these detoxification processes: GSTα, -μ, -π and -θ. They differ by distribution in tissue and their substrate specificities [93–98]. A better understanding of GST substrate specificity might help to fight cross-resistance patterns. To date, GSH overproduction and GST overexpression mechanisms are not well documented, no genic amplification being detected in resistant cells.

GSH and other GSH-related enzymes can mediate resistance using mechanisms other than GSH conjugates. For example, GSH can modulate DNA repair functions and thus control cisplatin resistance [99]; GSTμ can detoxify BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) by denitrosation [100]; glutathione reductase, glutathione peroxidase, γ-glutamyl transpeptidase and epoxyde hydrolase could also be involved in resistance phenotypes [101–103].

Alterations of glutathione metabolism are varied and not well understood. Moreover, other resistance mechanisms are often involved and can explain atypical cross-resistance phenotypes [104]. For example, it has recently been shown that an expression of MRP can be associated to glutathione-mediated detoxification: MRP is able to transport glutathione conjugates [105], suggesting that the transporter may represent a link between cellular resistance to some classes of cytotoxic drugs and glutathione-mediated mechanisms of resistance [45].

Reversion of glutathione-mediated resistance can be achieved by two kinds of compounds, since either depletion of GSH or inhibition of GST restores sensitivity to alkylating drugs. Two GST inhibitors have been tested: ethacrynic acid [106] and indomethacine [107]. Ethacrynic acid is not specific to a class of GST. The inhibition can be achieved by two different ways: it can covalently bind to a GST active site, thus inhibiting substrate binding, or it can form a stable complex with GSH that inhibits GST [108]. Phase I and II clinical studies of a combination of ethacrinic acid and thiotepa showed that resistance to this alkylating agent can be efficiently reversed [109]. Recently, studies have focused on more specific inhibitors such as indomethacine, which acts more specifically on GSTα [106]. Piriprost, a new GST inhibitor, has been described [110]. Buthionine sulfoxide (BSO) inhibits the first enzyme involved in GSH synthesis, γ glutamyl-cysteine synthetase. BSO treatment thus leads to decreased GSH levels or even to total depletion of intracellular GSH, blocking detoxification pathways [111].

Qualitative and/or quantitative alteration of drug targets

Drugs are specifically targeted against one or a small number of cellular components. Alteration of these components to avoid the effects of the drug yield efficient resistance mechanisms. Either the overexpression of the drug target allows it to fulfill its biological function even in the presence of the drug or point mutation(s) of the target affects its interaction with the drug, rendering it insensitive to the drug effect. Such resistance mechanisms are rapidly acquired when the drug has a unique cellular target or when the drug is an analogue of the target substrate, and have been described for resistance to almost all types of xenobiotics.

Resistance to cancer chemotherapy mediated by target alteration

Topoisomerase alteration. Topoisomerase II, which is an analogue of procaryotic DNA gyrase, is a homo-

dimeric enzyme of 170-kDa. It is essential for DNA replication since it modifies DNA topology by transient breakage of DNA and religation. As this process is occurring, a covalent intermediate complex is formed between a tyrosine residue of the enzyme and the 5′ phosphate group of one end of the DNA [112]. This cleavable complex is the target of various cytotoxic drugs, intercalating agents such as adriamycin, actinomycin D, m-AMSA and mitoxanthrone, and nonintercalating agents such as epipodophyllotoxines (etoposide and teniposide). These molecules are not direct inhibitors of topoisomerase II but bind to and stabilize the intermediate complex interfering with the cleavage/religation reaction. This results in DNA fragmentation and chromosomal aberrations and rearrangements.

In many cases, resistance implies either an alteration of topoisomerase II levels or expression of a mutated enzyme (for review, see ref. [113]). A decrease in the level of topoisomerase II, to the minimal amount required for cell division, reduces the number of DNA damages. Several mechanisms can lead to a change in the enzyme level: deletion of one allele of the gene, mutations in the promoter region or mutations that affect protein translation or stability. Moreover, topoisomerase II activity levels can also be modulated by post-transcriptional modifications. Point mutations on topoisomerase II, either in the vicinity of the ATP-binding site or in close contact with the active site, and/or post-translational modifications (methylation, phosphorylation) resulting in decreased interaction of the drug with the enzyme and/or the DNA, yield resistant cells [36, 114–117]. In some resistant cell lines, the resistance has been linked to differential expression of the two isoforms (α and β) of topoisomerase II, or with modified cellular distribution of the enzyme [98].

Topoisomerase I also modifies DNA topology and is necessary both for replication and transcription. Camptothecine (CPT) is an efficient topoisomerase I inhibitor. It stabilizes the reaction intermediate in which topoisomerase I is covalently bound to the 3′ terminus of the DNA phosphate backbone, resulting in higher levels of topoisomerase I-DNA adducts and in higher DNA breakage [112]. There are only a few cases of camptothecine resistance, most often linked to altered topoisomerase I levels. The greater the amount of topoisomerase I, the greater the number of lethal lesions produced [118]. The mechanism of downregulation of topoisomerase I by camptothecin is under investigation. Recently, a mutant of topoisomerase I with a higher affinity for DNA, that can cause resistance, was described. Some mutated enzymes less sensitive to inhibition and thus generating less DNA cleavage have been purified and studied (for reviews, see refs [98, 119]).

Modification of DHFR cellular contents. Dihydrofolate reductase (DHFR) is the cellular target of antifolate cytotoxic drugs such as methotrexate. Increased levels of DHFR allow the cell to overcome the effect of the drug [1]. Overexpression mechanisms are still not known, and as for MDR Pgp, DHFR overexpression happens before gene amplification. Moreover, an increase in DHFR activity is associated with the expression of a variant enzyme with reduced affinity for antifolates. In some cases, a further increase in the expression of mutated DHFR is combined with marked decreased expression of the wild-type enzyme. From the X-ray structure of human DHFR, residues Arg 22 and Phe 31 are involved in interactions with the drugs. Their substitution (Arg 22 Lys, Phe 31 Ser) in mutant enzymes probably affects drug binding. Structural differences between the antifolate molecules could account for the differential gene alterations in antifolate resistance.

