REVIEW ARTICLE

Drug Safety 1999; 21 Suppl. 1: 19-31
0114-5916/99/0001-0019/$06.50/0

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Preclinical *In Vitro* Cardiac Electrophysiology

A Method of Predicting Arrhythmogenic Potential of Antihistamines in Humans?

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Abstract

The cardiac action potential results from a dynamic balance between inward depolarising Na^+ and Ca^{2+} currents and outward K^+ repolarising currents. During a cardiac cycle, the resultant of repolarisation phase from all ventricular cells is represented by the QT interval of the surface ECG. Congenital long QT syndrome (LQTS) is characterised by polymorphic ventricular tachycardia sometimes with twisting QRS morphology (torsade de pointes) which, although usually self-limiting, can result in sudden cardiac death.

Acquired LQTS can be induced by a variety of drugs, including some non-sedative histamine H_1 receptor antagonists (astemizole, terfenadine). The Committee for Proprietary Medicinal Products of the European Union has recently proposed studying the action potential in *in vitro* heart preparations as a preclinical test for predicting the propensity of noncardiovascular drugs to induce malignant QT prolongation in humans.

The effects of several histamine H_1 receptor antagonists on the electrically evoked action potential have been evaluated in rabbit Purkinje fibres. In this preparation, astemizole (0.3 to 10 μmol/L) prolongs the duration of the action potential measured at the level where repolarisation is 90% complete (APD_{90}). This effect is dependent on drug concentration, incubation time, pacing frequency and K^+ or Mg^{2+} concentration. Astemizole also markedly depresses the rate of rise of the action potential (V_{max}). Terfenadine showed qualitatively similar, but quantitatively smaller, effects in this model. The histamine H_1 receptor antagonists cetirizine, ebastine, carebastine, loratadine and fexofenadine do not significantly affect APD_{90} at 1 μmol/L, but cetirizine and carebastine prolong it slightly at 10 μmol/L.

In conclusion, in rabbit Purkinje fibres, astemizole and terfenadine produce adverse electrophysiological effects at concentrations which may be achieved in the human myocardium in certain clinical situations. APD_{90} lengthening induced by carebastine and cetirizine is minor and occurs at concentrations that are very unlikely to be encountered clinically, since these drugs, in contrast to astemizole and terfenadine, do not accumulate in the myocardium.

Direct extrapolation of preclinical results to humans requires great caution, since malignant QT prolongations by terfenadine and astemizole are extremely rare clinical events. However, since prolongation of the QT interval often pre-

cedes the development of torsade de pointes, any significant delay in cardiac repolarisation produced by noncardiovascular drugs in preclinical, and particularly in clinical, studies should, in general, be considered to indicate a potential cardiac risk in humans. Its significance should subsequently be evaluated in appropriate studies in patients with conditions known to predispose to arrhythmias.

The Committee for Proprietary Medicinal Products (CPMP) of the European Union has recently published a document containing recommendations for assessing the potential of noncardiovascular drugs to prolong the QT interval in humans.[1] This official intervention has been prompted by numerous reported cases of sudden cardiac death associated with the use of drugs from various therapeutic classes. In particular, certain nonsedative histamine H_1 receptor antagonists, such as astemizole and terfenadine, can prolong the QT interval and, in the most serious cases, this effect can culminate in fatal ventricular fibrillation resulting from episodes of torsade de pointes. This cardiac adverse reaction has been clearly documented in attempts at suicide using overdoses of astemizole and terfenadine. It has also been observed in patients who have taken therapeutic doses of these drugs but in whom there were concomitant risk factors predisposing to arrhythmias. These included congenital or acquired long QT syndrome (LQTS), congestive heart failure, severe hepatic or renal dysfunction, bradycardia, electrolyte imbalance (hypokalaemia produced by potassium-wasting diuretics, hypomagnesaemia, acidosis) and certain drug regimens (e.g. agents interfering with drug metabolism).[2-4]

The preclinical studies proposed by the CPMP for determining the arrhythmogenic potential of a compound include *in vitro* and *in vivo* models, which are routinely used to demonstrate the effects of drugs on electrophysiological and electrocardiographic parameters. In particular, *in vitro* Purkinje fibre preparations from any laboratory animal species (e.g. rabbit, guinea-pig, dog or pig) are considered to be suitable experimental models provided that the major ionic currents underlying the investigated cardiac action potential do not differ substantially from those encountered in human cardiac tissues.[1]

The aims of this article are to discuss basic ideas concerning the cardiac action potential, congenital and acquired LQTS and *in vitro* preparations for studying the cardiac electrophysiological actions of drugs. Additionally, electrophysiological effects of some nonsedative histamine H_1 receptor antagonists are detailed and their clinical relevance is discussed.

