Pharmacokinetics of the Treatment of Fungal Infections

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In considering the suitability of a compound for antimicrobial therapy three factors must be taken into account: the antimicrobial activity of the compound; its absorption, distribution and excretion from the body, that is to say the drug’s movement in the body or ‘pharmacokinetics’; and its toxicity.

GENERAL PHARMACOKINETICS

Unless a drug is given intravenously, when it achieves an almost instantaneous concentration in the plasma, it is necessary to consider how much of an orally administered agent survives destruction by the acid and enzymes of the stomach, and what proportion is absorbed from the intestine into the plasma or lost in the faeces; if it is given intramuscularly, how much is absorbed from the injection site and how quickly.

When the agent reaches the plasma it may become bound to protein and thereby rendered inactive, as only the unbound fraction of the drug exerts an antimicrobial effect. Even while absorption is continuing to make the plasma level rise, elimination of the drug by metabolism and excretion begins. Some of the compound may be chemically modified or conjugated in the liver, liberating products which re-enter the plasma or are excreted via the bile into the gut where some may be reabsorbed and subsequently re-excreted in an 'entero-hepatic cycle'.

Some of the compound may be excreted by the kidney in the glomerulo-filtrate or by active tubular secretion, and some of that which appears in the tubules may be actively reabsorbed again—a process which, depending on the chemical nature of the compound, may be crucially affected by the pH of the urine. In this and other ways, the handling of the parent compound may differ from that of its derivatives or conjugates.

TISSUE DISTRIBUTION

There finally remains a residue of the agent which has left the plasma and found its way into the extracellular space. From the extracellular space it
penetrates the cells to reach any parasite that has taken up an intracellular position. On leaving the plasma, some of the agent may become fixed to cellular binding sites corresponding with those of the plasma proteins and, because the tissue sites are likely to be much more numerous, they may constitute the main reservoir of the drug. The bound drug (like that attached to plasma proteins) is in equilibrium with its free form and will be re-liberated as the concentration of the free drug falls with its uptake into lymphatics and capillaries.

ACCESS TO SPECIALISED ISSUES
There are particular problems of access to specialised tissues such as the meninges and the lungs which are often particularly involved in the treatment of systemic fungal infections. Passage of drugs from the plasma to the CSF by diffusion is usually very limited but may increase considerably in the presence of inflammation, when greatly increased permeability allows circulating agents and other blood constituents to reach the CSF by ‘bulk transport’. When inflammation is absent, as in many fungus infections, several therapeutic agents reach the CSF in very low concentrations so that the systemic administration of drugs must be supplemented by intrathecal injection. Some compounds, notably penicillins, can be actively transported out of the CSF by a mechanism resembling that of the proximal renal tubule, but this has not been described as occurring with antifungal agents.

Transport of drugs into the lungs is more complicated and less well documented. I have discussed elsewhere (O'Grady, 1968) the factors likely to influence the levels of antimicrobial agents reached at sites of infection in the lungs, but few meaningful direct measurements of the concentrations found in the course of therapy have been made. In general, it is reasonable to suppose that agents which diffuse readily elsewhere cross the alveolar capillaries with little difficulty, but access to the bronchial lumen appears to be limited unless gross inflammation facilitates the transport of plasma constituents.

Information about the pharmacokinetics of antifungal agents in humans is limited, making it necessary to supplement our meagre knowledge from animal experiments, which ignore species differences.

ANTIFUNGAL AGENTS
There are numerous antifungal agents available in the sense that many substances inhibit fungal growth or even kill fungi in vitro, but very few are useful for the treatment of systemic fungal infections in man. First, many of them are not very active, at least in comparison with the extraordinary activity of antibacterial agents such as penicillin. Secondly, the substances that
are active *in vitro* are often rapidly broken down or conjugated in the body and antimicrobially inactive compounds are released. Thirdly, a number of these substances are too toxic for systemic use in man.

The relatively few agents suitable for systemic rather than local use include various polyenes, particularly amphotericin B, and possibly hamycin; two synthetic agents, 5-fluorocytosine and clotrimazole; a natural product of a bacillus, saramycetin, and for dermatophyte infections, griseofulvin. The plasma levels reported with these drugs are shown in Table 1.

**Table 1. Plasma levels from conventional doses of antifungal agents (Louria, 1958; Utz et al., 1968; Crounse, 1963; Plempel et al., 1969; Witorsch et al., 1966; Koechlin et al., 1966).**

| Agent            | Dose (mg/kg) | Route | Plasma level (µg/ml) |
|------------------|--------------|-------|----------------------|
| Amphotericin B   | 1            | i/v   | 0.5-3.5              |
| Hamycin          | 20/day       | Oral  | Trace-0.1            |
| Griseofulvin     | 100          | Oral  | 1-2                  |
| Saramycetin      | 3-5          | s/c   | 2-13                 |
| Clotrimazole     | 40/day       | Oral  | 1                    |
| 5-fluorocytosine | 30           | Oral  | 10-40                |

Although our knowledge of their absorption, distribution, metabolism and excretion is far from complete, the antifungal agents currently available are a useful illustration of factors that should be studied in evaluating any antimicrobial agent. These are:

1. *In vitro* versus *in vivo* activity.
2. Selective toxicity, that is to say disparity between toxicity for microbial and mammalian cells.
3. The ‘heterogeneity’ of natural drugs and the effect of route of administration and of formulation changes on their behaviour.
4. The effects of accumulation and concentration in special organs or tissues.
5. Adaptive and compensatory breakdown of the drug.
6. Non-microbiological activity.

