

New Concepts in the Evaluation of the Pharmacokinetics Behaviour of Antibiotics

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แนวทางใหม่ในการประเมินเภสัชจลนศาสตร์ของยาต้านจุลชีพ

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ยาต้านจุลชีพที่ใช้รักษาโรคติดเชื้อจะให้ประสิทธิภาพได้เหมาะสมที่สุด ขึ้นอยู่กับปัจจัยหลายประการ ได้แก่ ตัวผู้ป่วยเอง ภูมิคุ้มกันของผู้ป่วย เชื้อก่อโรค และเภสัชจลนศาสตร์ของยา. ในปัจจุบัน พบว่าเภสัชจลนศาสตร์ของยามีความสำคัญเพิ่มขึ้นเรื่อยๆ และสามารถใช้อธิบายทฤษฎีการสำร่อย หรือความล้มเหลวของการรักษาได้ด้วย. โดยหลักพื้นฐาน ยาต้านจุลชีพจะต้องไปถึงจุดที่มีการติดเชื้อเพื่อการกำจัดเชื้อให้หมดไป. และจะต้องมียาอยู่ในกระแสเลือด น้ำเชื้อนั้นได้ถูกนำมาเข้าสู่วงไหลเวียนเลือดแล้ว. นอกจากนี้อัตราการซึมผ่านของยาเข้าไปสู่ตัวจุลชีพได้ร่ามากน้อย และการสะสมยาในตัวจุลชีพ หรือในเม็ดเลือดขาวก็จะเป็นปัจจัยสำคัญในการกำจัดเชื้อก่อโรคที่หลบซ่อนอยู่ภายในเซลล์ของเม็ดเลือดขาวด้วย. การค้นพบปรากฏการณ์ที่เชื่อมีการแปลงรูปร่างเมื่อระดับยาค่าต่ำกว่าระดับเริ่มเชื้อ โดยเฉพาะ ยากลุ่มมัยตาแลกเทมที่พบว่าจุลชีพถูกกินโดยเม็ดเลือดขาวได้ง่ายขึ้น. ยาประเภทที่ออกฤทธิ์เข้าไปในคีย์โพลัสสม หรือ RNA จะสามารถหยุดการแบ่งตัวของเชื้อได้หลายชั่วโมง. ข้อมูลทั้งหมดนี้เมื่อนำมาใช้ในการจัดการบริหารยา จะทำให้สามารถบริหารยาได้เพียงวันละครั้ง และยังมีการกระจายไปสู่บริเวณรอยโรคได้ดีขึ้น. (วารสารโรคติดเชื้อและยาต้านจุลชีพ 2537 ;11:37-43.)

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A correct antibiotic therapy must take into account some parameters related to the host, the infectious agent and the drug to be used. Since the beginning of the antibiotic era, much has been known with regards to microbiological, pharmacological and clinical aspects, which have been studied in depth. Therefore on the basis of the most recent acquisitions, the antibiotic treatment for many infectious diseases has been often

basically modified.

From the point of view of microbiological aspects, in the last years changing patterns in bacterial ecology and in the susceptibility of pathogens to antibiotics, as well as emergence of "new" microbial species have been observed. On the other hand, changing characteristics in the patient populations and disease evolution have been observed too.

Therefore the need either of new antibacterial drugs or of changing the so called "traditional" antibiotic schedules has been deeply felt. Search for new drugs has been expanding, and new antibiotic regimens have been adopted on the basis of bacteriological, pharmacological and pharmacokinetic criteria which were highlighted in recent years.

This paper reviews some concepts which have been for a long time the guidelines for antibiotic treatment. More recently new concepts have been introduced, often representing a revision of the so called traditional ones.

Bacteriological criteria

An antibiotic must be chosen on the basis first of its antibacterial spectrum of activity, which can be wide or narrow.

A wide spectrum of activity allows the use of the drug in many clinical situations, when mixed bacterial flora is present and particularly when bacteriological diagnosis is lacking. However, it is more appropriate, when possible, to use antibiotics with the most specific activity against the pathogen on the basis of the susceptibility tests, so that therapeutic results can be achieved, avoiding alteration of the normal resident bacterial flora. Nowadays, many bacterial species resistant to the common antibacterial agents are spreading out, because of the extended use of antibiotics, which can deeply modify bacterial ecology.

