Cardiac Side Effects

With the cost of drug development rising, identifying potential cardiac danger at a very early stage can prove to be a wise commercial decision. Dr Derek Terrar of Oxford Cardiac Pharmacology Ltd (OCP) and Oxford University provides a preclinical assessment of the cardiac risk of new drugs

Dr Derek Terrar is Research Director of Oxford Cardiac Pharmacology Ltd (OCP) and Reader in Pharmacology at Oxford University. He is one of the world's leading authorities on the testing of the influence of drugs on cardiac muscle contraction (including the possible role of cyclic ADP ribose and related substances on excitation-contraction coupling). He is also interested in pacemaker mechanisms and their regulation in the sino-atrial node, mechanisms of action of drugs used to treat cardiac arrhythmias (with particular reference to potassium channels and their cellular regulation) and mechanisms by which general anaesthetics exert harmful effects on the heart. Dr Terrar graduated in Zoology with an interest in biophysics and neurophysiology at University College London in 1969; he obtained his PhD in the Pharmacology Department at UCL in 1972. He has worked at The University of Miami Medical School and The London School of Pharmacy on neuromuscular transmission. Dr Terrar's work on cardiac myocytes was initiated at The Middlesex Hospital and has continued at Oxford since 1984.

Any new drug, whatever its intended target in the body, may have harmful effects on the heart. Cardiac side effects have caused the withdrawal of drugs that have reached the market or halted drug development in far too many recent cases. Regulatory authorities have given much attention to these problems, issuing guidelines that have evolved following feedback from pharmaceutical companies. Even without the intervention of regulatory authorities, it makes good commercial sense to investigate possible harmful effects on the heart at the earliest stages of drug development, so that lead compounds with the least likelihood of cardiac side effects can be identified and taken forward. Much attention has been given to prolongation of the QT/QTc interval of the ECG, and the tendency for a drug to cause this can be assessed with several preclinical approaches. The aim of this article is to discuss the advantages and disadvantages of available preclinical methods for identifying cardiac risk.

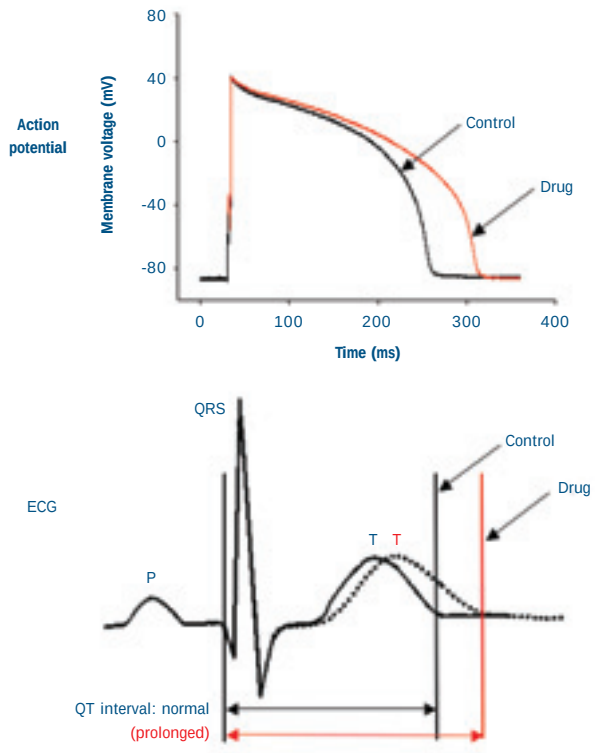
PREVIOUS CASES OF PROBLEM DRUGS

High profile examples of drugs that have encountered cardiac risk problems after reaching the market include terfenadine (an antihistamine), cisapride (a gastro-intestinal drug) and some fluoroquinolone antibiotics. In addition, anecdotal evidence indicates that many promising lead compounds in drug development have been abandoned because of cardiac side effects. In view of the acknowledged high costs of drug development and taking drugs to the market (counted in hundreds of millions of pounds), the wise commercial decision is to identify potential cardiac risks at a very early stage of development so that lead compounds with minimal risk can be taken forward.

QT PROLONGATION AND ITS CARDIAC EFFECTS

Regulatory authorities in the US, Europe and Japan have attributed great importance to the identification of cardiac risks and have co-ordinated their approaches through the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (1). Particular attention has been paid to possible drug effects to prolong the QT interval of the ECG (or more often the QT interval 'corrected' for the influence of heart rate, QTc, after application of appropriate formulae). This is because there is a marked correlation between excessive QT/QTc prolongation and the ability to produce potentially fatal torsade de pointes arrhythmias (2); an extensive study documenting this correlation

Figure 1: Prolongation of QT interval and action potential duration



has been provided by Redfern *et al* (3). The QT interval broadly reflects the time between the upstroke of the cardiac ventricular action potential and repolarisation (see Figure 1). In the latest ICH guidelines (ICH S7B available on the websites of the FDA and EMEA), it is said that "When ventricular repolarisation is delayed and the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including torsade de pointes, particularly when combined with other risk factors (such as hypokalemia, structural heart disease or bradycardia). Thus, much emphasis has been placed on the potential proarrhythmic effects of pharmaceuticals that are associated with QT interval prolongation."

