pit, approximately 1 cm long, which is located inside the nasal septum (Fig. 1). It is, however, distinct from the olfactory system as most of the compounds that stimulate the VNO have no odour, and it does not respond to many odorants.

Stimulation of receptors on the surface of the VNO by pheromones triggers an electrophysiological response, which transmits neural impulses to the hypothalamus. Kevin McCarthy, Senior Vice-President of Pherin, says: 'The VNO links the endocrine system to the nervous system via the hypothalamus. Synthetic compounds that stimulate the VNO might affect those emotions and physiological functions that are controlled by that area of the brain: from anxiety, aggression and fear to appetite and sexual motivation.'

While working at the University of Utah, Louis Monti-Bloch, now Vice-President of Research at Pherin, developed a multichannel system that delivers test substances into the VNO using a nasal spray and measures the voltage induced by depolarization of the receptor cells. The system delivers a continuous air stream containing discrete pulses of picogram quantities of pheromones and a plant oil vehicle to the VNO.

Many hundreds of synthetic pheromone analogues have now been tested using this system. Like natural pheromones, vomeropherins are species- and gender-specific. For example, the steroid vomeropherin pregna-4,20-diene-3,6-dione (PDD) is inactive in human females. In males, nasal delivery of nanomolar quantities of PDD results in a dose-dependent depolarization of the VNO receptors, followed by statistically significant decreases in cardiac and respiratory frequency and in the serum concentration of some hormones[3].

Testing in humans

As the effect of vomeropherins is species-dependent, they cannot be tested for efficacy in animal models. With the exception of toxicology tests, all preclinical research is carried out in human cell lines or using human volunteers. 'This is a blessing in disguise,' says McCarthy, 'as all the data we report will have been obtained from humans.'

Pherin has now reported encouraging results from a pilot study of their lead compound, PH94B, for acute anxiety disorders in women. This was a double-blind, placebo-controlled Institutional Review Board trial of female volunteers who scored highly on the Hamilton A diagnostic tool for anxiety and were not taking any other medication. All the women who received picomolar quantities of PH94B via a nasal spray showed a statistically significant reduction in their anxiety levels compared with controls, and no significant side effects were noted. Following these results, the FDA accepted Pherin's Investigational New Drug application for PH94B and Phase I clinical trial protocols are currently being designed, with trials expected to start before the end of 2001.

If PH94B and similar compounds prove effective in clinical trials, the VNO should offer several advantages as a drug delivery system. The vomeropherins are well tolerated, fast acting and active in very small quantities. Furthermore, this system affects the CNS through local stimulation of a sense organ and so compounds that are delivered in this way do not need to pass through the blood–brain barrier.

References

1 Døving, K.B. and Trotier, D. (1998) Structure and function of the vomeronasal organ. *J. Exp. Biol.* 201, 2913–2925

2 Berliner, D.L. (1996) Steroidal substances active in the human vomeronasal organ affect hypothalamic function. *J. Steroid Biochem. Molec. Biol.* 58, 1–2

3 Monti-Bloch, L. *et al.* (1998) Modulation of serum testosterone and autonomic function through stimulation of the male human vomeronasal organ (VNO) with pregna-4,20-diene-3,6-dione. *J. Steroid Biochem. Molec. Biol.* 65, 237–242

Drugs with a magnetic attraction to tumours

Janet Fricker, Freelance writer

An innovative new approach that couples anti-cancer drugs to a carrier system containing iron that can be targeted by magnets to the tumour site is showing promise in both animal models[1] and Phase I/II clinical trials.

Many anticancer drugs are limited by their toxicity profile and by development of multiple drug resistance. FeRx (San Diego, CA, USA) believes that its proprietary Magnetic Targeted Carrier (MTC) technology makes chemotherapy more effective by increasing the drug concentration at the tumour site, while limiting the systemic drug concentration. Their studies have initially concentrated on delivering doxorubicin to hepatocellular carcinomas, this drug being one of the

1359-6446/01/$ – see front matter PII: S1359-6446(01)01771-8

most widely used anti-neoplastic agents for treating this particular type of cancer. Current treatments of hepatocellular carcinoma using either single or multiple agents have been unable to produce a response rate of more than 25% and have had no significant impact on survival rates[2]. Meanwhile, surgical resection is usually only considered for those patients with good hepatic reserve and who present with a small single tumour located in an easily accessible region within the liver.

Mechanism of magnetic targeting

Magnetic targeting for drug delivery was pioneered by Kenneth Widder and Andrew Senyei while as students at Northwestern University (Chicago, IL, USA). In the early 1980s, they initially used external magnets to target drugs that had been bound to activated carbon mixed with iron. However, the approach only became feasible when they switched from ferrous oxide magnets to elemental iron magnets, which have a greater depth of penetration. MTCs (1–2 μm in size) can adsorb and desorb pharmaceutical agents such as doxorubicin. Adsorption is a simple and rapid process with the MTCs being mixed with the drug immediately prior to administration and then passed through a catheter into a hepatic artery branch upstream of the tumour.