Besides the antibacterial spectrum, the intrinsic antibacterial activity against the pathogen must be taken into account. When considering the "*in vitro*" Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC), the concentrations which approximately must be achieved at the level of blood, tissues and particularly of infectious focus in order to obtain therapeutic results can be desumed (1, 2). However, the antibiotic dosage regimen can be defined not only on the basis of antibacterial activity but also on the basis of pharmacokinetic behaviour. At the beginning of the antibiotic era dosage regimens were defined with empirical criteria: in fact achievement and maintenance of blood concentrations equal to or even superior to either MIC or MBC between doses were considered necessary for bacterial eradication and clinical cure. The more recent acquisitions in this field have deeply modified these concepts, as it will be reported later on.

Pharmacological and pharmacokinetic criteria

Antimicrobial agents can exert antibacterial activity only if the pharmacokinetic behaviour allows an adequate distribution in body fluids and tissues and the achievement of therapeutic concentrations at the infection site, without adverse effects.

Tissue levels, however, are not only related to blood levels, since other pharmacokinetic parameters, such as molecule physicochemical characteristics, serum protein binding, etc. may condition drug penetration into tissues and body fluids. Moreover some particular organism compartments, such as cerebro-spinal fluid, prostatic fluid, sputum, etc., are endowed with specialized transport mechanisms, which further condition the antibiotic penetration.

Drug penetration into the infection site can be prevented by some local features, such as fibrosis, thick walls endowed with low permeability to the drugs (abscess for instance), etc. Other conditions at the infection site (presence of purulent material, pH, oxygen pressure, etc.) can heavily hamper the activity of many antibiotics. It must be taken into account that drug concentrations which are reached at tissue level or at the infection site must be related to the MICs requested to inhibit the specific pathogen responsible of the infection. It can be deduced that the therapeutic efficacy of antibiotics relies on several parameters of different nature, that need to be carefully evaluated (3).

Traditional antibiotic treatment schedules

The so called "traditional" antibiotic treatment schedules relied on the blood achievable MICs. An antibiotic peak concentration 4 to 8 fold the MIC was considered adequate. Such a definition is to be thought arbitrary and empirical, due to the incomplete knowledges of pharmacokinetic behaviour of antibiotics at that time. In some severe clinical situation intravenous continuous infusion therapy was thought mandatory to maintain drug concentrations above such levels. On the other hand, as some antibiotics, mainly beta-lactams, are active against rapidly growing bacteria, the administration of the drug in fractional doses was believed more rationale, in order to allow bacteria to reach log phase growth in the interval between administrations; this strategy should guarantee a higher activity of the successive drug dose (1).

None of the two theories was experimentally proven in a definite way, even if the clinical practice has led to individualize which choice seems to be more adequate for a given pathological situation. Concerning the duration of treatment, it was considered necessary to prolong drug administration for a few days after clinical cure and defervescence, as therapy success was considered to be linked mainly to the maintenance of "therapeutic" concentrations for a long time.

An experimental approach devised to demonstrate which was the most adequate intervals between antimicrobial administration, was obtained in the forties by Eagle and other Authors (4-6); they put in evidence the so called "post-penicillin stationary phase". According to this finding, *Staph. aureus* and other gram-positive cocci, exposed "in vitro" to penicillin, do not regrow immediately when the antibiotic is removed, but they start to grow with a certain delay. This phenomenon was subsequently studied more in detail, and was demonstrated for many other antibiotics and defined "post-antibiotic effect" (7). Many "in vitro" models were prepared to simulate "in vivo" conditions: the antibiotic activity was studied gradually reducing its concentrations in function of the half life (8, 9). By this way it could be demonstrated that when antibiotic concentrations fall below MIC, bacteria stop their growth at a lesser extent than when they are exposed to constant concentrations. When the antibiotic is completely and rapidly removed, bacterial growth is completely inhibited for a certain time (different according to antibiotic and bacterial species), while when antibiotic concentrations are gradually reduced, the inhibition period is shorter and is followed by a period in which bacterial division process is prolonged. The experimental demonstration of this phenomenon led to consider not necessary to maintain antibiotic blood concentrations always above the MIC, between the dose intervals, but it was considered enough to maintain the drug levels above the MIC for half an interval between administrations; in this way it could be taken advantage of the post-antibiotic effect.

