starpharma

DENDRIMERS FOR ENHANCED DELIVERY OF SMALL MOLECULE AND BIOLOGICAL DRUGS

CONTROL SOLUBILITY, HALF-LIFE, TOXICITY AND TARGETING

The use of macromolecule conjugates to enhance performance of drugs is currently an area of intense research for both small molecules and biological therapeutics. Starpharma has been applying its dendrimer technology to address important challenges in drug development, an approach which is now gaining favour with professionals in the industry.

Developing a new drug is a complex task in which many different parameters must be optimized together, including potency, toxicities, bioavailability, biodistribution, clearance rates, formulability and defensible patent position.

There are a limited number of variables available to address all of these constraints in a single molecule. For this reason conjugating drugs to macromolecular "vehicles" is increasingly being considered as a means of breaking the problem into manageable pieces. For example, one may choose to optimise potency in the structure of the drug itself, but leave control of biodistribution to a vehicle, such as a conjugated macromolecule. In this note we will describe how Starpharma's dendrimer-conjugate technology allows this division of labour between drug and vehicle to be achieved in practice.

FOUR KEY APPLICATIONS

Starpharma has focused on four key drug delivery objectives:

Increase Solubility – by conjugating the drug to a dendrimer construct, very large increases in drug solubility have been achieved.

Control Half-Life – the dendrimer can protect drugs from degradation and inhibit renal clearance. The half-life of drugs have thereby been substantially increased.

Control Off-Target Toxicity – different dendrimers can keep drug away from different tissues. This has been used to reduce dose-limiting toxicities.

Target Organ, Tissue or Receptor – dendrimers have been used to actively or passively target a payload drug to particular destinations.

WELL-DEFINED, VERSATILE, SYNTHETIC MACROMOLECULES

Starpharma's dendrimers are highly-branched macromolecules with a well-defined structure. Starting with a core molecule, branching lysine units are repeatedly added in layers (or "generations"), until the desired structure is reached *(figure 1)*.

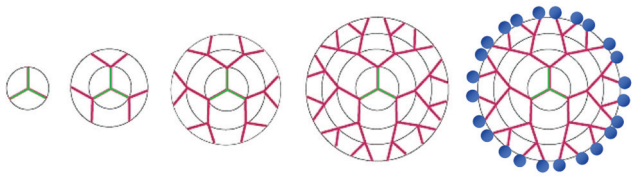

Figure 1: *A dendrimer is a macromolecule with a well-defined highly-branched, 3D structure. It is synthesised in spherical layers, by adding monomers onto a core. For most of Starpharma's pharmaceutical dendrimers the monomer of choice is lysine, which is biofriendly and low-cost.*

The many surface points of attachment can then be functionalised with one or more groups depending on the intended application *(figure 2)*.

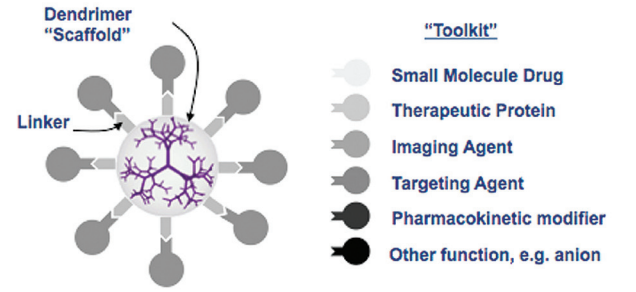

Figure 2: *The surface of the dendrimer can be modified to achieve a wide range of functionality. Typically one or more functional groups are used on 32 to 64 attachment sites.*

Starpharma has taken its polylysine dendrimers into the clinic in the form of an anti-viral vaginal gel, VivaGel®, currently in Phase II studies. VivaGel® is the subject of a licensing agreement with SSL, the owner of the Durex® condom brand as a microbicidal condom coating. VivaGel® is manufactured under cGMP and its structure is closely related to many of the dendrimers that Starpharma uses for drug delivery applications.

