Part III.IV Antileishmanial Drug Design and Delivery

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The History of Drug Design

Drug design as a concept has been established for some 95 years. It was the founding concept of chemotherapy, as proposed by Paul Ehrlich (1891), that man should develop "magic bullets" (drugs) aimed at the "targets or receptors" (microbes).

Ehrlich's concept of chemotherapy soon became a reality although his earliest attempts in 1891, involving the use of methylene blue as an antimalarial agent, were not widely accepted. It was not until 1907, when he used benzo-purpurin to treat mal de caderas successfully that the success of chemotherapy became assured.

The apocalypse of wonder drugs such as May and Baker's Compound 693 and the plethora of antibiotics realised thereafter, have indeed allowed man to develop "magic bullets" aimed at the "target" microbes. Many useful antimicrobial agents have therefore been discovered in the past 95 years using the so-called empirical drug development procedures, including some 60 years ago, the antileishmanial activity of the antimonials (see Brahmachari, 1941).

The Empirical Development of Drugs: Opportunity and Luck?

Clearly the empirical development of chemotherapeutic agents has been of enormous benefit to mankind, but it is not however the most expedient or rational route for drug development. The empirical approach relies heavily on a combination of opportunity and luck, as well as massive synthesis and screening efforts.

The classical example of opportunity and luck occurred in 1907 when seizing upon an observation that some organic arsenical compounds possessed antimalarial activity, Ehrlich synthesised more than 600 arsenical compounds and screened them for antitrypanosomal and antisyphilitic activity. Only number 418 was found to possess useful antitrypanosomal activity and none of the series possessed antisyphilitic activity. Some years later as luck would have it the series was rescreened and one of the series was found to possess moderately useful chemotherapeutic activity against syphilis.

Even by current standards 2 active compounds out of 600 would be considered to be highly successful. The empirical drug design approach is therefore largely opportunistic and most unfortunately very inefficient. Through the use of current technologies however it is now possible to design drugs via a non-empirical or rational route.

Lateral Design of Drugs: Empirical Discovery and Rational Development

Several putative new antileishmanial drugs, such as the inosine analogs, some of which are presently under clinical trial, have been realised via a combination of the empirical and non-empirical approaches. This semi-empirical or lateral approach involved the empirical discovery of Allopurinol's antileishmanial activity and the subsequent rational development of active analogs (see e.g. Rainey *et al.*, 1985). The lateral approach is however limited by the relatively few active drugs which have been discovered by the classical empirical methods. The most rational approach to drug design is therefore the non-empirical route outlined in the previous contribution (Part III:III). The non-empirical approach aims to identify and exploit molecular differences in the biochemistry of the parasite and the host.

Non-empirical Drug Design: At the Atomic Level

Of the potential differences so far identified (see the previous presentation), I would like to focus on one particularly unique and exquisite target, which is by far the most advanced, in terms of characterisation and development, for chemotherapeutic attack.

The glycosome is a unique subcellular organelle found in all the clinically important species of *Leishmania* (Hart & Opperdoes, 1984), as well as in all members of the Trypanosomatidae so far studied (e.g. Opperdoes *et al.*, 1984). It contains many essential pathways involved in catabolism (glycolysis, glycerol metabolism and oxidation of fatty acids), anabolism (ether lipid and pyrimidine biosynthesis) and anaplerotic function (carbon dioxide fixation). The enzymes which constitute these essential pathways must therefore be at striking variance to their mammalian host counterparts.

For example, the topogenesis of the glycosomal polypeptide must be completely different from the mammalian cytosolic counterpart, as the glycosomal polypeptide must contain a translocation sequence to be directed to the organelle. The differences in the atomic structure between the glycosomal and the mammalian counterpart polypeptides could therefore be used to design highly specific antitrypanosomatid drugs.

Antiglycosomal Drug Design: Fact or Fantasy?

The fact that in the history of chemotherapy virtually no clinically useful drugs have ever been "designed to order" may suggest an element of fantasy in the proposition to design antiglycosomal drugs.

Considerable progress has, however, been made towards this goal (Hart *et al.*, 1985). Several glycosomal polypeptides have been purified to homogeneity (Misset & Opperdoes, 1984) and crystals grown for X-ray crystallography studies (e.g. Wierenga *et al.*, 1984). The amino acid sequence needed to build the atomic models using the molecular coordinates have been obtained using nucleotide sequences and the genetic code (e.g. Michels *et al.*, 1985). Unique features in the atomic

structure will soon be exploited to design putative antglycosomal compounds.