1. Cardiac Action Potential and ECG

The cardiac action potential is the integrated electrical activity of excitable heart cells and results from several small inward and outward currents contributed by numerous pathways (probably more than 15) that include ionic pumps and exchangers and, most importantly, voltage-gated ion channels. Each of these mechanisms possesses individual voltage-dependent and kinetic properties. The characteristic forms of the action potential measured in different parts of the heart are determined by cyclical changes in the intensity of inward depolarising, and outward repolarising, currents. Hence, the initial depolarisation phase of the action potential is attributable to the predominance of inward Na^+ and Ca^{2+} currents (I_{Na}, I_{Ca}), whereas the repolarisation phase can result from several outward K^+ currents (I_K: I_{Kto} – transient outward; the 3 components of delayed rectifier: I_{Kur} – ultrarapid delayed K rectifier; I_{Kr} – rapid delayed rectifier; I_{Ks} – slow delayed rectifier; I_{K1} – inward rectifier). Repolarisation reflects both increases in K^+ current and the inactivation or reduction of inward currents. The plateau phase of the action potential that immediately follows the initial depolarisation is the outcome of a dynamic, transient equilibrium between inward and outward ionic currents.

Figure 1 depicts an idealised profile of an action

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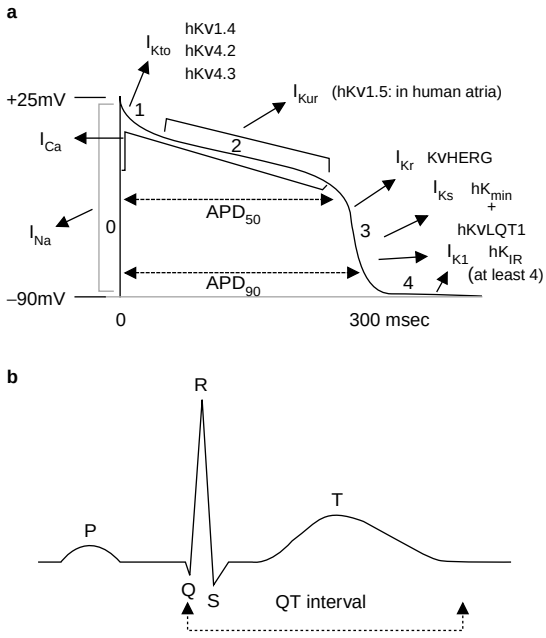

Fig. 1. (**a**) Idealised morphology of the action potential measured under *in vitro* conditions in a cardiac ventricular myocyte with the 5 phases (0, 1, 2, 3 and 4) used to describe this electrical event and the main ionic currents mediating each phase. (**b**) Idealised normal surface ECG pattern during a cardiac cycle. The P wave is due to depolarisation of atrial myocytes, the QRS complex to the ventricular depolarisation phase and the T wave to the repolarisation phase of ventricular cells. **APD_{50}, APD_{90}** = action potential duration at 50 or 90% complete repolarisation, respectively; **hK_{IR}** = human inward K^+ rectifier channel; **hK_{min}** = human K^+ channel subunit; **hKv** = human voltage-dependent K^+ channel; **hKvLQT1** = human voltage-dependent K^+ channel mediating I_{Ks}; **I_{Ca}** = inward Ca^{2+} current; **I_{Kto}** = transient outward K^+ current; **I_{Kur}** = ultrarapid delayed K^+ rectifier current; **I_{Kr}** = rapid delayed K^+ rectifier current, **I_{Ks}** = slow delayed K^+ rectifier current; **I_{K1}** = inward K^+ rectifier current; **I_{Na}** = inward Na^+ current; **KvHERG** = HERG voltage-dependent K^+ channel mediating I_{Kr}.