Thymidylate-synthase alteration. The major cellular target of 5-fluorouracyl (5-FU) is thymidilate synthase (TS). The 5′-monophosphate-5-fluorouridine is the activated form of 5-FU which interacts with TS, resulting in cell death through thymidine deficiency. Again, there are two resistance mechanisms. The first one is an alteration of the affinity of TS for the drug through a single amino acid substitution, resulting in a lower number of 5-FU-TS complexes. The second one is increased TS activity. Some compounds (leucoverine, methotrexate, etc.) have been shown to revert resistance, and are under investigation as promotors of 5-FU cytotoxic effects [98].

Resistance to antimalarial antifolates: DHFR mutation

Antifolates currently used for prophylaxis or treatment of malaria are pyrimethamine and cycloguanil, the active metabolite of proguanil. They have a higher affinity for the parasite bifunctionnal enzyme dihydrofolate reductase thymidilate synthase (DHFR-TS) than for human DHFR. DHFR-TS inhibition leads to parasitic thymidine deficiency.

Substitution of Ser 108, in the DHFR domain, by asparagine or threonine is responsible for resistance to pyrimethamine or cycloguanil, respectively [120–123]. While the substitution of residue 16 in DHFR-TS specifically confers resistance to cycloguanil, additional mutations of residues 51, 59 or 164 could be responsible for cross-resistance to both drugs and for higher resistance levels [124]. Some multiresistant *P. falciparum* strains that have been isolated displayed a variant DHFR with residues 108 and 164 both substituted [73, 125]. Molecular modelling of these substitutions from known tridimensionnal structures of DHFR suggests that the residues mutated in resistant strains, and particularly Ser 108, are located in the vicinity of the active site and participate in drug binding. Their substitution may affect the affinity of the enzyme for the drugs.

Antiviral resistance by target alteration

The three major classes of inhibitors of the HIV-1 viral cycle are nucleoside analogue inhibitors of the viral reverse transcriptase (RT), nonnucleoside inhibitors of RT and viral protease inhibitors. The main factors that influence in vivo resistance of HIV-1 to these compounds are the high rate of viral replication, high viral mutation rate, viral load in infected patients and rapid turnover of the viral population. Loss of susceptibility to these drugs appears to be due to RT or protease amino acid substitutions. Substitutions found in HIV-1-resistant strains have been reviewed by Kimberlin et al. [126]. The high error rate of HIV-1 RT suggests that misincorporation is responsible for the high frequency of mutation, enabling HIV-1 to rapidly acquire drug resistance. This marked diversity among HIV-1 genomes is likely to be associated with natural drug resistance found in HIV-1 strains before any treatment [127].

Resistance to nucleosidic RT inhibitors. Nucleosidic RT inhibitors act by mediating premature termination of nascent viral DNA. Current treatment of HIV-1 infections with single antiviral drugs yields only short-term benefits because of the emergence of drug-resistant strains. AZT (3′-azido-3′-deoxythymidine) was used intensively as soon as its antiviral efficiency was demonstrated. Resistance can develop within 6 to 12 months in HIV-1 positive patients with advanced disease, but develops more slowly during treatment of earlier-stage disease [128, 129].

As few as three to five point mutations of the RT gene can lead to high-level resistance [126, 130–132]. These substitutions are located in the N-terminal portion of RT, which is responsible for polymerase activity and nucleotide binding. HIV-1 becomes gradually resistant by the stepwise accumulation of these specific mutations: Met 41 Leu, Asp 67 Asn, Lys 70 Arg, Thr 215 Phe or Tyr, Lys 219 Gln. Resistance level is dependent on the combination of mutations acquired [128]. However, it was shown that substitution of residue 215 alone is able to confer high-level resistance to AZT. AZT resistance is associated with resistance to nucleotide inhibitors with a 3′-azido group such as 3′-azido-2′,3′-dideoxyuridine (AZdU), but not to other RT inhibitors.

In contrast to AZT, strains resistant to 2′-deoxy-3′-thiacytidine (3TC) emerged rapidly through a single mutation at codon 184 (Met 184 Val or Met 184 Ile) in the RT gene [130]. In addition to 3TC resistance, these substitutions suppress AZT resistance conferred by mutations of codons 41 and 215 [133]. These resistance 'suppressor' mutations lead to a marked effect of AZT-3TC combination therapy on viral development. However, a recent study among patients treated with this combination detected cross-resistance patterns linked to the substitution of residues 184 and 215 of RT, occasionally associated to the substitution of residue 333 [133].

Strains resistant to 2′,3′-dideoxyinosine (ddI) have been isolated in patients treated with this drug after the development of resistance to AZT. Resistance to ddI is associated with resistance to 2′,3′-dideoxycytidine (ddC), whereas sensitivity to AZT is increased [134, 135]. Leu 74 Val or Met 184 Val substitutions are sufficient to acquire this phenotype but are associated, in clinical isolates, to Thr 215 Tyr substitution which, in this particular context, does not confer resistance to AZT [134].

A set of five mutations in the RT polymerase domain has been identified for their ability to confer resistance to combination therapy with AZT and ddI or ddC. These mutations – Ala 62 Val, Val 75 Ile, Phe 77 Leu, Phe 116 Tyr and Asn 151 Met [133, 136, 137] – are able to confer resistance to various antiviral 2′,3′-dideoxy nucleoside analogues. Enzymatic analyses of mutated RT revealed an enzymatic efficiency comparable to wild-type RT but marked differences for inhibition constants leading to drug resistance.