The duration of the cardiac action potential in ventricular muscle, and therefore the QT interval, depends on a delicate balance of ionic currents flowing during the plateau (including depolarising currents, such as Ca^{2+} currents, persistent Na^+ current, Na^+-Ca^{2+} exchange current and repolarising currents through a variety of K^+ channels). In the context of QT prolongation, by far the most attention has been given to the hERG K^+ channel (so called because it is the human form of a K^+ channel coded by a gene related to the gene for the ether-a-go-go K^+ channel, discovered in the fruit fly, Dresophila, used extensively in pioneering K^+ channel studies). Most (if not all) drugs that have been found to be associated with torsade de pointes arrhythmias and QT prolongation reduce ionic current through hERG K^+ channels, either by direct blockade, or in a few instances by reducing hERG channel expression at the cell surface.

The simplest *in vitro* screen for the potential of new chemical entities (NCEs) to cause QT prolongation associated with torsade de pointes arrhythmias is therefore to test for an effect on hERG K^+ channels. This is commonly done in tissue-cultured cell lines that express hERG channels (often human embryonic kidney, HEK, or Chinese hamster ovary, CHO, cells). The most direct and informative approach is to investigate the log (concentration)-effect curve for blockade of K^+ currents through hERG channels under voltage-clamp (patch-clamp) conditions. (Voltage-clamp methods are used to control the voltage across the cell membrane with an appropriate negative feedback circuit; this allows precise control of the opening and closing of ion channels, such as hERG K^+ channels that depend on membrane voltage, and are therefore described as 'voltage-gated'. Patch-clamp is one type of voltage-clamp.) Automated patch-clamp methods allow medium- to high-throughput assays to be done (4). The various commercially available systems for automated patch-clamp have particular advantages and disadvantages, though it is striking that at a recent Ion Channels in Drug Discovery symposium (satellite of The Biophysical Society meeting in February 2006) many speakers using these methods said that they checked their most promising compounds with the 'gold standard' of manual patch-clamp techniques.

Another approach using hERG K^+ channels that allows high-throughput assays is the use of fluorescence methods and conventional multi-well plate readers. The most successful of these procedures are binding studies, where a ligand known to bind to the hERG channel is made fluorescent, and the ability of a new chemical entity to interact with the hERG channel is assessed from its tendency to compete with the fluorescent ligand for hERG channel binding. This approach is useful provided that there is overlap between the binding sites for the new chemical entity and the fluorescent ligand. Recent antibody-based methods are being evaluated.

INFORMATION FROM HERG CHANNELS

Can hERG assays tell us all that we need to know? No, but they are a very useful first step. The first problem with concentrating only on hERG channels is that a new chemical entity might act at a variety of other ion channels. This problem will be returned to later. The second problem is that the hERG channel protein is not as simple as it might first appear. There is an accessory protein, MiRP, that is thought to regulate the hERG channel when it is in place in a cardiac myocyte. In addition, it has recently been discovered that there are at least two forms of the hERG channel (1a and 1b as alternate transcripts of the hERG1 gene). The K^+ current carried by hERG channels in cardiac myocytes is referred to as I_{Kr} (for rapidly activating) and is now thought to be minimally composed of hERG1a and hERG1b subunits (5). Whether this complexity affects interactions with new chemical entities remains to be determined. Currently available commercial assay systems use cell lines expressing only hERG1a. A further complication is that hERG channel proteins contain sites for regulation by protein kinases, and it is possible that phosphorylation of hERG proteins by these kinases may alter drug binding. This complexity is already sufficient to raise doubt about whether it is safe to rely on a

simple hERG assay alone, though it is worth mentioning that there may be yet more features of the functional hERG/I_{Kr} channel in its cardiac environment that remain to be discovered.