potential generated by a ventricular myocyte and the main ionic currents and ion channels mediating each phase of this electrical event in the human ventricle.[5,6] It should be noted that qualitatively and quantitatively different currents may contribute to the action potential generated by cells in other parts of the heart. For example, in the human atria, a sustained K^+ current (I_{sus}) partly carried by an hKv1.5 channel (one of the ultrarapid component of the delayed outward rectifier current) intervenes in the early repolarisation process. Thus, excitable cardiac cells contain many channels and, in particular, multiple subtypes of K^+ channels, each of which may be a target for a genetic disease or for the wanted or unwanted action of a drug.

The surface ECG reflects the sum of the synchronised electrical activities (action potentials) generated by all cardiac cells during a heart cycle. The P wave of the ECG results from the depolarisation of the atria. The beginning of the Q wave and the termination of the T wave indicate, respectively, the initiation and the end of ventricular repolarisation. The QT segment of the ECG, as such, or in a form corrected (QTc) by the heart rate value by using, for example, the formula of Bazett ($QT_{Bc} = QT/\sqrt{RR}$) or of Fridericia ($QT_{Fc} = QT/\sqrt[3]{RR}$) as well as its dispersion (wider QT interval in any 2 of the 12 standard ECG leads), are clinically used markers of the cardiac repolarisation process. Although QT interval duration represents the sum of both ventricular depolarisation (QRS intervals) and ventricular repolarisation (QT minus QRS), QT prolongations very rarely result from widening of QRS (an exception to this rule is provided by the effects of the class I antiarrhythmics such as encainide or flecainide).[7] However, it should be noted that QT interval measurements are an approximate estimation of the time required for ventricular repolarisation, since the beginning of the Q and the end of T waves cannot always be determined with accuracy. Furthermore, the values of QT interval can change depending on autonomic drive to the heart and the selected ECG lead to measure it and heart rate (the correction formula by Bazett is inaccurate, since it under- or overestimates the value of QT for low and high rates, respectively). In conditions of stable sinus rhythm, a QTc value greater than 430 (adult males) to 450 (adult females) msec (or up to 550 msec according to some authorities) and a QT dispersion value greater than 40 to 60 msec are generally considered abnormal.[4,7,8]

In conclusion, despite a number of constraints against the accurate determination of the QT inter-

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val, this parameter is a valuable tool for detecting wanted and unwanted effects of drugs on cardiac repolarisation and for studying the factors and clinical conditions that may increase or predispose to an increase in its magnitude.

2. Congenital and Acquired Forms of LQTS

The shape of the cardiac action potential can be profoundly altered even by small changes in any of the ionic currents that contribute to it. Hence, any drug- or disease-provoked increase in inward current or decrease in either one or more K^+ channel currents can diminish the intensity of the net outward current responsible for the repolarisation process and thereby cause a phenotypical prolongation of the action potential.

The hereditary forms of LQTS are generally autosomal, dominant, familial disorders characterised by indications of prolonged ventricular repolarisation in the ECG and a propensity for polymorphic tachycardia, sometimes with twisting QRS morphology on either side of the ECG isoelectric line. This is referred to as torsade de pointes and is usually self-limiting, but can lead to episodes of recurrent syncope and sudden death. These events often affect young, otherwise healthy individuals, particularly during conditions of psychological and physical stress. The delay in the repolarisation process favours early and late afterdepolarisations (particularly at the level of the Purkinje conducting system). These are produced by premature openings of Na^+, and L-type Ca^{2+} channels, which further worsen the lengthening of the action potential, a trigger for torsade de pointes.[9]

Genetic analyses have demonstrated that the genome of individuals afflicted with LQTS is such that they have defective genes encoding for abnormal proteins constituting cardiac ionic channels. To date, 3 main specific mutations in cardiac ion channel genes (*KVLQT1*, *HERG*, *SCN5A*) have been clearly identified.[10-12] Furthermore, the same gene may be the target of different mutations affecting different individuals, suggesting a high degree of polymorphism in the inheritance of certain cardiac channels.[10-12]