Resistance to nonnucleoside inhibitors. Nonnucleoside RT inhibitors such as neviparine, pyridinone, Cl-TOBO and BHAP are a structurally diverse group of compounds that, despite this variability, are functionally homologous. They bind to a small pocket located near the active site of RT, at residues 103, 181 and 188, and produce a premature dissociation of the enzyme-DNA template-primer complex. Residues 181 and 188 belong to a very conserved domain among various RTs and are essential for enzymatic activity. In resistant clinical isolates, RT is modified in one to three of these residues within the inhibitor binding pocket. Thus, Tyr 181 Cys substitution induces a 100-fold increase in the minimal inhibitory concentration of neviparine [138, 139] and resistance to Cl-TIBO [140]. Combinations of substitutions at residues 103 and 181 or 103 and 188 could be responsible for cross-resistance to all nonnucleoside RT inhibitors [141]. Other substitutions in two domains of RT, residues 100 to 108 and 181 to 190, have been detected in HIV-1-resistant isolates. All these mutations probably alter the enzyme's ability to interact with the drug.

If cross-resistance patterns to several nonnucleoside inhibitors are often encountered, most often the strains remain very sensitive to nucleoside analogues. In this regard, the combined use of several antiviral agents remains the most promising method of treating HIV-1 infections [142, 143].

Resistance to HIV-1 protease inhibitors. The proteolytic cleavage of Gag and Gag-Pol precursor protein into functional proteins, which occurs during HIV-1 infection, is the target of a set of new drugs, the antiviral protease inhibitors. Inhibition of protease activity results in production of noninfectious immature viral particles. Because the clinical efficiency of HIV-1 RT inhibitors is limited by the emergence of resistant viral variants, protease inhibitors have now entered clinical studies.

Similarly, under selective pressure, HIV-1 strains have evolved to acquire resistance to these new compounds through mutations of the protease gene. Various amino acid substitutions are associated with the decrease in antiviral efficacy of the inhibitors. The most common substitutions observed in resistant strains are Leu 10 Arg, Met 46 Ile, Leu 63 Pro, Val 82 Thr and Ile 84 Val. However, it has been shown that only three substitutions in combination (Met 46 Ile, Leu 63 Pro and Val 82 Tyr) are able to confer resistance to three different compounds (MK639, XM323 and A-80987), the loss of only one of these mutations abolishing resistance. Various levels of resistance were observed depending on both amino acid substitutions and the inhibitor used. The cross-resistance to six inhibitors requires an additional mutation of residue 84 [144]. The comparison of three-dimensionnal structures of the mutated and wild-type proteases shows that slight changes due to the mutation can explain the decreased affinity for the inhibitors [145]. In the case of the protease inhibitor Ro31-8959, only three mutations of the protease gene are found in resistant isolates. The mutation Gly 48 Val plays an initial role in determining the resistance pattern, while two other mutations (Leu 90 Met and Ile 54 Val) can be found in combination with the first one [146].

Unlike RT inhibitors, resistance to protease inhibitors was found to be associated to various amino acid substitutions depending on the resistance level and the drug used. The genotypic basis of this is complex and not fully understood at this time, and the phenotypic influence of individual substitutions awaits the results of further genetic studies. Still, the resistance profiles described to date suggest that multitherapy with different protease inhibitors holds promise for treating HIV-1 infection.

Antibiotic Resistance by target alteration

In bacteria, target alteration means that the regular target is overexpressed or is partially or totally replaced by a variant form which is able to fulfill its cellular function while it is insensitive to antibiotics. Such a variant can also be acquired by the bacteria through conjugation or transduction of a new gene.

Resistance to the main antituberculosis drugs by target modification. Recently, a new outbreak of tuberculosis in developed countries has been linked to the emergence of resistant *Mycobacterium tuberculosis* strains. Many isolates became resistant to a wide range of antituberculosis drugs and caused several fatal outbreaks in both HIV-1-positive and HIV-1-negative individuals. In the United States, 13% of new cases are found to be resistant to at least one of the five front-line antituberculous drugs. Two drugs are mainly used clinically to fight tuberculosis: rifampicin and isoniazid. Both are implicated in resistance phenomena.

The bacterial target of rifampicine is RNA polymerase. It inhibits transcription through binding to RNA polymerase β subunit [147]. Point mutations in the *rpoB* gene that codes for RNA polymerase β subunit have been detected in three small domains, in the central part of the gene. These mutations affect only 8 residues in a 23-residue fragment, the most often substituted residue being Ser 531. These residues may form the antibiotic-binding site and their substitution may induce conformational changes in this pocket since variant enzymes have a lower affinity for rifampicin [148–151].

Isoniazid (INH) was first reported to be effective against *M. tuberculosis* in 1952. INH-resistant strains were isolated almost immediatly after it began to be used. At present, about 20% of the *M. tuberculosis* strains isolated in New York City hospitals are resistant to INH. It was recently demonstrated that INH inhibits the biosynthesis of cell wall fatty acids, specifically the characteristic mycolic acids of mycobacteria (fig. 2C). INH binds to Inh A, a protein essential to fatty-acid elongation. This enzyme preferentially reduces long-chain substrates (12–24 carbons), which is consistent with its involvement in mycolic acid biosynthesis. In INH-resistant strains, the *Inh A* gene is mutated. The mutation Ser 94 Ala alone is sufficient to significantly reduce the level of inhibition of mycolic acid biosynthesis by INH. This substitution may affect the steady-state binding of NADPH, which happens prior to INH binding during the inhibition process, via a reduction of the enzyme affinity for the reduced nucleotide [151, 152]. Overexpression of Inh A, by mutations in the 5′ regulatory region of the *Inh A* gene or by genic amplification, can also be responsible for resistant phenotypes [153].

