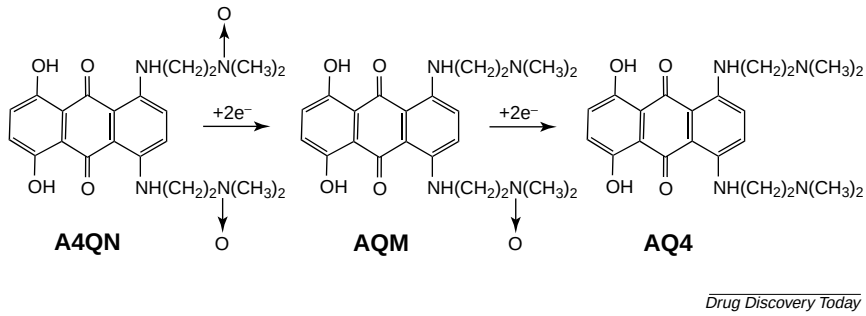

Figure 1. AQ4N undergoes bioreduction to produce the toxic AQ4 (Ref. 1). AQ4 is 1,4-*bis*[{2-dimethylaminoethyl}-amino]5,8-dihydroxyanthracene-9,10-dione. AQ4N is the di-*N*-oxide of AQ4.

(Oxford, UK). In the first instance, AQ4N is being given in conjunction with standard radiotherapy. 'The drug has the potential to reduce the radiation dose and side-effects, but the main reason for its use is to kill more cells,' explains Steward. Each patient receives two intravenous injections of AQ4N; one dose 14 days before radiotherapy and another immediately beforehand. Steward anticipates that the trial will run for 6–12 months. Further preliminary trials that examine the use of AQ4N in conjunction with chemotherapy and its dosage are also being planned. If AQ4N is successful Steward believes that it will be commercially available in 3–4 years.

Reference

1 Patterson, L.H. and McKeown, S.R. (2000) AQ4N: a new approach to hypoxia-activated cancer chemotherapy. *Br. J. Cancer* 83, 1589–1593

Depleting cholesterol to make sex safer

Martina Habeck, Freelance writer

Researchers have discovered that removing cholesterol from cell membranes inhibits the infectious ability of HIV. They hope that β-cyclodextrins (BCD), which can deplete cells of cholesterol, could provide a new microbicide for use against sexual transmission of the disease.

HIV begins its infection by binding to the CD4 receptor and to a co-receptor (chemokine receptor sites CCR5 or CXCR4) on the host-cell surface. Attachment to the receptor and co-receptor enables HIV to fuse with the host-cell membrane and to empty its contents into the cell so that it can replicate. The virus can also spread to other cells through fusion of an infected cell with an uninfected cell (Fig. 1). When newly formed virus particles bud from the surface of the host cell, they take a part of the host-cell membrane with them to form the viral envelope.

Lipid rafts

The HIV envelope excludes CD45, a highly expressed leukocyte surface protein, whereas it incorporates other host membrane-proteins that are far less abundant. In an attempt to explain this phenomenon, James Hildreth and his team at Johns Hopkins University School of Medicine (Baltimore, MD, USA) proposed that HIV-1 budding does not occur at random membrane sites, but at specific regions known as lipid rafts, which also exclude CD45 (Box 1). Confocal fluorescence microscopy and virus phenotyping using monoclonal antibodies supported their hypothesis, demonstrating that lipid-raft-associated molecules, such as the glycosylphosphatidylinositol (GPI)-linked proteins Thy-1 and CD59 and the ganglioside GM1, are incorporated in the viral envelope, whereas molecules specific for other membrane regions are excluded[1].

Encouraged by their findings, the scientists investigated whether lipid rafts are involved in other aspects of HIV-1 biology. Lipid rafts are particularly rich in cholesterol, which is important for many biological functions, including fusion.

Box 1. Lipid rafts

Cell membranes contain ordered microdomains enriched with glycosphingolipids and cholesterol. These lipid assemblies are thought to form so-called rafts that serve as moving platforms for a variety of cellular events, such as membrane trafficking, signalling and cell adhesion[a]. Rafts exert their function by separating or concentrating specific membrane-proteins. Among the molecules included in lipid rafts are glycosylphosphatidylinositol (GPI)-linked proteins, whereas molecules such as the membrane phosphatase CD45 or cadherin E are excluded.

a Simons, K. *et al.* (1997) Functional rafts in cell membranes. *Nature* 387, 569–572