THE ALTERNATIVES

One alternative to hERG channel assays in cell lines is to record the I_{Kr} ionic current from cardiac ventricular myocytes. Although it might be thought difficult to investigate the I_{Kr} current in isolation because of the existence of so many other ion channels in the heart, it is possible to take advantage of its rapid activation and to record I_{Kr} as a 'tail current' (activated by appropriate voltage-clamp protocols) with negligible contamination from other currents. In principle, this could be done in human cardiac ventricular myocytes, though questions of ethics and availability of healthy tissue prevent this from being carried out on anything but a very small scale. Investigating drug effects on I_{Kr} recorded from, for example, guinea-pig or rabbit ventricular myocytes, remains a worthwhile alternative. The I_{Kr} channels in the cardiac environment will have their full complement of subunits and regulatory proteins. Although guinea-pig and rabbit ventricular action potentials show slight differences from one another and from humans, this is largely because of the different balance of expression of the variety of ion channels, while the properties of I_{Kr} are remarkably similar.

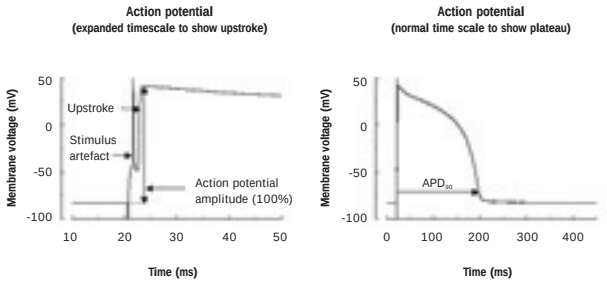
INTERACTION OF NEW CHEMICAL ENTITIES

Returning to the question of ion channels other than the I_{Kr} channel, it is clear that new chemical entities could interact with many other ion channels, underlying the cardiac action potential. Some drugs are selective for a particular channel, while others have several simultaneous effects (and the balance of actions on different channels may vary with drug concentration). For example, cisapride and terfenadine can prolong action potentials at low concentrations, as a consequence of hERG/I_{Kr} channel block, and have additional possibly harmful effects at high concentrations. It was pointed out above that the QT/QTc interval reflects action potential duration, and since there is a requirement to predict the propensity of a new chemical entity to prolong QT/QTc, it follows that measurements of action potential duration in isolated cardiac muscle provide useful predictive information. There are many possible *in vitro* methods for recording cardiac action potentials, with the emphasis on measurement of cardiac repolarisation. A frequently used measure is APD90, which is the action potential duration measured between the time of rapid upstroke and the time when repolarisation reaches 90 per cent back towards baseline (or, in other words, back to 10 per cent of the maximum amplitude of the action potential, measured from the resting membrane potential to the action potential peak – see Figure 2). This is a simple measure that is normally very well correlated with QT interval. Drugs that have a selective effect to block I_{Kr} currents prolong APD90 and QT/QTc. Drugs could prolong APD_{90} by selective block of other K^+ currents (such as I_{Ks} or I_{K1}) and could be equally dangerous (and would certainly prolong QT/QTc), but would be missed in simple I_{Kr}/hERG assays. More recently, other more complex measures of drug effects on action potentials have been advocated, such as 'triangulation'. Some drugs, particularly those with effects on many ion channels may alter the shape of the action potential; triangulation may be said to occur if the difference between APD_{30} (time for 30 per cent repolarisation) and APD_{90} is increased by the drug. Other estimates of triangulation rely on the ratio of APD_{90} and APD_{50} (action potential duration at 50 per cent). There is some debate about the relative merits of triangulation or simple changes in APD_{90} in predicting the propensity of a new chemical entity to cause torsade de pointes arrhythmias. However, both are easily measured and I would argue that the effects of new chemical entities should be tested on cardiac action potentials, and substantial changes in either of these parameters is cause for serious concern about potential cardiac toxicity.

MAKING PREPARATIONS

One of the oldest preparations available for recording cardiac action potentials is the multicellular Purkinje fibre. These Purkinje fibres are part of the system for rapid conduction of the action potential 'impulse' to allow all regions of the ventricular muscle to be speedily activated, thus permitting synchronisation of contraction. When the heart is dissected, they can be easily seen as white thread-like structures running across the inside surface of the ventricles (where the ventricular muscle is relatively pink). In the past, the Purkinje fibre was a very popular preparation, largely because of the large size of the cells and relative lack of contraction in a tissue that is designed to conduct the impulse at maximum speed; these properties of the Purkinje fibre allow the tips of 'sharp' microelectrodes to be inserted and kept in place intracellularly more readily than is the case in most ventricular muscle preparations. However, the QT/QTc interval reflects action potential duration in the large bulk of working ventricular muscle, rather than the longer duration action potentials in the Purkinje system, and it therefore seems preferable to choose a ventricular tissue model to test for drug effects. A counter-argument is that perhaps the Purkinje fibre system is more sensitive to action potential prolongation by drugs (and might be a source of arrhythmias), but even if this were to be the case, it seems the slow drug access of drugs to cells in the centre of such a preparation is a

Figure 2: Measuring APD_{90}



severe disadvantage of the multicellular Purkinje fibre (and may have been the reason why effects of terfenadine and cisapride were difficult to detect in this preparation). Healthy individual myocytes can be isolated from Purkinje fibres, and if a specific effect on this conducting tissue is warranted, I would argue that it should be carried out on Purkinje fibre isolated myocytes.