Resistance to quinolones by alteration of drug targets: DNA Gyrase and topoisomerase IV. DNA gyrase and topoisomerase IV, two homologous proteins with decatenating activity, are both targets of the antibacterial quinolones [154]. Both enzymes play the same critical role in DNA replication as topoisomerases do in eucaryotic cells. In a similar fashion, a covalent intermediate is formed between the enzyme and DNA. Quinolones bind to this complex to produce a stable ternary complex so that DNA can neither be religated nor replicated. At higher concentrations, double-stranded DNA breaks are released from trapped gyrase and/or topoisomerase IV complexes.

In many Gram-negative strains, and in particular in *Escherichia coli*, the primary target of quinolones is DNA gyrase [155]. A small number of point mutations in the *Gyr A* gene are responsible for quinolone resistance [156, 157], but the single Ser 83 Leu substitution in the Gyr A subunit suffices for bacterial resistance. All

the substitutions have been mapped in the vicinity of the tyrosine involved in DNA binding and result in low affinity of quinolones for the DNA gyrase-DNA complex [18]. Two mutations in the *Gyr B* gene have been described, but their contribution to resistance is not clear [158, 159]. Resistance is a stepwise process, and higher resistance levels can be achieved through combinations of mutations at different Gyr A or topoisomerase IV sites [160, 161].

In contrast, the primary target of quinolones in Gram-positive bacteria such as *Staphylococcus aureus* is topoisomerase IV [155]. Thus, low-level resistance occurs through changes in one of the two subunits of topoisomerase IV, while gyrase modifications yield additional resistance [162]. The Ser 80 residue of topoisomerase IV of *S. aureus*, which corresponds to Ser 83 of Gyr A, is substituted by Phe or Tyr in both high- and low-level quinolone-resistant isolates [163]. The Glu 84 Lys substitution also contributes to resistance [164].

MLS and tetracycline resistance mediated by ribosome modifications. Ribosomes are the target of tetracyclines, macrolides, lincosamides and streptogramin B through different inhibition mechanisms. Tetracyclines inhibit protein biosynthesis by binding strongly to the ribosomal 30S subunit, thus hindering the attachment of amino-acyl transfer RNAs (tRNAs) to the ribosomal A site. Cytoplasmic proteins Tet (O), Tet (M) and Tet (Q) are supposed to interact with ribosomes and make them insensitive to tetracyclines. The complete mechanism by which ribosomes are protected is still unknown [80].

Macrolides, lincosamides and streptogramin B (MLS) are structurally unrelated antibiotics that display the same antibacterial mechanism. They block protein synthesis by inhibition of the peptidyl-transferase activity of the ribosomal 50S subunit. Ribosomal protection-mediated resistance is widespread. Most of the ribosomal RNA (rRNA) nucleotide alterations are point mutations and base methylations within domain V of 23S RNA. Most often, resistance implies synthesis of an enzyme responsible for N^6-methylation or N^6-N^6-dimethylation of an adenine of ribosomal 23S RNA. Mutations within domain V (drug-binding site) or II (increased expression of the E peptide) of 23S rRNA have also been described [165]. These modifications of ribosomes result in decreased affinity for MLS. These mechanisms of resistance are not totally understood, and various genes could be involved [166, 167].

***β*-lactam resistance through PBP modification.** The penicillin-binding proteins (PBPs) are the targets for *β*-lactams. These enzymes are essential for bacterial cell wall synthesis since they perform both transglycosylation and transpeptidation reactions of peptidoglycan biosynthesis. *β*-lactams are irreversible inhibitors of PBPs, binding covalently to the catalytic serine residue of the transpeptidase active site. Thus, bacteria can become resistant by an alteration of the PBP bacterial pattern [168, 169], by point mutations of PBPs, overexpression of one PBP or acquisition of new PBPs. Such a mechanism of resistance is especially important in Gram-positive bacteria in which the evolution of *β*-lactamases or decrease of permeability are uncommon.

Each bacterial species possesses a set of seven to nine different PBPs with distinct enzymatic activities. This means that *β*-lactams do not have a single target, since all PBPs can be inhibited to various extents. Thus, in some resistant strains, several enzymes are mutated. However, the alteration of only one PBP is most often sufficient to confer resistance, since the activity of the altered PBP will compensate for the ones inhibited by the antibiotic. In fact, the substitutions encountered preserve normal cellular enzymatic activity, while the affinity for antibiotics is markedly reduced [170, 171]. For example, the substitution of only two residues of streptococcal PBP 2B (Thr 252 Ala and Glu 282 Gly) may be responsible for some conformational changes in the enzyme structure which result in a decreased affinity for *β*-lactams [172, 173].

Overexpression of unmodified PBP with a naturally low affinity for *β*-lactams can also account for bacterial resistance. This is generally the result of mutations in the regulatory elements. The high amount of PBP then allows the antibiotic effect to be overridden [174, 175]. Acquisition of new PBP with a very low affinity for antibiotics leads to high-level resistance [170, 176, 177]. The acquired gene can also result from recombination events between homologous genes. In such a case, this gene encodes a 'mosaic protein' with low affinity for *β*-lactams [173, 174].

Whatever the alteration of PBPs responsible for resistance, one or a small number of PBPs are able to ensure cellular functions essential for the biosynthesis of the bacterial cell wall.

Vancomycin resistance via the biosynthesis of a new peptidoglycan precursor. Vancomycin is one of the most recent antibiotics to enter clinical treatment, so resistance phenomena are still seldom but occur increasingly. Antibacterial activity of glycopeptides (vancomycin and teicoplanin) is the consequence of their binding to the D-Ala-D-Ala extremity of the peptidoglycan precursor uridine diphosphate-N-acetylmuramic pentapeptide (UDP-MurNac-pentapeptide). These *N*-acyl-D-Ala-D-Ala glycopeptide complexes block the subsequent transglycosylation and transpeptidation which attach and cross-link the incoming disaccharyl peptide to the growing peptidoglycan layer.