Later on the relevance of specific and non-specific mechanisms of host defense in the therapeutic response was demonstrated (10). In fact in the immunocompetent host they co-operate in the eradication of infection. Their determining importance became more evident when the course of infection in subjects affected with various kind of immunodepression could be followed. In this situation it is of importance to determine whether or not

antibiotics can directly interfere with specific and non-specific defense mechanisms. Many "in vitro" studies where performed on this topic, often with conflicting results (11, 12, 13). A few studies, however, performed to demonstrate a possible negative interference of antibiotics on the natural defense mechanisms "in vivo", did not confirm this hypothesis. In fact the complex "in vivo" reactions hardly allow to individualized either a positive or a negative interference on the complicated processes which follow an infection or a pharmacological intervention. In some cases, however, some beta-lactams and particularly cefodizime, a third generation cephalosporin, show a positive interference, even "in vivo", on some phagocytic functions (14).

Formulation of "up-to-date" antibiotic treatment schedules

So far the so called "traditional" concepts, which were considered of paramount importance in the formulation of antibiotic therapy were briefly exposed. The "up-to-date" antibiotic treatment schedules rely on new and old concepts, the latter critically reviewed according to the most recent acquisitions.

Nowadays bacteriological criteria tend to be more comprehensive: the antibacterial activity of an antibiotic is defined according the MIC₅₀ and MIC₉₀, that is to the MICs for 50% and 90% of clinical isolates. In this way the epidemiological situation regarding susceptibility of bacterial species in a definite geographic area or environment (e.g. hospital) is taken into account. These parameters are particularly useful to establish an empiric therapy in a more rationale way when susceptibility testings are lacking and it is possible to rely only on the epidemiological situation relative to a definite area. This aspect is particularly important not only for community-acquired infections, but also and mainly in hospital setting, where the very common and often unduly use of antibiotics exerts a selective pressure on the resident bacterial population contributing to the emergence of bacterial species with multiple resistances to the most commonly used antibiotics. In the evaluation of bactericidal activity more attention is now paid to the killing rate, as, of course, the more rapid is the bactericidal effect the more efficacious is considered the antibiotic activity.

The epidemiological studies concerning the diffusion of antibiotic-resistant bacterial strains both in community and in hospital setting have been increasing in the years;

in this way the present diffusion of resistance is usually well known, so that a more rationale empiric therapy can be established, whenever requested. The diffusion of plasmidic resistance is of course wide and dangerous as it easily diffuses even among different bacterial species. Resistance to beta-lactams is quite widespread and is usually mediated by beta-lactamase production. Nevertheless also resistance due to modifications of the antibiotic target (for instance of PBPs, that are proteins which bind beta-lactams; of DNA-girase, the enzyme that binds to quinolones, etc.) or to modifications of bacterial cell permeability, though less frequent, are quite important for some bacterial species. In recent years the significance and value of "post-antibiotic effect" has been revised. Even if its real value in clinical practice has not yet been fully understood, its "*in vitro*" demonstration has conditioned the determination of intervals between antibiotic administration in therapy. The results so far obtained seem to confirm its influence on the duration of "*in vivo*" antibiotic activity. To confirm that the "*in vivo*" antibiotic efficacy is due not only to the achievement of MIC at blood and tissue level, but involves other factors besides those so far described, that is the "post-antibiotic effect" and the cooperation of defense mechanisms, also the role of the so called subinhibitory concentrations must be considered (that is antibiotic concentrations below MIC) (15). Such concentrations (mainly those of beta-lactams) are in position to promote evident structural modifications in bacterial cells, mainly in the cell wall, which induce the formation of filaments which can not divide. Such filaments are more easily uptaken by phagocytes and killed by polymorphonuclear cells. Moreover many antibiotics at subinhibitory concentrations are able to reduce bacterial adherence to endothelial cells; this circumstance is of paramount importance to allow penetration of microorganisms into tissues where they cause infection (16).