A growing number of pharmaceutical companies are now working with Starpharma: the company has announced collaborations with Lilly, GSK's Stiefel Laboratories, and Elanco. A number of additional collaborations with undisclosed partners are also under way.

Below we give some examples of well-known drugs that we have modified with dendrimers to achieve different objectives.

INCREASE SOLUBILITY

Paclitaxel is a cancer drug well known for its poor aqueous solubility (<1µg/ml). When conjugated to a dendrimer construct a 9000 fold increase in solublised Paclitaxel is achieved *(Figure 3).*

Paclitaxel, no dendrimer Solubility 0.8 µg/ml

Paclitaxel with dendrimer Solubility >37 mg/ml

Figure 3: *By conjugating Paclitaxel to a dendrimer construct its aqueous solubility is enhanced 9000-fold.*

The structure is designed to release the API in the body so that it can achieve its intended pharmacological effects
(See Inset Box "Linkers – controlling where the drug is released").

CONTROL HALF-LIFE

The half-life ($t_{½}$) of a drug can be substantially enhanced by attaching it to a dendrimer construct. For example attaching methotrexate (MTX) to different members of a family of dendrimer constructs leads to a range of clearance rates in rats (figure 4), ranging from 24 minutes (free MTX), to nearly 24 hours shown in this figure, and 50 hours in a related construct.

A comparable result was achieved with doxorubicin (DOX): a $t_{½}$ of approximately 30 minutes for free DOX was extended to 34 hours for a DOX-dendrimer construct.

Linkers – controlling where the drug is released

Some drugs continue to function whilst attached to dendrimers, whereas others need to be released before they can work. Starpharma uses different kinds of linker between the dendrimer and the drug to control when and where drug is released, for example:

"Permanent" Linker

If the drug is found to work satisfactorily when attached to the dendrimer, then there may be no need to release it, e.g. insulin.

Hydrolytically Unstable Linker

Sometimes sustained release of the active may be all that is required, with no preferred release location. In this case a linker that breaks down with a certain half-life in aqueous solution may be the correct choice.

Acid-Labile Linker

Newly forming tumors are often hypoxic, and therefore have a low pH. By using a linker that is stable at neutral pH, but unstable at lower pH it is possible to preferentially release drug at a tumour.

Enzymatically Cleaved Linker

It is possible to preferentially release a drug molecule at the location where a specific enzyme occurs by connecting the drug to the dendrimer with a linker that is subject to enzymatic degradation. Such locations include tumours where certain enzymes are commonly over-expressed.

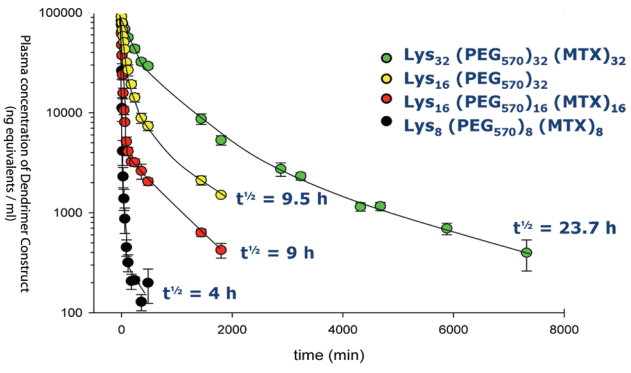

Figure 4: *Attaching small molecule drugs to dendrimer constructs allows control of drug half-life. In this study the half life of methotrexate (MTX) was extended from 24 minutes to 24 hours. For a different methotrexate construct a 50 hour half life was achieved.*

The approach is applicable both to small molecules and biological therapeutics. In *figure 5* the activity of insulin is prolonged so that glucose levels are suppressed longer in mice receiving insulin-dendrimer construct compared with mice receiving insulin alone.