Three distinct resistance phenotypes have been described: Van A, Van B and Van C. They differ by resistance level and the genetic determinism of resistance (inducible or constitutive, plasmidic or chromosomic). These phenotypes result from the expression of different proteins but the overall principle is conserved. At least seven proteins are involved in this resistance

mechanism, which consists in an alteration of the metabolic pathway of peptidoglycan precursors that results in the synthesis of a modified precursor, without affinity for glycopeptides but able to participate in peptidoglycan growth. This set of proteins can be acquired by the bacteria, or enzymes involved in normal precursor biosynthesis can be mutated to modify their activity [178].

Van A-inducible phenotype which corresponds to high-level resistance to both vancomycin and teicoplanin has been fully studied. Genic elements of resistance are most often carried on the Tn 1546 transposon made of transposition elements, two genes involved in resistance regulation (*van R* and *van S*), three structural genes directly involved in resistance (*van A*, *van H* and *van X*) and two genes encoding accessory proteins (Van Y and Van Z) which are not essential for resistance [179]. Van R and Van S make up a typical two-component regulatory system that is involved in signal transduction and transcriptional regulation of the structural genes. Together, Van H and Van A synthesize D-Ala-D-Lac rather than D-Ala-D-Ala. Van H is a D-hydroxy acid dehydrogenase which converts pyruvate into D-lactate. Van A is a D-Ala-D-Lac depsipeptide ligase homologous to D-Ala-D-Ala ligases of Gram-negative bacteria [180]. Thus, D-lactate is used by this enzyme to form the D-Ala-D-Lac depsipeptide, which is then complexed to UDP-MurNAc-tripeptide to produce an altered peptidoglycan precursor. Vancomycin binds to this precursor with 1000-fold lower affinity compared with the normal precursor. Van X and Van Y play an indirect role in resistance by decreasing the amount of regular precursor [181]. Van X is an essential D-Ala-D-Ala dipeptidase unable to hydrolyse D-Ala-D-Lac [182]. The more than 10^5-fold difference in catalytic efficiency of Van X for hydrolysis of D-Ala-D-Ala vs. D-Ala-D-Lac leaves D-Ala-D-Lac intact for subsequent incorporation into peptidoglycan. Van Y is not essential for resistance but reinforces Van X activity through its DD-carboxypeptidase activity [183], which catalyses the hydrolysis of the D-Ala-D-Ala extremity of normal precursors. The overall mechanism of resistance is summarized in figure 6.

Recently, it was shown that the Van C protein, involved in Van C phenotype and certainly encoded by a chromosomal gene, has an activity homologous to that of Van A. It mediates the synthesis of a D-Ala-D-Ser depsipeptide which is used to elaborate another type of altered precursor of peptidoglycan without affinity for vancomycin [184]. It was proposed that Van X could be a potential target to restore vancomycin sensitivity. This idea is under investigation [182].

Figure 6. Peptidoglycan biosynthesis in vancomycin-sensitive (*A*) or -resistant (*B*) bacteria.

Nonactivation of the drug

While some therapeutic agents are reactive molecules, others need to be activated to become efficient. Thus, the failure of activation of the prodrug minimizes therapeutic effects of the drug.

Resistance to nucleoside analogues used in cancer chemotherapy

The simplest uracyl derivative, the 5-fluoro-uracyl (5-FU) is one of the most widely used cytotoxic drugs. This molecule is not active by itself but needs to be converted to 5-fluorouridine 5′-monophosphate (FdUMP) inside cells to be effective. Resistance to this compound can be mediated by an alteration of each step of activation, such as inhibition of orotate phosphoribosyl transferase, uridine phosphorylase or uridylate kinase activities, resulting in too low a concentration of active drug.

In the same way, a set of kinases activates cytosine arabinoside (Ara-C), an analogue of deoxycytidine, into

aracytidine monophosphate (CMP), and into aracytidine diphosphate and finally into aracytidine triphosphate which is the active form of the drug. The major mechanism of resistance consists in a decrease of the deoxycytidine kinase involved in the first step of phosphorylation [98].

Purine analogues such as the 6-mercaptopurine (6-MP) and the 6-thioguanine (6-TG) are first converted into monophosphate ribonucleosides by hypoxanthine guanine phosphoribosyl transferase (HGPRT) and then activated into deoxyribonucleotides. The inability of HGPRT to initiate activation results in resistance to these drugs [98].

Resistance to the antiviral drug acyclovir, by modification of thymidine kinase

The target of acyclovir is the RT of HIV-1 but in several resistant viral strains the RT is not altered while the thymidine kinase is. This enzyme is responsible for the activation of the drug by phosphorylation. Thus, resistance results from a deficiency in activated drug. TK is mutated in a way that alters its substrate spectrum: whereas acyclovir is not phosphorylated, TK conserves its biological functions [185, 186].

Resistance to antituberculosis drugs, INH and pyrazinamide

As previously described, INH inhibits biosynthesis of cell wall mycolic acids. However, INH is a prodrug that requires activation to unstable electrophilic species. Deletions or point mutations in the *M. tuberculosis Kat G* gene, which encodes a unique catalase peroxidase, results in the acquisition of INH resistance. This enzyme oxidizes INH to form reactive intermediates which can be quenched with added nucleophiles. Mutations in the vicinity of the active site or deletions make the enzyme less efficient in activating the drug [151, 187]. Rouse and Morris's results [188] hint that genetic alterations other than complete deletion of *Kat G* are prevalent among INH-resistant mycobacteria. Until recently, it was not clear how the bacteria adapt to the loss of Kat G activity in detoxification of organic peroxides generated by the normal biochemical processes of the bacteria. It was shown by Sherman et al. [189] that the loss of Kat G activity is correlated with the overexpression of the *AphC* gene, which encodes for an alkyl hydroperoxidase that appears to compensate for Kat G.