Although rapidly inactivating Na^+ currents are primarily responsible for the initial upstroke of the action potential, a persistent late component of this current can sometimes contribute to the plateau phase. The mutant *SCN5A* gene at the LQT3 locus on chromosome 3 encodes an abnormal Na^+ channel α subunit protein. The mutant channel phenotypes show dispersed reopenings and prolonged bursts, which lead to lengthening of the tail portion of the inward Na^+ current, an effect that can cause prolongation of the action potential.[10]

Several defective genes also encode for the α subunit, which intervenes in the formation of a K^+_{HERG} (human erg-related gene) channel carrying I_{Kr} and of the α (K_{vLQT1}) subunit, which coassemble with the β subunit (hK_{min}) to constitute the K^+ channels carrying the I_{Ks} current (fig. 1). The mutant *HERG* and the *KVLQT1* genes responsible for the LQT2 and LQT1 are, respectively, located on chromosomes 7 and 11. All these mutations and others (LQT4 and LQT5) that affect cardiac repolarisation channels lead to either a reduction in the tissue density of normally expressed K^+ channels, to abnormally operating channels or to assembly of nonfunctional channels. Thus, their ultimate consequence is a decrease in the amplitude of the outward current leading to a prolongation of the QT interval of the ECG.[10,11]

In summary, in patients with LQTS, the fine equilibrium between inward and outward currents responsible for the plateau phase of the action potential is shifted in the inward direction and this leads to action potential lengthening which can culminate in torsade de pointes, particularly in the presence of concurrent risk factors favouring cardiac dysrhythmias.

Cardiac repolarising outward K^+ currents, and particularly HERG I_{kr}, can be either a primary desirable, or a secondary unwanted, site of action for certain members of numerous clinically used drug classes. For instance, the therapeutically relevant property of class III antiarrhythmics (*d*-sotalol, dofetilide) is blockade of repolarising K^+ channels.

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The resulting prolongation in the action potential duration produces a useful increase in cardiac refractoriness. This increase in refractoriness is exploited therapeutically for suppressing ectopic activity, which acts as a trigger for re-entrant electrical circuits. However, when the effects of these drugs in prolonging the action potential duration become very marked, torsade de pointes may ensue. Additionally, class III antiarrhythmics can lengthen action potentials of midmyocardial cells and Purkinje fibres to a greater extent than those generated by subendocardial myocytes. The resultant dispersion in ventricular refractoriness disturbs the smooth synchronisation of the overall repolarisation process. This condition favours arrhythmogenic re-entrant mechanisms which, in turn, can initiate ventricular fibrillation.[13]

Many other currently used drugs (antihistamines – astemizole and terfenadine; antipsychotics – haloperidol, thioridazine; antibacterials – erythromycin; antifungal agents – ketoconazole; an agent for treating incontinence – terodiline; an agent with prokinetic activity – cisapride) can block K^+ channels, in general, and the rapid component of the delayed rectifier K^+ channel current (I_{Kr}), in particular. This may result in serious cardiac consequences for the cardiac repolarisation process already mentioned.[5,14]

It is important to mention that abnormalities in systems responsible for cardiac electrogenesis other than ionic channels could lead to prolongation of the repolarisation phase of the cardiac action potential. For example, the electrogenic Na^+/Ca^{2+} exchanger is a major contributor to the plateau phase of the cardiac action potential and provides a major source of the current that gives rise to the QRS complex of the ECG. It is thus possible that certain patients affected by congenital or acquired LQTS may also suffer delayed cardiac repolarisation as a result of abnormalities in the function of the Na^+/Ca^{2+} exchanger.