Papillary muscle is another commonly used preparation for recording cardiac action potentials. It has the advantage that it shows the ventricular action potential waveform, but again it is a multicellular preparation and drug access to central cells is often problematic.

Another preparation, available since 1978, is the isolated cardiac ventricular myocyte. Clearly a ventricular myocyte shows the ventricular action potential waveform that gives rise to the QRST components of the ECG, and for which there is a good correlation between action potential duration and QT/QTc (see Figure 1). Drug access to isolated ventricular myocytes is excellent, allowing the action potential prolongation effects of low concentrations of terfenadine and cisapride to be readily detected (in contrast to experiments in multicellular Purkinje fibres, as mentioned above). Some might be dissuaded from using isolated ventricular myocytes for action potential assays, since in the ICH S7B document they are referred to as "technically challenging" and "more fragile" than multicellular preparations, though the document does acknowledge that isolated ('disaggregated') ventricular myocytes "minimise diffusional barriers to the site of action". However, action potentials can be stably recorded from isolated ventricular myocytes using either 'sharp' microelectrodes or patch electrodes. Another useful parameter that can readily be recorded from isolated ventricular myocytes is action potential upstroke ('maximum rate of depolarisation' reflecting opening of voltage-gated Na^+ channels). Routine methods are now available for isolation/disaggregation of myocytes and for reliable electrical recording from these cells. A further advantage of the isolated myocyte is that very small volumes of test substance can be used.

Action potentials can also be recorded in whole isolated hearts (such as those from guinea pig or rabbit, chosen because action potentials in these species are broadly similar to those recorded from human ventricles, and in preference to adult rat and mouse, where ventricular muscle action potentials show atypical waveforms). The hearts are most commonly used in the Langendorff configuration, in which the perfusing solution is applied via the aorta, so that the valves at the base of the aorta shut to allow perfusion through the coronary circulation. This preparation has the advantage that the condition of the muscle most closely approximates that in the whole animal, and drug access is moderately good since it is via the coronary circulation (though not quite as speedy as with the isolated ventricular myocyte). Recording with intracellular microelectrodes is not practical over long periods because of muscle contraction, although monophasic action potentials can be readily recorded with appropriate electrodes. Monophasic action potential recordings give a good indication of action potential duration, but absolute measures of membrane potential are not possible, and action potential upstroke cannot be reliably measured. Most commonly, these preparations are used with spontaneous initiation of the heartbeat from the sino-atrial node (though stimulation via the atrio-ventricular node is possible after ablation of the sino-atrial node), and drug-induced instability can be a useful additional index of potential cardiac toxicity.

Finally QT/QTc measurements can be made on whole animals. However, most, if not all, anaesthetics have effects on action potential duration and QT interval (with a variety of actions on the underlying ion channels), and so tests for drug effects on QT/QTc are best done with implanted electrodes and Holter monitoring after recovery from anaesthetic. Analysis of data from ambulatory animals can be challenging.

CONCLUSION

In summary, many preclinical methods are available to assess the risk of cardiac toxicity, particularly the risk of QT prolongation and torsade de pointes arrhythmia. A minimum strategy is to test for effects on the hERG K^+ channel and for effects on action potential duration, preferably in an isolated ventricular myocyte. The hERG channel test can provide useful high-throughput information, though our developing understanding of the complexity of I_{Kr} channels in a cardiac environment shows that this may not detect the full story even for this one channel. Ventricular myocytes provide a useful test system, both for studies of I_{Kr} in a cardiac environment and for investigation of drug effects on ventricular action potentials, ensuring that additional harmful actions on cardiac ion channels other than I_{Kr} are not missed. ◆

The author can be contacted at
derek.terrar@pharm.ox.ac.uk

References

1. **http://www.emea.eu.int/pdfs/human/ich/042302en.pd**

2. **Dessertenne F, La tachycardie ventriculaire a deux foyers opposes, *Arch Mal Couer Vaiss* 59: pp263-272, 1966**

3. **Redfern WS et al, Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development, *Cardiovascular Research* 58: pp32-45, 2003**

4. **Kiss L *et al*, High throughput ion-channel pharmacology: planar-array-based voltage clamp, *Assay Drug Dev Technol* 1: pp127-35, 2003**

5. **Jones EMS *et al*, Cardiac I_{Kr} channels minimally comprise hERG 1a and1b subunits, *The Journal of Biological Chemistry* 279: pp. 44,690-44,694, 2004**