Pyrazinamide (PZA), an analogue of nicotinamide, was used to treat tuberculosis in the early 1950s and is now used in combinatory therapy as it considerably shortens the treatment period when used in combination with INH or rifampicin. In all the strains resistant to PZA, the PZAse activity has been lost. This enzyme, which is a nicotinamidase, degrading nicotinamide into nicotinic acid, converts PZA to the bactericidal pyrazinoic acid. A single mutation of nucleotide 169 leading to the substitution His 57 Asp, or of nucleotides 288 or 162 leading to premature termination, are sufficient to abolish PZAse activity. In the absence of this enzymatic activity, the prodrug PZA is not activated and has no effect on mycobacteria [190].

Drug inactivation

Inactivation of the drug directly results in a deficiency of active drug at the target level. A wide range of drugs can be inactivated, usually leading to high-level resistance. An additional factor that may increase the resistance level is that the altered drug is most often pumped out of the cell and competes with active drug entry.

Degradation of a cytotoxic drug adriamycin

Zhang et al. [191] have shown that eucaryotic cells can become resistant to adriamycin by degradation of this drug before it reaches its cellular target. A reductase appears to be responsible for the enzymatic alteration of adriamycin. Some glycosidic cleavages may also inactivate the drug.

Moreover, it was described recently that resistance to adriamycin can, in some cases, be linked to the overexpression of a tissue-type transglutaminase (TGase). This calcium-dependent enzyme catalyses the cross-linking of adriamycin to the γ-carboxamide group of glutamine residues in some proteins. Thus, adriamycin is trapped and loses its cytotoxic activity [192].

Antibiotic inactivation

Drug inactivation, by hydrolysis or by formation of inactive derivatives, is the most common mechanism of resistance to β-lactams, aminoglycosides and chloramphenicol [193]. The different ways of inactivation are frequent even if they most often result from gene transfer and cannot derive from point mutations of essential genes. Generally, the inactivation gene is disseminated from one bacteria to another by conjugation or transformation, and that transfer is often followed by the evolution of this enzyme to adapt its substrate spectrum.

***β*-lactam hydrolysis.** β-lactam antibiotics represent the major class of antibacterial agents and are widely used to treat various infections. The β-lactam moiety of the antibiotic molecules is structurally homologous to the D-Ala-D-Ala extremity of the peptidoglycan precursor that is the regular substrate of PBPs.

The major resistance mechanism to these antibiotics, in Gram-negative bacteria, consists in the overexpression

of a periplasmic β-lactamase that inactivates the drug by hydrolysis of the β-lactam ring [168]. Various β-lactamases have been described in a wide range of bacterial species [194]. These enzymes differ by their substrate spectrum. However, they are able to rapidly evolve to adapt their spectrum of hydrolysable antibiotics according to selective pressure. In this way, more than 30 variants of TEM-1, the most widespread β-lactamase among Gram-negative bacteria, have been described. They differ from the wild-type enzyme by different combinations of one to five point substitutions among eight residues (Gln 39, Glu 104, Arg 164, Gln 205, Ala 237, Gly 238, Glu 240 and Thr 265) in the vicinity of the active site (fig. 7) [195, 196]. The effect of some mutations on catalysis has been studied, but is not always clear. For example, the role of Glu 104 Lys substitution only shows when combined with another substitution [197]. However, it was shown that Gly 238 Ser substitution has a direct effect on the active-site conformation, leading to its enlargement and thus, to better access of third-generation cephalosporins that can then be hydrolysed [198]. A highly similar structural effect was strongly suggested for the Arg 164 Ser substitution [199].

Thus, the successive clinical use of several generations of β-lactams has lead to the successive appearance of resistance phenomenoms linked to new β-lactamases.

Inhibition of β-lactamases by clavulanic acid or sulbactam, used in combination with a β-lactam antibiotic, restores bacterial sensitivity. However, the clinical use of these inhibitors was rapidly followed by the emergence of resistant bacteria expressing a β-lactamase insensitive to inhibition [200, 201]. Thus, more than 10 variants of TEM-1 avoid the clavulanic acid effect [202]. Substitutions in inhibitor-resistant TEM β-lactamases (IRT) are different from those observed in enlarged

