Stealthy polymers target drug-resistant tumour cells

Joanna Owens, joanna.owens@current-trends.com

A combination of micelle-mediated delivery and focused ultrasound could defeat multidrug resistance in cancer cells and reduce the undesirable effects of chemotherapy. Natalya Rapoport and colleagues at the University of Utah (Salt Lake City, UT, USA), and others previously[1], have shown that capturing drugs in a polymer micelle increases their uptake by multidrug resistant (MDR) cancer cell lines. Effective chemotherapy regimens are frequently hindered by the resistance of tumour cells to one or more drugs; for example, bladder cancer, the seventh most common cancer in the USA, is virtually untreatable by chemotherapy. Pluronic™ polymer micelles (BASF, Mt Olive, NJ, USA) have been previously shown to hypersensitize MDR cells resulting in the increased uptake of drug[1], but Rapoport's research shows that this method of drug delivery to MDR cells can be further enhanced by focused ultrasound at tumour sites[2].

Evasive polymers

An important feature of the micelles used in the work by Rapoport and colleagues is their ability to evade the immune response. Each Pluronic micelle consists of a central hydrophobic core of polypropylene oxide (PPO) and two side-blocks of polyethylene oxide (PEO) that self-assemble in different solutions to form the micelle structure (Fig. 1). The Pluronic nanoparticles (10–20 nm in diameter) are not recognized by the reticular endothelial system of the cell, and thus can target tumour cells without provoking an immune response. Rapoport suggests that this property is possibly a result of the steric nature of the CH_2OCH_2 structure being similar to that of water. She also points out that the dynamics of these PEO chains means that the micelles prevent opsonization (the coating of micelles with specific antibody), and likens this function to that of a revolving door, in that the chains move too quickly to allow opsonization to occur.

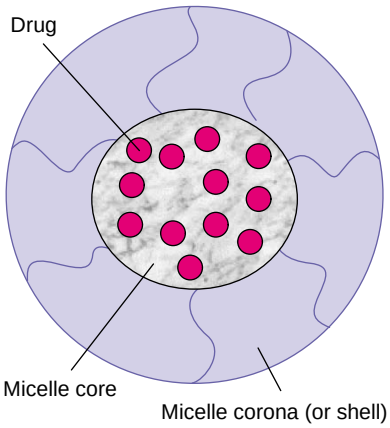

Figure 1. Schematic representation of pluronic micelle structure. Each Pluronic micelle consists of a central hydrophobic core of polypropylene oxide and two side-blocks of polyethylene oxide that comprise the shell, which self-assemble in different solutions.

Targeting MDR cells

So how do MDR cells become hypersensitized to drugs delivered by Pluronic micelles? One possibility is that internalization of the micelles reduces the respiration rate of both MDR and drug-sensitive cells. Because cells often achieve their drug resistance via the efflux of drugs through energy-dependent transporters, the reduction in respiration impairs this mechanism, and results in hypersensitivity to chemotherapeutic agents. Indeed, this hypothesis has been proven both by Rapoport and by Kabanov and colleagues at the Nebraska Medical School (Omaha, NE, USA) by measuring the respiration rate of MDR cells in culture. This was achieved by several methods. Rapoport and coworkers used a lipophilic nitroxide probe that localizes to the cell and mitochondrial membranes, and can be used to measure bioreduction, that is, the rate of electron transport. Meanwhile, Kabanov's group measured ATP production directly. Both groups are now collaborating to study the effects of ultrasound on ATP production in tumour cells.

A second possibility is that the cytoplasmic vesicles of MDR cells, in which chemotherapeutic agents such as anthracycline accumulate, are more readily permeabilized by Pluronic micelles than the vesicles of drug-sensitive cells. This means that the anti-cancer drug is released more readily from the vesicles to its target, the nucleus. Using a fluorescent probe conjugated to the micelle surface, Rapoport and colleagues studied the uptake of the micelles into vesicles by fluid-phase endocytosis. Two interesting observations were made. First, drug uptake was rapid – 80% of total uptake occurred during the first seven minutes of exposure. Second, the release of Pluronic micelles from the vesicles occurred at a lower micelle concentration in MDR cells than in drug-sensitive cells, as shown by a reduction of fluorescence in the vesicles and an increase in fluorescence in the cytoplasmic environment.