In the case of doxorubicin and methotrexate it was necessary for the API to be released from the construct to function.
For insulin, release was not required. *(See Inset Box "Linkers – controlling where the drug is released").*

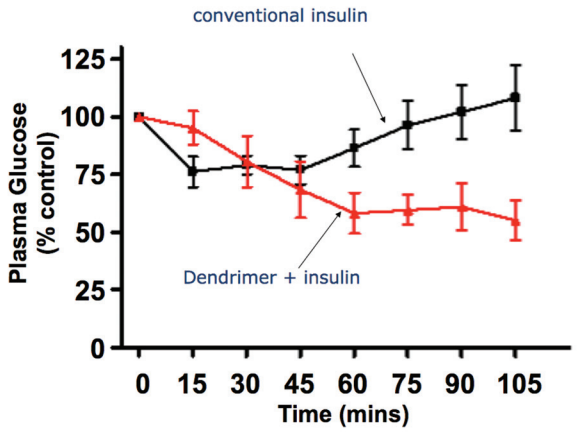

Figure 5: *The half-life of biological molecules can also be extended using dendrimers. In this case the effective duration of activity of insulin is seen to be prolonged in mice compared to insulin alone.*

CONTROL TOXICITY

Damage to heart muscle can be a dose limiting toxicity for cancer drug doxorubicin *(Figure 6a)*. However, when doxorubicin is attached to a suitable dendrimer construct with preferential release of drug near a tumor *(See Inset Box "Linkers – controlling where the drug is released")*, much less free drug reaches the heart, and cardiac damage is reduced, *(Figure 6c)*, whilst drug efficacy is maintained at the tumour *(Figure 6d)*.

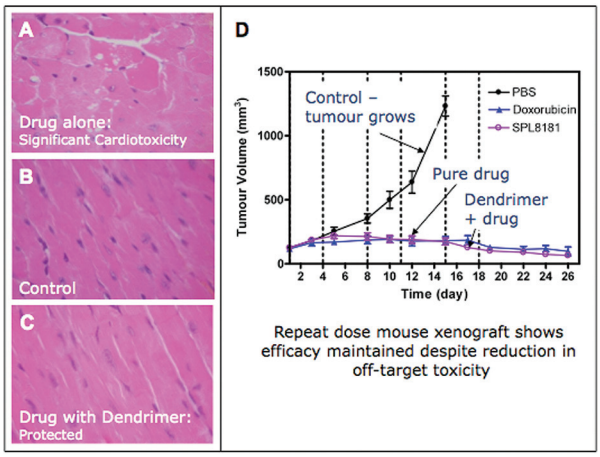

Figure 6: ***(A)*** *Cardiac tissue damage can be a dose limiting toxicity for doxorubicin.* ***(B)*** *Image of undamaged cardiac muscle where negative control (PBS) is administered.* ***(C)*** *When DOX is conjugated to dendrimer, heart tissue no longer becomes damaged despite retention of efficacy against tumour as shown in xenograft study* ***(D)****.*

TARGETING

There are two mechanisms that may be advantageously employed to control the destination of drugs when attached to a dendrimer.

Active Targeting – Adding a suitable targeting molecule to the construct, such as an antibody, allows the dendrimer to carry a payload to a target receptor. In *Figure 7* this is achieved for a payload of gadolinium, allowing visualization in an MRI scanner.
The payload could equally have been a small molecule API however.

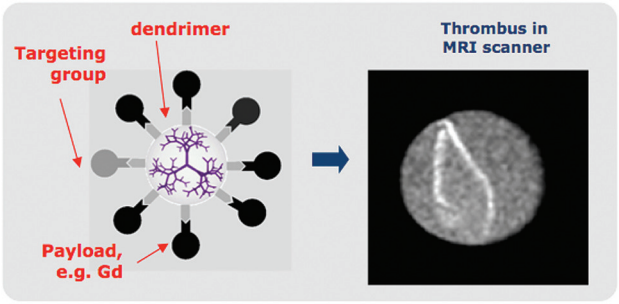

Figure 7: *Left: By associating a dendrimer with a targeting group (e.g. an antibody) multiple "payload" molecules can be delivered to a molecular target. Right: Here the antibody fragment targets certain activated platelets, and the payload is successfully delivered to a blood clot. In a control study without the correct antibody no such delivery was achieved, showing that the targeting in the image above was specific in nature. (Work conducted in collaboration with the Baker IDI Institute)*

Passive Targeting – Even in the absence of any targeting group, tailoring the size and physico-chemical properties of the dendrimer can achieve preferential accumulation in target tissues or organs *(Figure 8)*.