In conclusion, K^+ channel mutations underlie certain forms of congenital LQTS and, because the native K^+ channels are also direct or indirect pharmacological targets for many currently used drugs, the products of the same native or mutated genes appear to be responsible for both the congenital and acquired forms of LQTS. It is tempting to speculate that patients responding in an exaggerated or unexpected manner to certain drugs, thereby acquiring LQTS, may carry cardiac channel abnormalities which have no pathological consequence for normal life but may predispose them to the adverse effects of drugs with the potential to prolong QT.[10] This may be attributable to at least 2 distinct mechanisms. The drug can reveal the phenotype of an underlying genetic disease either by reducing further the impaired function of a mutated channel or by altering the function of a native channel that is essential for a normal cardiac repolarisation process in individuals with a mutated channel.[10]

Finally, additional endogenous factors recognised to worsen adverse effects associated with long QT intervals and large QT dispersion include diminished baroreflex sensitivity, reduced heart rate variability due to a shift of the sympathovagal balance towards a sympathetic predominance, and the occurrence of ventricular early or late afterpotentials.[15]

3. *In Vitro* Study of the Action Potential in Ventricular Tissues from Experimental Animals

The CPMP has proposed the use of cardiac papillary muscle and/or Purkinje fibre preparations as an approach to assessing the likelihood of a drug causing QT prolongation in humans.

Technically, the action potential profile is measured by using glass microelectrodes to penetrate into the membrane of single cells of cardiac tissues. These multicellular preparations used for this purpose are mounted in isolated tissue chambers, perfused with a warm (35 to 37°C), oxygenated (95% O_2/5% CO_2) physiological salt solution (e.g. Tyrode) and then paced via bipolar silver electrodes to deliver generally square wave electrical pulses of approximately 1 msec duration, twice threshold intensity (20 to 150μA) and 1Hz frequency. After an appropriate equilibration period (generally 30 to 60 minutes) to achieve steady-state conditions, action potentials are recorded from

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a cell, using standard glass microelectrodes (filled with 3 mol/L KCl, with a resistance range of 30 to 50 Mohm) connected to an appropriate amplifier.

Under normal conditions, the resting transmembrane potential of a functional ventricular myocyte or a fibre of the specialised conducting system lies within –80 and –95mV. On excitation, from a threshold potential of about –70mV, a rapid regenerative depolarisation due to Na^+ influx into the cell develops and shifts the membrane potential to a value within the range +25 to +35mV (phase 0 of the cardiac cell action potential) [fig. 1]. Thus, measuring the rate of depolarisation, which is given by the first derivative (dV/dt) of the phase 0 of the action potential (V_{max}: V/sec), provides information on the function of Na^+ channels with an increase in V_{max} indicating an enhanced channel function and a reduction reflecting an impaired channel function.

In addition to the Na^+ conductance, a second inward depolarising current becomes activated at membrane potentials of around –50mV and persists throughout the plateau phase of the action potential. This is initially due to extracellular Ca^{2+} entering the myocyte through slow Ca^{2+} channels and subsequently to additional ionic pathways (e.g. Na^+/Ca^{2+} exchanger) that contribute substantially to the elevation in cytosolic Ca^{2+} concentration (fig. 1). Outward currents overcoming inward ones are essentially carried by K^+. Thus, the duration of the action potential measured at selected points (25, 50 and 90%) of the repolarisation process (APD_{25}, APD_{50}, APD_{90}; msec) are parameters providing an overall estimation of the function of the Ca^{2+} and K^+ channels (fig. 1). An additional parameter that can be estimated from APD values is the slope of the terminal repolarisation phase, the approximate value of which is given by the ratio (R) of APD_{50} over APD_{90}. Thus, if R increases, the final repolarisation is delayed. In order to compare the effects of a group of drugs on terminal repolarisation, the ratios obtained before (R_b) and after (R_a) tissue incubation with the drug are calculated and the additional ratios (R_b/R_a) are analysed. An R_b/R_a value lower than 1 indicates that the drug increases the repolarisation time, whereas a value greater than 1 indicates that the final repolarisation process is accelerated. This parameter is useful when the changes produced by drugs in APD_{50} and APD_{90} are of different magnitude (fig. 2).

The resting potential (E_m:mV) is also a routinely measured parameter in electrophysiological studies. Its value corresponds to the transmembrane potential (~ –90mV) at full repolarisation. It provides information on the function of the inward rectifier K^+ channel carrying the I_{K1} current, which acts to clamp the cell at the resting potential. Hence, when the transmembrane potential of the resting cell is less than –90mV, the cell is said to be in a partially depolarised state.