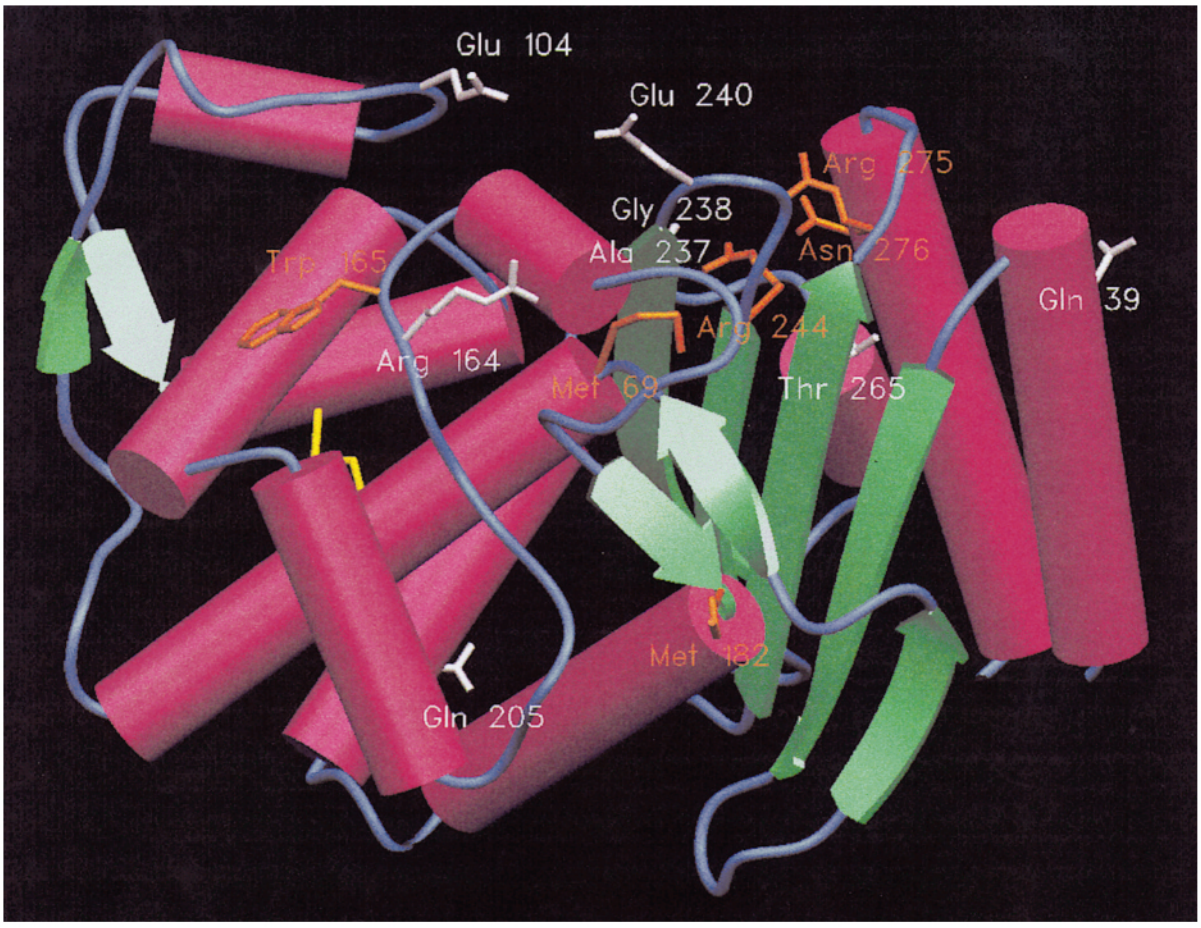

Figure 7. Three-dimensional structure of TEM-1 β-lactamase (Protein Data Bank access code: 1BTL). Residues mutated in extended-spectrum enzymes are shown in white and the ones involved in inhibitor resistance are shown in orange. Graphics were prepared with SETOR [Evans S. V. (1993) J. Mol. Graphics. **11:** 134–138].

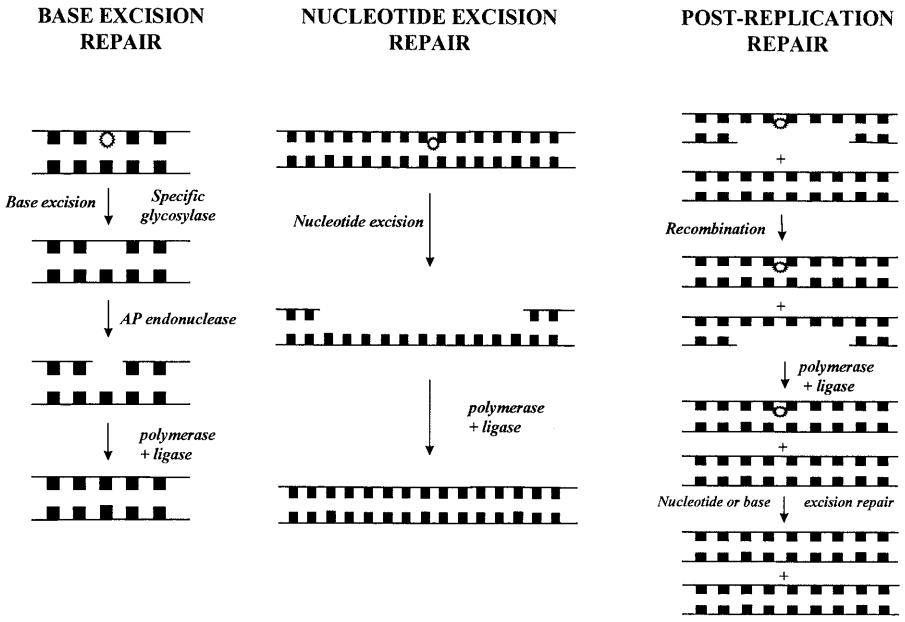

Figure 8. The three major pathways of DNA repair. In base excision repair, phosphodiester bond cleavage can be performed by AP endonucleases (5′ to the AP site) or the AP lyase activities of some glycosylases (3′ to the AP site). Postreplication repair can proceed by reinitiation or by template switching (see ref. [209] for a review).

spectrum β-lactamases, since IRTs differ from TEM-1 by one to three substitutions among six residues (Met 69, Trp 165, Met 182, Arg 244, Arg 275 and Asn 276, fig. 7). Met 182 Thr and Trp 165 Arg substitutions do not seem to have any effect on TEM-1 inhibition. However, it was shown that mutations Met 69 Ile, Val or Leu [203], Arg 244 Cys, Ser or Thr [203] and Asn 276 Asp [204] clearly reduce clavulanic acid inhibition of TEM-1. These substitutions probably modify the active-site topology and displace a water molecule essential to the inhibition pathway.

Pursuit of structure-function studies of TEM-1 could lead to nonhydrolysable β-lactams or efficient inhibitors of β-lactamases.

Aminoglycoside inactivation. Inactivation is the major means of resistance to aminoglycosides. It consists in a chemical modification of the antibiotics by acetylation, adenylation or phosphorylation. The target of this class of antibiotics is the ribosome, and the chemical alteration of aminoglycosides considerably decreases ribosomal affinity for these drugs.