*In vitro versus in vivo Activity*

The important distinction between *in vivo* and *in vitro* activity is well illustrated by the substituted hydroquinolines, several of which are potent antifungal agents *in vitro*. Some years ago Dr R. E. M. Thompson and I examined a large number of substances which appeared to have potential as agents for
the treatment of systemic fungal infections (Thompson and O'Grady, 1959). In these experiments a suspension of *Candida albicans* was injected into the thigh of the mouse where it invaded the muscle and excited a considerable inflammatory response, producing a swelling of the thigh that could be measured with calipers (O'Grady, 1966). Comparison of the size of the lesions which developed in treated and untreated animals provided a measure of antifungal activity *in vivo* which corresponded well with the established value of the compounds in human therapeutics. One of the compounds examined, 5-nitro-8-hydroxyquinoline, (nitroxolin), had a very wide antimicrobial spectrum including *Candida albicans*, for which the minimum inhibitory concentrations were 2 to 3 μg/ml, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Despite this activity *in vitro*, however, treatment of the mouse thigh Candida lesion with nitroxolin produced no effect whatsoever (O'Grady and Smith, 1966). The reason for this disparity is that in the body the compound is treated like a phenol and is rapidly converted into microbiologically inactive conjugates.

**Systemically Active Agents**

In the course of the study on nitroxolin we again demonstrated the marked activity of nystatin, discovered by Hazen and Brown in 1951 and since shown to be a member of a very large group of 'polyene' antibiotics. The polyenes, very few of which have come into clinical use, are so called because their large circular molecules contain numerous double bonds. Pimaricin, which has four double bonds, is a tetraene, amphotericin B, which has seven double bonds, a heptaene, and so on (Lampen, 1966).

Polyenes differ in their antifungal activity and Table 2 compares the activity of three that are in clinical use: hamycin, nystatin and amphotericin

|                         | Minimum Inhibitory Concentration (μg/ml) |
|-------------------------|------------------------------------------|
|                         | Hamycin | Nystatin | Amphotericin B |
| *Candida albicans*      | 0.01    | 1.0      | 0.04           |
| *Candida tropicalis*    | 0.01    | 0.5      | 0.01           |
| *Aspergillus niger*     | 0.4     | 0.8      | 1.0            |
| *Microsporum canis*     | 0.01    | 0.5      | 0.05           |
| *Trichophyton mentagrophytes* | 4.0   | 3.0      | 0.7            |

B. Amphotericin B and hamycin are substantially more active than nystatin against *Candida* and a *Microsporum*, but hamycin is less active than nystatin and very much less active than amphotericin against *Trichophyton*.
Selective Toxicity
None of these polyenes has any effect on bacteria because they unite with sterols, which are important constituents of the fungal cell membrane but not of the bacterial cell membrane (Lampen, 1966). The effect of the union of the polyene and cell membrane sterol is to increase the permeability of the cell, causing loss of potassium, sugars and other small molecules, and making it impossible for the integrity of the milieu interieur to be maintained. This is also the basis of the toxic effect of amphotericin B which produces haemolysis of red cells and damages the peculiarly sensitive cells of the proximal renal tubules.

Routes of Administration
Nystatin was extremely effective when given systemically to mice suffering from the experimental Candida thigh lesion but had no effect when given by mouth (O'Grady and Thompson, 1961). Amphotericin B was as effective as nystatin when given intramuscularly and also had some effect when given by mouth. Partial absorption of amphotericin B from the gut has been demonstrated in man (Louria, 1958) but the degree of absorption is insufficient for the agent to be used by this route for the treatment of systemic infections.

Hamycin is comparatively well absorbed when given orally, provided the preparation is in a suitable form (Utz et al., 1968).

This raises the question of the importance of the composition of the pharmaceutical preparation. We tend to think that preparations contain what is written on the label and nothing more and that those with the same name and dosage are exactly equivalent. But substances like antibiotics, which are produced by natural fermentation processes, are commonly contaminated by other compounds introduced, used or produced in the course of manufacture. Although a product has a guaranteed degree of activity, it may contain a fraction, however small, of obscure composition and properties that could contribute to the activity, or more likely, to adverse effects. Moreover,
in order to make tablets, stable oral suspensions or preparations for injection, it is often necessary to mix the product with binding, dispersing or stabilising agents. Apart from any effect these additives may have, the way in which the crystals are milled and the tablets compressed can exert a considerable influence on the plasma levels obtained from oral administration of the compound. Table 3 shows the marked effect on the plasma levels of giving griseofulvin in regular or finely milled forms. The effect of meals in depressing the plasma levels of orally administered drugs is well known; Table 3 also shows how the simultaneous administration of fat greatly increases the plasma concentrations of griseofulvin.

