

Permeability assays

One of the challenges in developing a new drug is finding a compound that not only alters the disease target but also is soluble and can permeate the cell membranes. It is poor permeability kinetics that often cause a drug candidate to fail. To address this problem, researchers have focused on the development of cell-based assays that allow them to quantify the migration of compounds across cell membranes. Techniques such as Caco-2 cell assays model gastrointestinal tract (GIT) absorption, while RBE4 cell assays model permeability across the blood-brain barrier (BBB).

Unfortunately, cells can be difficult to work with, and it is not always possible to increase assay throughput to facilitate the screening of larger compound libraries. Much effort has gone into the development of in vitro permeability assays that avoid these complications. One such test is the parallel artificial phospholipid permeability assay (PAMPA), first described by Manfred Kansy and colleagues at F. Hoffman-La Roche in Basel, Switzerland (*J. Med. Chem.* **1998**, *41*, 1007–1010). In PAMPA, the wells of a 96-well dish are filled with buffered solutions containing the test compounds. These are then overlaid with a filter plate that has been impregnated with phospholipids. A second solution is placed over the filters, and the permeation of the test compounds into this solution is determined.

PPH patients live longer

Patients with a rare and deadly lung disorder known as primary pulmonary hypertension (PPH) have reason to hope that an FDA-approved medication can help them live longer with an improved quality of life. Intravenous use of the drug epoprostenol (marketed as Flolan) was shown to increase three-year survival rates by up to 80% and nearly double exercise tolerance, according to a study conducted by researchers at Chicago's Rush-Presbyterian-St. Luke's Medical Center (*Circulation* **2002**, *106*, 1477–1482).

PPH has an incidence of about two in a million. It affects women more than men, and usually occurs when patients are in their 30s or 40s. Patients experience an abnormal rise in the blood pressure of the pulmonary arteries. This rise is linked with changes in the small pulmonary blood vessels that increase resistance to blood flow. When these blood vessels become more resistant to the blood flow, the heart's right ventricle is forced to work harder for enough blood to travel through the lungs. The lungs thicken and become dysfunctional, resulting in death on average within 2.8 years.

Alex Avdeef and his colleagues at plon (Woburn, MA, www.pion-inc.com) recently developed a PAMPA assay that models the GIT permeability of drugs, and they presented their findings in August at the IBC Drug Discovery Technology 2002 conference in Boston. The researchers tested 40 different phospholipid combinations and found that the one largely based on soy lecithin—the predominant lipid was phosphatidyl-

choline—performed best. They then tested their assay using a variety of steroids and compared the permeation results with those obtained using a Caco-2 assay or derived from actual GIT studies. They found that their PAMPA assay was able to mimic steroid transport in the GIT better than most of the Caco-2 assays. Furthermore, the PAMPA assay can be performed faster and more

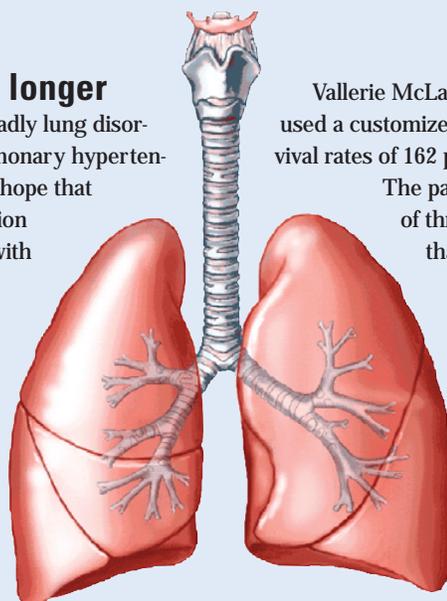
Vallerie McLaughlin and her colleagues used a customized database to analyze survival rates of 162 patients with severe PPH.

The patient group was composed of three times more women than men and had a mean age of 42. Along with a continuous infusion of epoprostenol through a portable infusion pump, patients were given treatments such as anticoagulants and diuretics for their condition. Throughout the study, each patient was periodically monitored with heart catheterizations, and researchers established baseline predictive values for the individual's exercise tolerance, heart function level, right atrial pressure, and response to the vasodilation agent adenosine.

Results showed that the one-year survival rate was 88%, two-year survival was 76%, and three-year survival was 63%, compared to 59%, 46%, and 35%, respectively, for similar patients in an NIH registry who were not receiving epoprostenol treatment.

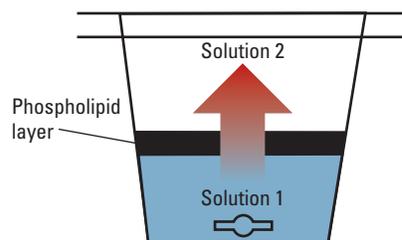
“Our data demonstrate that we can dramatically improve survival in patients afflicted with PPH,” says McLaughlin.

—JULIE L. McDOWELL



cheaply than an equivalent Caco-2 assay. The researchers are continuing their work and are hoping to next develop a PAMPA assay that will model the BBB.

—RANDALL C. WILLIS



Well construction for the PAMPA assay.