In conclusion, the changes produced by drugs in any of the electrophysiological parameters used to describe quantitatively the unicellular cardiac action potential reflect alterations in one or more of the many ionic currents that underlie this electrical event. However, it should be emphasised that the study of the action potential using a simple microelectrode is generally insufficient to determine the precise ion channel current(s) affected by the compound under investigation. Such information can, nevertheless, be obtained by using the patch clamp recording techniques. This allows study of single ion channels and is especially appropriate when measuring drug effects in human cardiac cells or in cloned human cardiac ion channels which should be preferentially expressed stably in mammalian cells, although an effect on one or more single channels could well be modified or nullified in whole tissue preparations or *in vivo* conditions by opposing effects on other channels and homeostatic mechanisms. Nevertheless, the nullified adverse effect may manifest itself again under particular pathological conditions.

4. Experimental Variables to Consider When Determining *In Vitro* Drug-Induced Electrophysiological Effects

The *in vitro* electrophysiological effects of a drug may depend on several experimental vari-

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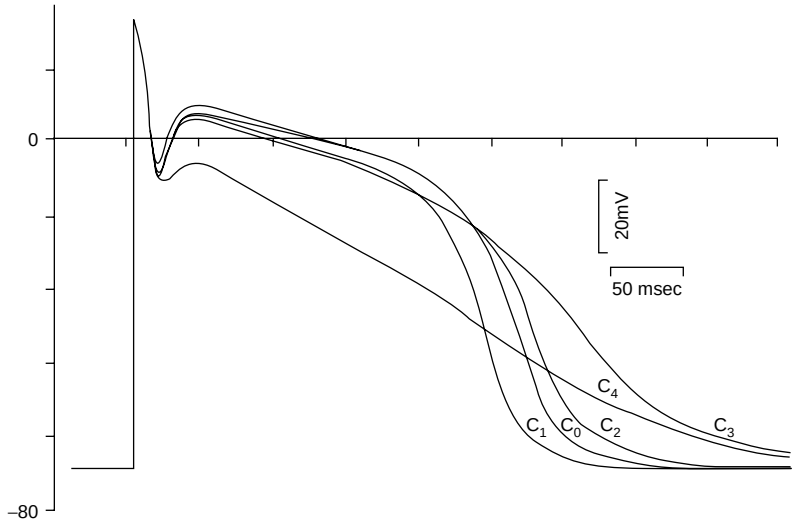

Fig. 2. Characteristic effects of three concentrations (C_2, C_3, C_4) of astemizole (0.1, 1 and 10 μmol/L, respectively) and its vehicle [C_1: dimethylsulfoxide (DMSO) 1%] on the shape of the action potential profile in rabbit Purkinje fibres paced at 1Hz and bathed in a standard salt solution (Tyrode). C_0 is the action potential measured under baseline conditions.

ables, such as concentrations investigated, incubation time, pacing frequency of the preparation, concentrations of ions (K^+, Mg^{2+} or H^+) in the bath solution and baseline values of electrophysiological parameters.[16-18] Thus, the selection of appropriate experimental variables (such as low stimulation frequency or low K^+ concentrations) is sometimes essential for revealing and quantifying adequately the possible electrophysiological effects of a drug. Failure to do so may sometimes explain differences in results reported by independent investigators. Although the values of experimental variables necessary for clearly observing the effects of a compound on electrophysiological parameters may be far from the physiological ones (and thus considered by some investigators to be of reduced scientific interest), it should be kept in mind that the main purpose of safety cardiac electrophysiology studies is to determine the potential risk for a drug to provoke QT interval prolongation, not only in healthy humans but, most importantly, in patients with cardiac problems who may be prescribed such a drug.

Results with astemizole obtained in studies of rabbit Purkinje fibres will be used to exemplify the role of certain experimental variables on V_{max} and the prolongation of the action potential repolarisation phase (APD_{50} and APD_{90}). As discussed above, the latter effect is considered to have the potential to predict the occurrence of drug-induced QT prolongation in humans.