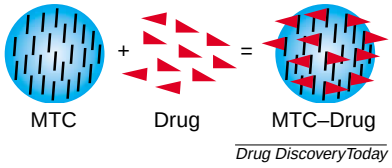

Figure 1. Drugs are adsorbed to the surface of the Magnetic Targeted Carriers (MTCs) by directly binding to the activated carbon component of the particle. The MTCs then localize the attached drug through retention in a magnetic field. The attraction of the MTCs to the magnetic field is due to the elemental iron component of the particle.

The magnetic field is powerful enough to cause the MTC–DOX to extravasate through the capillary bed into the targeted tissue. 'The elegance of our approach is that, rather than using biological properties, we are using the physical force of the magnetic field to pull the delivery vehicles into the tumour. This enables us to overcome problems such as positive pressure in tumours and multiple drug resistance pumps,' says Jacqueline Johnson, President and CEO of FeRx.

Johnson adds that several histological animal studies have been conducted to ensure that extrusion does not cause damage to the tissue. Little is known about the process of extrusion, although it is believed that MTCs are pulled through some kind of pore/channel opened by the force of the magnet. Once in the tumour, there are many other substances that have a higher affinity for carbon than the drug, causing it to be displaced.

'Once out of the blood vessels, there is no mechanism for MTC–DOX to migrate back into the circulation. The magnetic field has gone and the pores are closed,' says Johnson. The fact that MTC–DOX does not dissipate has been confirmed by MRI studies through visualization of the iron component of the particle. At the *Annual Scientific Meeting of the Society of Cardiovascular & Interventional Radiology* (San Diego, CA, USA in March 2000), Scott Goodwin, Chief of Vascular and Interventional Radiology at UCLA Medical Center (Los Angeles, CA, USA) presented data on 16 patients who received a single dose of MTC–DOX via intra-arterial infusion. MRI visualization after 28 days showed that the particles remained at the targeted site with no redistribution.

The story was somewhat different in preclinical studies, in which 18 swine were divided into six groups, three experimental groups that received a single dose of the MTC–DOX preparation and three corresponding control groups[1]. The extent of any embolization over 28 days was examined using angiography and showed gross and microscopic lesions in groups receiving >75 mg MTC, with or without doxorubicin. However, Johnson says that: 'In the preclinical studies, we were targeting healthy tissue, but because tumours have a lot of new vasculature laid down, they tend to be leaky and embolization has not been found to be a problem in humans.'

Clinical trials

FeRx is currently undertaking a Phase I/II escalating single-dose cohort study to determine the safety, tolerance, maximum tolerated dose and pharmacokinetic profile of MTC–DOX with intrahepatic delivery. 'As the carrier system targets the tumour so effectively, the weight of the patient is not relevant, nor is their liver volume,' says Johnson. In addition, FeRx is conducting separate Phase I/II trials in metastatic liver cancer. 'In this trial, we will give the treatment regardless of the primary tumour type, record the outcome and then go back and see if we get a better response from one type of primary tumour compared with another,' says Johnson.

The next step is to explore using the MTC delivery platform to target the

radioisotope, Yttrium 90, to a precise area of tissue and prevent systemic damage. 'The success of this project is important because there is much less variation in sensitivity between tumours with radiotherapy than with chemotherapy,' says Johnson. In addition to doxorubicin, MTCs can bind to other anti-cancer agents such as paclitaxel, 5-fluorouracil deoxyribonucleoside (FUDR), 5-fluorouracil, methotrexate, camptothecin and thalidomide. FeRx also plans to use the technology to deliver FUDR for gastrointestional metastatic liver cancers.

Alan Vernook, Professor of Clinical Medicine at The University of California at San Francisco (San Francisco, CA, USA) comments: 'The real issue with this treatment is that it can deliver regional therapy with virtually no toxicity and nothing in the data points to there being any problems. I am not convinced that doxorubicin is the best treatment, but there are many other drugs that can be explored. At present it takes a number of hours to infuse the treatment and target it. But once they know that it is safe, they can start to make changes to improve the efficiency.'

While the initial focus for the technology is on cancer therapy, FeRx hopes to eventually investigate its use for immunosuppressants such as cyclosporin A and 6-mercaptopurine, antifungals such as amphotericin B, and genetic vectors such as plasmids, antisense oligonucleotides and viruses, all of which will bind to MTCs[3,4].

References

1 Goodwin, S.C. *et al.* (2001) Single-dose toxicity study of hepatic intra-arterial infusion of doxorubicin coupled to a novel magnetically targeted drug carrier. *Toxicol. Sci.* 60, 177–183

2 Allgaier, H.P. *et al.* (1998) Hepatocellular carcinoma: non-surgical treatment. *Schweiz. Randsch. Med. Prax.* 87, 1466–1470

3 Goodwin, S. *et al.* (1999) Targeting and retention of magnetic targeted carriers (MTCs) enhancing intra-arterial chemotherapy. *J. Magnetism Magnetic Mater.* 194, 132–139

4 Rudge, S. *et al.* (2000) Preparation, characterization, and performance of magnetic iron-carbon composite microparticles for chemotherapy. *Biomaterials* 21, 1411–1420