1359-6446/01/$ – see front matter PII: S1359-6446(01)01822-0

Rapoport comments that: 'This is a very important finding, that the vesicles of MDR cells are much more susceptible to the effect of Pluronic micelles than those of drug-sensitive cells.' Rapoport and colleagues are now testing the efficacy of micelle-encapsulated drugs administered intravenously to animal models, and hope that other micelle forming polymers will have the same effect and that the drug will not be taken up significantly by non-cancer cells.

Ultrasound technology

It is thought that MDR cells, even those that comprise small metastases, will take up micelle-encapsulated drugs at any location in the body. Rapoport and colleagues have shown that ultrasound can be used to induce the release of the drug from micelles at the tumour site and to enhance its intracellular uptake by permeabilizing the tumour-cell membranes, thus reducing misdelivery to non-cancer cells. This also capitalizes on another potential benefit of Pluronic micelles: their ability to re-assemble. Once the drug has been released after ultrasound treatment, the micelles are not completely destroyed and they re-assemble, potentially taking any drug that was not taken up by the cell to another tumour site. Rapoport found that by using pulses of ultrasound, the release of drug was the same as with continuous ultrasound exposure, but that the likelihood of re-encapsulation was increased. Further, it has been previously observed that pulsed exposure to ultrasound is less damaging to tissue than continuous exposure.

Rapoport anticipates that in addition to chemotherapy, this delivery system might be used for gene or antibody therapy and vaccine delivery. However, she stresses that Pluronic micelles might not be the best delivery vehicles for this technology, and she has started to collaborate with researchers in Japan who have developed more stable micelles.

Future research

Rapoport and coworkers will continue to investigate the mechanism of resistance in MDR cells, in addition to carrying out studies in animal models to optimize this combined technology. Several questions need to be addressed:

- When is the most effective stage of treatment to apply ultrasound?
- What exposure time should be used?
- How many ultrasound cycles should be used?
- What is the optimum power intensity required to achieve efficacy without tissue damage?

Rapoport believes that the answers to most of these questions *in vitro* have been addressed by their studies, but that these parameters need to be studied in animal models *in vivo*. The commencement of *in vivo* studies should determine the elimination kinetics of the micelles *in vivo*. Rapoport says that Pluronic micelles in cells are rapidly released into a micelle-free environment, and that probes will be used to track the exact distribution and excretion of the polymers in animal models. However, despite the progression into animal studies, she stresses that 'any possible cancer treatment based on the findings would be years away'. Furthermore, she points out that the use of ultrasound to enhance delivery is restricted to certain organs: for example, delivery to the brain would not be possible because ultrasound cannot penetrate the skull, and similarly, the lung comprises too much air for ultrasound to penetrate effectively.

Rapoport's group are also embarking on studies with a paramagnetic analogue of doxorubicin, Ruboxyl, which was synthesized at the Institute of Chemical Physics (Moscow, Russia) and is in Phase II clinical trials in Russia for use against various cancers, particularly bone sarcoma. This compound is a useful reporter molecule for studying the uptake and localization of the micelle–drug nanoparticles in the cell because of its fluorescent and paramagnetic properties, which allow tracking of the compound *in vitro*. This work will be undertaken as part of a collaboration between Rapoport's group and the Institute of Chemical Physics.

Rapoport is now collaborating with Kabanov's group and comments that Kabanov initiated experiments with Pluronic micelles and has considerable experience in using Pluronic in animal models; further, Supratek (Montreal, Quebec, Canada) is using research by Kabanov, and that of Alakhov's group (University of Quebec, Laval, Quebec, Canada), to develop Pluronic formulations of an anti-cancer drug. However, this formulation is not targetable. Rapoport's model makes it targetable and, therefore, Supratek is interested in it. Rapoport hopes that collaborations with large pharmaceutical companies will follow.

References

1 Venne, A. *et al.* (1996) Hypersensitizing effect of Pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res.* 56, 3626–3629

2 Marin, A. *et al.* (2001) Acoustic activation of drug delivery from polymeric micelles: effect of pulsed ultrasound. *J. Control. Release* 71, 239–249