**Dosage**

Unfortunately, the drug with which we have had the greatest clinical experience, amphotericin B, is not sufficiently well absorbed when given orally and is damaging to the tissues when given by intramuscular injection, so that it must be given intravenously. It is the only compound in regular therapeutic use for which the dosage is fixed by toxicity rather than by the patient’s response. Through fear of its toxic effects, especially on the kidneys, it has been the usual practice to begin with very small doses, which are increased gradually until the patient begins to show signs of toxicity. Such titrations to toxicity in the patient bear no relation to the microbiological needs of the situation and attempts have been made to devise more rational systems of therapy. There are reasons for believing that clinical response to an antimicrobial agent should result if a plasma concentration of twice the minimum inhibitory concentration (MIC) for the infecting organism is achieved, but it is not known how often or for how long such concentrations must be maintained. It has been shown that by giving amphotericin, by rapid intravenous infusion once a day, sufficient to produce a plasma level twice the MIC of the infecting organism as determined by *in vitro* measurements, results equivalent to those produced by giving the patient the maximum tolerated dose can be obtained with a smaller total dose and less reaction (Drutz et al., 1967).

**Specialised Distribution**

The agents so far discussed are used systemically for the treatment of rare generalised fungal infection. The systemic agent most widely used is griseofulvin which owes much of its success in the treatment of dermatomycoses to the peculiarity of its pharmacokinetic behaviour. Griseofulvin is characteristically accumulated by keratinising epithelium and there is very little dispersal from the sites of uptake of the drug. This is well illustrated by the appearance of hairs suffering from trichophyton infection after the institution
of treatment with griseofulvin. There is an extremely sharp demarcation line between the abnormal distal part of the hair and the proximal part which, in growing, has taken up griseofulvin and, after eradication of the fungus, shows a completely normal appearance. The same localisation is responsible for the relatively slow transportation of griseofulvin through the skin. As the epidermal cells advance and gradually keratinise, griseofulvin is carried across the skin but does not reach the surface for 30 to 50 days.

**Adaptive Elimination**

Griseofulvin shows another pharmacokinetic property of great general interest. When drugs are given repeatedly—as they usually are—compensatory and adaptive mechanisms (located particularly in the microsomal enzymes of the liver) may be stimulated so that metabolic elimination of the compound is accelerated. As a result, the peak plasma concentrations obtained from successive doses and the half-life of the drug progressively decline. As the enzymatic processes for the disposal of agents are common to several different compounds, patients treated with one drug may subsequently show an enhanced capacity to dispose of another. This is illustrated by the effect of prior barbiturate administration on the disposal of griseofulvin (Busfield et al., 1963).

**Non-microbiological Effects**

Griseofulvin is also active outside its normal field of application. When testing many compounds against mouse thigh candidiasis we found (O'Grady et al., 1963) that griseofulvin substantially reduced the size of the lesions despite the fact that it had no measurable effect in vitro on Candida albicans. It transpired that this suppression was due to a peripheral cortisone-like effect of griseofulvin, which probably plays a part in the very rapid defervescence in inflammation that occurs in the treatment of trichophyton infections. This anti-inflammatory effect has been put to some use clinically, particularly in France, in conditions quite unrelated to fungal infections, such as dermatomyositis, although our own small experience has not impressed us with the value of the drug for this purpose.

In this brief review I have attempted to draw attention to some of the more important factors that affect the distribution of drugs in the body and to emphasise the many variables that may influence the final concentration of antifungal agent available to act on the parasite at the site of infection.

**References**

Busfield, D., Childs, K. J. and Tomich, E. G. (1963) *Lancet*, 2, 1042.

Crounse, R. C. (1963) *Archives of Dermatology*, 87, 179.
General Practitioner

At the beginning of the nineteenth century 'the physician of the poor in all cases, and of the rich when the distress or danger is not very great' was the apothecary. By the Apothecaries' Act of 1815 he became the licensed doctor. This great reform in medical services was applauded later by Wakley, the first editor of the Lancet, for whom six fighting words were better than one. By 1829 he could write 'In what estimation were held the solid and well-founded claims to public confidence of the general practitioners of England in comparison with the empty and delusive titles of St. Andrew's and Aberdeen doctors.' He went on, 'The encroachments made upon the interests and privileges of the profession by the out-patient system of our hospitals and by the fraudulent concocters of infirmaries and dispensaries have, of late, engaged much of our attention. These abuses constitute an evil of enormous magnitude and press upon the general practitioners with a force at once cruel and destructive.'

So the general practitioner got his name, but a few months later Wakley was at it again; the name was wrong. 'How monstrous a title for men of learning and character whose minds are stored with the richest treasures of the sciences.' To Wakley the term general practitioner was out almost as soon as it had come in. 'A more clumsy, a more vulgar or a more inapplicable expression could not be found.'