Half-life

For a long time drug dosage and interval between administrations have been determined, besides the bacteriological considerations, only on the basis of this pharmacokinetic parameter. However it has been ascertained that its determination offers a limited view of the behaviour of the drug in the body, where many different processes can influence its permanence in it. Today it is preferred to refer to the so called "mean residence time" that is the mean time necessary for

unmodified drug to pass through the body, taking into consideration all kinetic processes (bioavailability, absorption, distribution, metabolism, etc.) and not only the blood level curve. By this way a more precise evaluation of the time during which a drug remains in the body is obtained: when antibiotic is no longer detectable in blood after a certain interval from administration, that does not necessarily mean that it has been already eliminated, since it may have a more prolonged permanence in tissues. The behaviour of some among the new antibiotics, showing prevalent localization at tissue level has confirmed the necessity of this kind of evaluation.

Volume of distribution

This term refers to hypothetic volume of body fluid required to dissolve the total amount of drug at the same concentration present in blood. Drugs with high volume of distribution, such as rifampicin, macrolides, chloramphenicol, etc. distribute largely in tissues, both in intracellular and extracellular space. Other drugs, such as beta-lactams, aminoglycosides, etc. have low volume of distribution and reach only the extracellular space (17). Each one of the two categories of drugs has defined clinical indications: drugs with low volume of distribution will be effective in the treatment of infections due to pathogens with extracellular localization, while they will not be active in infections due to obligate intracellular pathogens. In the latter situation antibiotics capable to reach therapeutically active concentrations inside cells must be employed (18). However, since such antibiotics can reach active concentrations also in the extracellular fluid, they will be effective also against bacterial population located there.

Serum protein binding

The importance of serum protein binding in determining both antibacterial activity and passage of the antibiotic in extravascular fluids has been long debated (19, 20, 21). Antibacterial activity is developed only by the free fraction of the drug. Binding is reversible: protein-free fraction and protein-bound fraction are in equilibrium, so that a portion of free drug will be available at rate which is in function of stability and extension of binding. Only unbound fraction can reach interstitial fluid: the degree of penetration is determined by the ionization form of circulating drug, being the ionized form non diffusible and the non-ionized form diffusible. As consequence, drugs, even if extensively bound to serum proteins can reach high tissue concen-

trations if the free fraction is in non-ionized form which has the capability to penetrate tissues, allowing a prompt availability of an other non-ionized free fraction of drug. The capacity of antibiotics to bind to proteins of extravascular fluids and tissues is a further factor in position to modulate the pharmacokinetic behaviour since the bound fraction of drug can constitute a kind of reservoir from which the free drug is slowly released in blood stream (22).

Concentration in tissues and infectious focus

The capacity of antibiotics to penetrate in tissues has been always considered a fundamental prerequisite for their therapeutic activity. The degree of penetration, as it has been shown in the preceding paragraph, is determined by the physico-chemical and pharmacokinetic properties of the drug. Further conditions exist that control drug penetration and distribution in tissues. In the so defined "non specialized" tissues, the passage of antibiotics takes place by passive diffusion: in this situation serum/tissue concentration ratio is easily predictable. The so called "specialized" tissues and sites, such as central nervous system, retina, prostate, etc., are endowed with not fenestrated capillaries: the passage in these sites occurs by active transport. Therefore serum/tissue concentration ratio is more complex and conditioned by several factors.

Tissue is constituted by cells and interstitial fluid: drug lipophily determines the degree of intracellular penetration. Non lipophilic drugs (e.g. beta-lactams) do not penetrate cells, but are confined in the extracellular space, where concentrations correlate with serum concentrations. Very lipophilic drugs (e.g. tetracyclines and macrolides) and some amphoteric drugs (e.g. fluoroquinolones) penetrate quickly into cells and accumulate in them. Tissue levels of the latter drugs may be higher than serum levels (as it can be expected from the large volume of distribution) and serum concentrations are often lower than those of non lipophylic drugs (23, 24).

Ratio among levels reached in extracellular and intracellular fluid differs according to the body district. Traditionally the determination of tissue concentrations of antibiotics is carried out on tissue homogenate where it is not possible to evaluate if the drug fraction is located in interstitial fluid or in cells. This method gives only a rough indication of the entity of presence of the drug in tissue, but it leads to an incorrect evaluation of tissue/serum concentration ratio. In fact when infection

is not intracellular, it is located at interstitial space level. Therefore it is important to know how much antibiotic reaches such a space. Some experimental models feasible in animals and men, such as the production of suction or cantharidine blisters in which the collected fluid is roughly similar in composition to the interstitial one, can provide some useful informations. In fact, antibiotic levels in blister fluid, after administration by systemic route, offer a more reliable information about the active concentrations that can be reached at the infectious focus. Blister fluid concentration ratio of beta-lactams and aminoglycosides is equal or lower than 1, while ratio is higher in the case of some antibiotics (e.g. macrolides) or chemotherapeutic agents (e.g. fluoroquinolones) (25, 26).