Three types of enzymes perform these modifications: acetyltransferases (AAC), adenyltransferases (ANT) and phosphotransferases (APH). Many enzymes exist in each type. They have been classified according to their substrate spectrum, and thus the resulting resistance profile and site of antibiotic modification [205, 206]. In many resistant clinical isolates, a unique bifunctionnal enzyme (acetyl- and phosphotransferase) has been described. It results from the transcription and translation of a hybrid gene that may be obtained by the fusion of two distinct resistance genes [193].

Inactivation of other antibiotics. Like aminoglycosides, chloramphenicol can be inactivated by acetylation or diacetylation by a chloramphenicol acetyltransferase (CAT). Over 10 different enzymes have been described. They could have evolved from an ancestor enzyme to increase their affinity for the antibiotic [207].

Various other antibiotics can be altered (193) by phosphorylation or by glycosylation (erythromycin, rifampicin), by acetylation (streptogramin A), by nucleotidylation (lincosamides), by reduction (erythromycin) or by degradation (fusidic acid, erythromycin) [208–210]. Ribosylation of rifampicin has also been described [211]. Moreover, an enzyme sharing homology with monooxygenase confers low-level resistance to rifampicin through its degradation [212]. More recently, a secreted enzyme responsible for the inactivation of fusidic acid has been identified and purified. This enzyme is a highly specific esterase that modifies fusidic acid to its inactive lactone derivative [213].

Enhanced DNA repair function

DNA is the target of various cytotoxic drugs. Through direct or indirect binding to DNA, these drugs produce DNA alterations and genomic lesions leading to cell death. Simple alkylating agents covalently bind DNA,

most often on guanine N^7 or O^6 atoms. The two reactive groups of the bifunctionnal alkylating agents allow these drugs to form intra- or interstrand cross-links. Metallic derivatives, such as cisplatin, are also able to form DNA cross-links. Cisplatin generally disrupts DNA by inducing intrastrand cross-links between N^7 atoms of two adjacent guanines and to a lesser extent interstrand cross-links and monoadducts. Other cytotoxic drugs are able to noncovalently intercalate into DNA. Although all these interactions with DNA are potentially lethal, differences in the extent of their repair will affect the magnitude of cell death. An inverse relationship exists between cellular repair and cytotoxic sensitivity [214].

DNA repair processes are very complex and depend on the lesion to be repaired. Their regulation can involve up to 200 different genes. They have a major impact in cancer chemotherapy failure since they are involved in the resistance to a high number of cytotoxic drugs, especially the ones generally not affected by MDR phenotype [215]. The three major mechanisms of DNA repair are damage reversion, damage excision and postreplication repair (fig. 8) [95].

Damage reversion is the simplest biochemical pathway to restoring DNA structural integrity. O^6-alkylguanine DNA alkyltransferase is the major contributor to resistance to alkylating agents [216]. It transfers alkyl groups from O^6-guanine to a cysteine of the enzyme, preventing mutations [217, 218].

Reversion of this mechanism of resistance has been considered. Inhibition of the alkyl transferase results in significant potentialization of the cytotoxic effects of drugs. Various O^6-alkyl-guanines have been tested, the O^6-benzyl-guanine being the most efficient inhibitor [217]. However, such treatment may induce tumour development, since a cellular function of alkyltransferase is the cellular prevention of carcinogenic effects of numerous molecules [217].

Damage excision consists in base excision by a specific glycosylase in the case of specific base damages, followed by nicking of the DNA and elimination of the abasic site which is then repaired by a polymerase and a ligase, or polynucleotide excision which repairs a wide range of DNA lesions. In this case, nicking of the DNA on both sides of the lesion results in the excision of a 22- to 36-mer oligonucleotide, the gap being then repaired by a polymerase and a ligase. Numerous enzymes are involved in these resistance mechanisms, such as DNA glycosylases, polymerases and ligases. Their expression levels are positively regulated in the resistant cells.

Postreplication repair allows the repair of severe DNA lesions such as intra or interstrand cross-links. If unrepaired before replication, these lesions will result in replication blocks. The cells will reinitiate DNA synthesis past the lesions, using a downstream alternative origin of replication on the same chromosome that will approach the DNA damage from the other side, overcoming the blocks and leaving a small single-stranded gap. This gap induces a repair mechanism that will involve recombination events (daughter-strand gap repair) or template-strand switching [219].

The first step of repair is the same for all these mechanisms. It consists in recognizing of DNA damage, which involves proteins whose function is not always clear.

Introduction of DNA repair inhibitors in therapeutic regimens may help to improve therapeutic strategies. Several studies are under investigation to discover such chemosensitizers. The inhibition of specific enzymes such as DNA polymerase α by amphicolin or topoisomerase II may decrease repair activity [98]. Ara-C produced a synergistic toxicity with cisplatin apparently by increasing the formation of cisplatin-induced DNA cross-links. However, a relatively high dose of Ara C is required, and its distribution is tissue-specific. The more stable derivative F-Ara-A has different cytotoxic effects mediated by the inhibition of key enzymes of DNA metabolism. It seems to be an effective inhibitor of DNA repair, since it enhances the cytotoxicity of cisplatin at clinically achievable concentrations [220].

Conclusion

Numerous factors affect the efficiency of chemotherapeutic drugs, including pharmacological factors that prevent an adequate drug exposure at the site of action: the treatment used (drug administration regarding concentration and time) and morphologic considerations (absorption, metabolism, vascularity and tissue oxygenation) [221] play an important role in determining the success or failure of chemotherapy. However, beside these pharmacological factors, which can be accounted for by the use of adapted regimens, different cellular mechanisms are responsible for low- to high-level resistance.

Eucaryotic cells, parasites, viruses and bacteria have evolved a number of different ways to circumvent the toxic effects of numerous structurally and functionally unrelated drugs. Surprisingly, mechanisms responsible for resistance to various xenobiotics appear to be conserved throughout the living world.

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