1359-6446/01/$ – see front matter PII: S1359-6446(01)01981-X

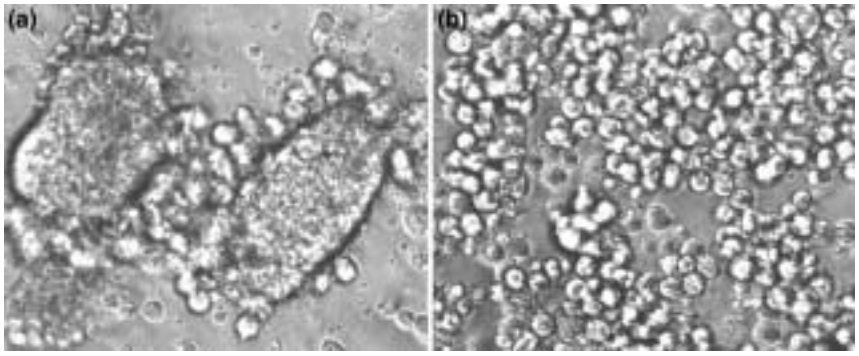

Figure 1. (a) Control cells infected with HIV fused with nearby uninfected cells, forming giant cells called syncytia. By contrast, **(b)** shows that BCD-treated cells formed no syncytia at all. Cells used were CD4-positive human T-cells. Kindly provided by James E.K. Hildreth. (Cells are T-cell lines, typically 12–20 μm diameter).

Cholesterol also plays an important role in the entry of some viruses, such as Semliki Forest and sindbis viruses.

Hildreth and colleagues[2] depleted cells of cholesterol by exposing them to BCD, a sugar derived from the partial breakdown of starch. The inside of the cyclodextrin ring is hydrophobic, which enables cyclodextrins to pull the fatty cholesterol molecules off the cell membranes and to encapsulate them within their cavity. Hildreth says, 'We found that BCD treatment makes primary cells, taken directly from humans, and laboratory cell lines resistant to both the cell–cell transmission of HIV and to infection by free viruses.' The team also observed that the expression of the co-receptors CXCR4 and CCR5 was reduced, probably because the receptors need cholesterol for their stability.

A novel microbicidal candidate

These findings raise hopes that a cholesterol-depleting compound, such as BCD, might have the potential to serve as a microbicide, which could be topically applied to the vagina or rectum to prevent HIV transmission. New technologies like this are urgently needed for protection from HIV and other sexually transmitted diseases (STDs). Research into this area has accelerated during the past 10 years and several potential microbicides are being evaluated clinically. Polly Harrison, Director of the Alliance for Microbicide Development (Silver Spring, MD, USA), points out that no microbicide has, to date, completed the advanced clinical trials that would support a claim for anti-HIV efficacy, and so it is crucial that all new strategies for developing such products be explored. She is, therefore, interested in this new approach. 'It seems to be a plausible strategy and I really hope there will be support for pursuing it.'

It is early days, however, and Harrison highlights several questions that need to be answered by future studies: for example, whether the treatment is safe and reasonably effective; whether the product is acceptable to potential users; and whether it remains *in situ* for a reasonable period of time.

The team at Johns Hopkins have already tested BCD as a vaginal microbicide in mice carrying human immune cells. At a recent symposium, they reported that BCD reduced HIV infection via the vagina by 95%. There were no side effects compared with the microbicidal compound nonoxynol-9, a spermicide that is currently in Phase III trials, which killed almost all the vaginal cells[3]. Evidence from Hildreth's laboratory also suggests that BCD might be effective against other STD pathogens.

Hildreth concludes, 'BCD is a great microbicide candidate. It has been in use for some time as a drug carrier and food additive. Therefore, we do not expect to see any major toxic effects if the compound is applied topically. I would not recommend its use in HIV treatment, however, because a systemic application could lead to adverse effects. After all, cholesterol has important signalling functions in immune cells.'

Hildreth and his team are now working on the formulation of a BCD-containing cream and are conducting further animal studies to confirm the safety and efficacy of their approach. If all goes well, they will be able to start human trials within 1–2 years.

References

1 Nguyen, D.H. *et al.* (2000) Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. *J. Virol.* 74, 3264–3272

2 Liao, Z. *et al.* (2001) Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1. *AIDS Res. Hum. Retroviruses* 17, 1009-1019

3 Hildreth, J.E.K. (2001) Adhesion molecules, lipid rafts and HIV pathogenesis. *HIV Pathogenesis Keystone Symposium*, 28 March-3 April 2001, Keystone, CO, USA (oral presentation 038)