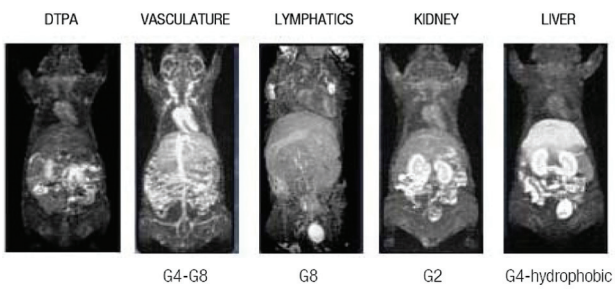

Figure 8: *Passive targeting to tissues or organs can still occur in the absence of specific targeting groups. The left image shows the distribution of a non-dendrimer contrast agent control. The other four images are different dendrimer-gadolinium complexes, showing how different dendrimers target different tissues. (Image Courtesy of M Brechbiel, NIH)*

STRENGTHS OF DENDRIMERS FOR DRUG DELIVERY

- The payload for small molecule drugs is typically 20-40% w/w.
- A good level of purity / monodispersity can be achieved.
- Although some dendrimers naturally migrate towards the liver, others do not. This means that both the liver and other organs can be addressed using dendrimers.
- Dendrimer formulations tend to have relatively low viscosity
- Dendrimers are synthesised using standard chemical processes.
- A long shelf life observed in ambient and accelerated stability studies of dendrimer product VivaGel® (>2 years ambient)
- Dendrimer constructs often yield a readily soluble powder when freeze dried.
- PEG's string-like form means that it can still be cleared through the kidney quicker than would otherwise be expected from it's moleular weight ("reptation").
  The branching 3D structure of a dendrimer can be used to obstruct this clearance mechanism. On the other hand it is possible to make smaller dendrimers that are excreted reasonably quickly through the kidney, if required.
- Dendrimer breakdown products are primarily natural lysine which is readily processed by the body.
- Dendrimers have a covalent structure. This contrasts with liposomes which are often considered metastable so that they can eventually rearrange to form planar bi-layers.

BEYOND PHARMACEUTICALS

Starpharma is also pursuing applications of dendrimer technology beyond pharmaceuticals, e.g. in cosmetics, coatings and agrochemicals. Starpharma would be pleased to discuss the application of its dendrimer technology to any sector, pharmaceutical or otherwise.

About Starpharma

Starpharma Holdings Limited (ASX:SPL, OTCQX:SPHRY) is a world leader in the development of dendrimer technology for pharmaceutical, life-science and other applications. SPL has two operating companies, Starpharma Pty Ltd in Melbourne, Australia and DNT, Inc in the USA. Products based on Starpharma's dendrimer technology are on the market in the form of diagnostic elements and laboratory reagents through licence arrangements with partners including Siemens and Merck KgA.

The Company's lead pharmaceutical development product is VivaGel® (SPL7013 Gel), a vaginal microbicide designed to prevent the transmission of sexually transmitted infections, including HIV and genital herpes. In September 2008 Starpharma signed a full licence agreement with SSL International plc (LSE:SSL) to develop a VivaGel® coated condom. SSL manufactures and sells Durex® condoms, the market-leading condom brand worldwide.

Starpharma also has agreements with Lilly, Elanco, Stiefel Laboratories (a GSK Company), and Unilever as well as many research collaborations with some of the world's leading organisations.

To discuss the potential use of of dendrimer technology in your application, please contact:

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