However, when infection is present, other factors interfere in the behaviour of the antibiotic in tissues and at infectious focus: it is not yet completely elucidated how infection and inflammation can interfere in pharmacokinetic behaviour of antibiotics. However it is ascertained that increased hematic flow and inflammation favour penetration of antibiotics in tissues and infectious focus. On the contrary when abscessual cavity is delimited by a non vascularized capsule, antibiotics can not reach infectious focus. Other factors such as pH, oxygen tension, redox potential, can modify the activity of antibiotics either in positive or negative direction. Local necrosis and abscess formation definitely contribute to decrease antibiotic activity.

Renal and biliary excretion

Elimination route of antibiotics may influence their activity in some body districts, their toxicity and interference with other drugs. Main routes of elimination are the renal and biliary ones.

When biliary elimination route is prevailing, besides a high activity in infections of biliary tract and parenchymal infections, a certain incidence of intestinal disturbances has to be expected. In fact the elevated intraluminal concentration of antibiotic, particularly if it is active against anaerobes, produce an unbalance of normal intestinal flora and consequently intestinal troubles. Hepatic metabolism can produce several metabolites, endowed or not with antibacterial activity and with different toxicity in comparison to original compound. Often, if the drug is metabolized by microsomal hepatic system, interference with metabolism of other drugs can occur. As consequence increased degradation of a concomitantly administered drug is

obtained or, on the contrary, its blockade. Diminished or increased blood levels of the drug, respectively will result: two situations that can lead to drug ineffectiveness or to toxic effects. Knowledge of entity and type of metabolism as well as of elimination of drugs by biliary route allows to adequately modify dosage in case of hepatic insufficiency. Moreover it is possible to predict interference with other drugs at prevalent hepatic metabolism. In such cases it may be necessary to monitor blood levels to determine whether they are in the range necessary to assure efficacy.

Severe insufficiency of renal function imposes to reduce dosage of the antibiotic or to extend interval between doses, when drugs at prevalent renal excretion are employed. In fact the reduced elimination implies prolongation of half-life and increase of blood and tissue levels of the antibiotic, with risk of accumulation and toxic phenomena. However, according to the drug and its pharmacokinetics, reduction of dosage will be necessary at different levels of renal failure: for instance, if the drug is excreted both by renal and hepatic route, dosage adjustment is necessary only when renal function is severely deteriorated since elimination through liver may represent a vicarious route for the elimination through kidney. On the contrary for drugs at prevalently glomerular excretion and without possibility of hepatic elimination, such an adjustment has to be adopted when the renal insufficiency is of slight and moderate degree.

In conclusion, the widening of knowledge on bacterial biology, on multifaceted aspect of antibacterial activity and pharmacokinetic behaviour of antibiotics, has led to identify new approaches in the formulation of therapeutic schemes. They have found application mainly for new antibiotics the peculiar pharmacokinetic characteristics of which have been put in evidence. Long half-life, higher penetration in tissues and cells require shorter period of treatment. The most recent macrolides, (dirithromycin, clarithromycin and particularly azithromycin) represent an example of new trends in treatment schedule.

The new concepts have led to the revision of already established schedules of antibacterial therapy with well known antibiotics. For instance the therapy of some uncomplicated urinary infections consists in one single dose of antibiotic, surgical prophylaxis has been shortened to 24 hours, tuberculosis is treated by "short course" or "intermittent" administration of antituberculous drugs, etc.

The present review on the evolution of concepts that have been for years the basis of antimicrobial therapy, demonstrates how they have been modified on the basis of a series of new acquisitions. However the evolution continues: "new antibiotics", "new pathogens", "new patients" will probably require further modifications of treatment modalities, in order to render therapy more adequate to the continuously evolving therapeutic needs.

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