It has been shown, for example, that a major difference between the atomic structure of the glycosomal enzymes and their mammalian counterpart is the occurrence of two clusters of positively charged amino acid residues 40Å apart. These positive clusters may well be involved in the essential topogenesis of the glycosomal enzymes and could therefore serve as excellent targets against which to model putative new drugs.

The element of fantasy in antileishmanial and antglycosomal drug design is therefore very small; in fact, within the next few years the first ever non-empirical development of antiprotozoal drugs could easily be achieved.

Antileishmanial Drug Delivery: Aiming to Kill an Intracellular Parasite

It is reasonable to assume that antileishmanial chemotherapy is hampered by the intracellular nature of the parasite. The efficiency of both existing and puta-

tive drugs could therefore benefit considerably from the development of a specific drug delivery system.

Both the relatively high dose levels required to treat unresponsive cases and the untoward host cytotoxicity could be eradicated. Mathematical model predictions suggest that the present 10mg/Kg dose level of stibogluconate could be reduced to as little as 10µg/Kg, that is, assuming 100% efficiency in targeting and comparable antileishmanial pharmacokinetics parameters.

Ideally, an antileishmanial drug delivery system would transport the drug from the site of administration to the infected macrophages, where it would be accumulated, via a receptor-mediated process, in the parasitophorous vacuole. The prodrug-carrier conjugate would then be degraded to release the active drug for accumulation by and activity against the parasite.

Antileishmanial Drug Delivery: Via Lipoprotein Targeting

Progress has recently been made towards the development of an antileishmanial drug delivery system, in that

1. Lipoprotein Prodrug Complex
D = Drug, L = Linkage, T = Targeting Ligand
2. Lipoprotein Receptor
- 3, 4, & 5. Receptor Mediated Uptake
- 7, 7, & 8. Fusion with Parasitophorous Vacuole
9. Accumulation of Prodrug Complex
10. Accumulation of Free Drug in Amastigote

Figure 1. A schematic diagram of the proposed model for antileishmanial drug delivery: via lipoprotein targeting.

a potential new receptor-mediated process has been identified. I have demonstrated the occurrence of low density lipoproteins (LDL) and modified low density lipoproteins (mod-LDL) on the surface of infected macrophages, and have used this receptor-mediated uptake and degradation process to accumulate prodrug complexes in the parasitophorous vacuole.

LDL and mod-LDL entrapped drug as a carrier was shown to have improved efficiency of certain drugs (Hart, manuscript in preparation). The fatty acid derivative of the drug Daunorubicin, for example, has been used to demonstrate a 100–200 fold increase in antileishmanial activity when administered as an LDL or mod-LDL complex.

Figure 1 shows the overall scheme of the proposed lipoprotein targeting model. It is presumed that the observed degradation of the lipoprotein facilitates the liberation of the prodrug conjugate in the parasitophorous vacuole and that the recently described β -oxidation system of the parasite (Hart & Coombs, 1982) facilitates the uptake and subsequent activation of the free drug from the fatty acid conjugate.

LDL and mod-LDL therefore have great potential as drug carriers; they possess a hydrophobic core, hydrophilic surface, and an apoprotein moiety which are ideal for exploitation in the transport of prodrug complexes. It is also possible that lipoproteins could be implicated in the delivery of liposome entrapped drugs to the reticuloendothelial system, since under certain physiological conditions, lipoproteins have been suggested to exchange phospholipids and contents with liposomes (e.g. Scherphof *et al.*, 1983).

The receptor-mediated uptake and degradation of lipoproteins in *Leishmania* infected macrophages has been shown to result in the accumulation of cholesterol in infected macrophages, and probably accounts for the highly translucent vacuoles associated with *Leishmania*

infected macrophages. A rather striking similarity exists, therefore, between the aetiology of leishmaniasis and arteriosclerosis, as both are associated with the accumulation of cholesterol in macrophages, and both result in highly translucent vacuolation.

Conclusion

It would seem therefore, that 100 years after the first observations of *Leishmania* (Cunningham, 1885) and 95 years after the foundation of chemotherapy (see Ehrlich, 1910), that we are at last technologically capable of realising non-empirically designed antileishmanial "magic bullets" which can be aimed specifically at the intracellular "targets" – the amastigotes.

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