5. Electrophysiological Effects of H_1 Histamine Receptor Antagonists

In rabbit Purkinje fibres, astemizole produces concentration-dependent changes in the shape of the action potential. In particular, the highest concentration studied reduces the rate of repolarisation so markedly that the action potential assumes a triangular shape (fig. 2).

After a 30-minute superfusion of the Purkinje fibres with astemizole, the APD_{50} and APD_{90} increase in a concentration-dependent (0.1 to 10 μmol/L) manner. In these experiments, 1 and 10 μmol/L concentrations of astemizole also de-

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pressed the depolarisation rate (V_{max}) of the action potential (fig. 3).[17]

A study of the kinetics of the onset of the electrophysiological effects of a small concentration (0.3 μmol/L) of astemizole indicates that the prolongation of the action potential fails to attain steady-state conditions even after 3 hours of incubation. Interestingly, this concentration produces a selective, marked augmentation of APD_{50} and APD_{90} without modifying V_{max}[17] (fig. 3). This indicates that astemizole is more potent in inhibiting the function of Ca^{2+} and K^+ channels than it is in impairing Na^+ channel function.

Reducing the pacing frequency of the Purkinje fibre preparation increases the duration of the action potential and decreases the depression of V_{max} produced by astemizole. At a very low pacing frequency the magnitude of the astemizole-induced prolongation of the action potential is greater than that measured at high frequencies, whereas the reverse holds true for V_{max} (fig. 3). Such phenomena are a characteristic feature of most of the known class III antiarrhythmic agents and reflect the dependency of the drug action on the frequency of channel opening (reverse use dependence).[5]

The influence of reducing the stimulation frequency on the prolonging effect of astemizole on the action potential is clearly potentiated by decreasing the extracellular KCl concentration (fig. 4).

Figure 5 shows the effects in APD_{90} produced by 1 and 10 μmol/L concentrations of astemizole, terfenadine, ebastine, carebastine, cetirizine, fexofenadine, loratadine and vehicle (dimethylsulfoxide; DMSO). The results reported were measured at the end of a 30-minute superfusion of paced (1Hz) rabbit Purkinje fibres with a standard salt solution (4.0 mmol/L KCl and 0.5 mmol/L $MgCl_2$). Each of the 3 concentrations (0.1, 1 and 10 μmol/L: results for the 0.1 μmol/L are not represented) of the compounds studied was superfused for 30 minutes before increasing the concentration rapidly to the next highest in the series. Of all the compounds studied, only astemizole and terfenadine produced a significant prolongation of the action potential at 1 μmol/L concentration. At 10 μmol/L, the effects of these compounds increased slightly, and carebastine and cetirizine slightly but significantly prolonged the action potential. The other compounds studied did not significantly affect this parameter (fig. 5).

DMSO slightly but significantly decreased APD_{90} when compared with the corresponding value measured in control preparations (data not shown) at the end of 60 and 90 minutes of perfusion (fig. 5).

Astemizole and terfenadine also produced concentration-dependent decreases in V_{max} (38.3 ± 9.5% and 25.4 ± 4%, respectively at 10 μmol/L). Ebastine at 10 μmol/L, but not at 1 μmol/L, produced a small significant reduction in V_{max} (14.2 ± 3.1%), which was not seen with carebastine, cetirizine or loratadine.

6. Discussion

Health authorities of the European Community are now alerted to the fact that drug-induced prolongation of the QT interval (acquired LQTS) is associated with an increased risk of fatal ventricular arrhythmic events (torsade de pointes). This serious cardiac problem can be experienced also by individuals with congenital LQTS, which stems from unfavourable mutations in genes coding for subunits that are essential for the correct functioning of cardiac ionic channels.[10-12] Since in these patients the probability of torsade de pointes is high, the prolongation of the QT interval by noncardiovascular drugs is currently viewed as an undesirable adverse effect and the risks it presents to humans should be carefully assessed. For this reason, the CPMP has recently demanded that new noncardiovascular medicinal products be routinely subjected to appropriate preclinical tests to determine whether they adversely influence cardiac electrogenesis.[1]

The extrapolation of undesirable cardiac electrophysiological effects of a compound to humans should satisfy a few reasonable criteria. Firstly, the mechanism affected by the test compound should have a counterpart in the human heart. This may

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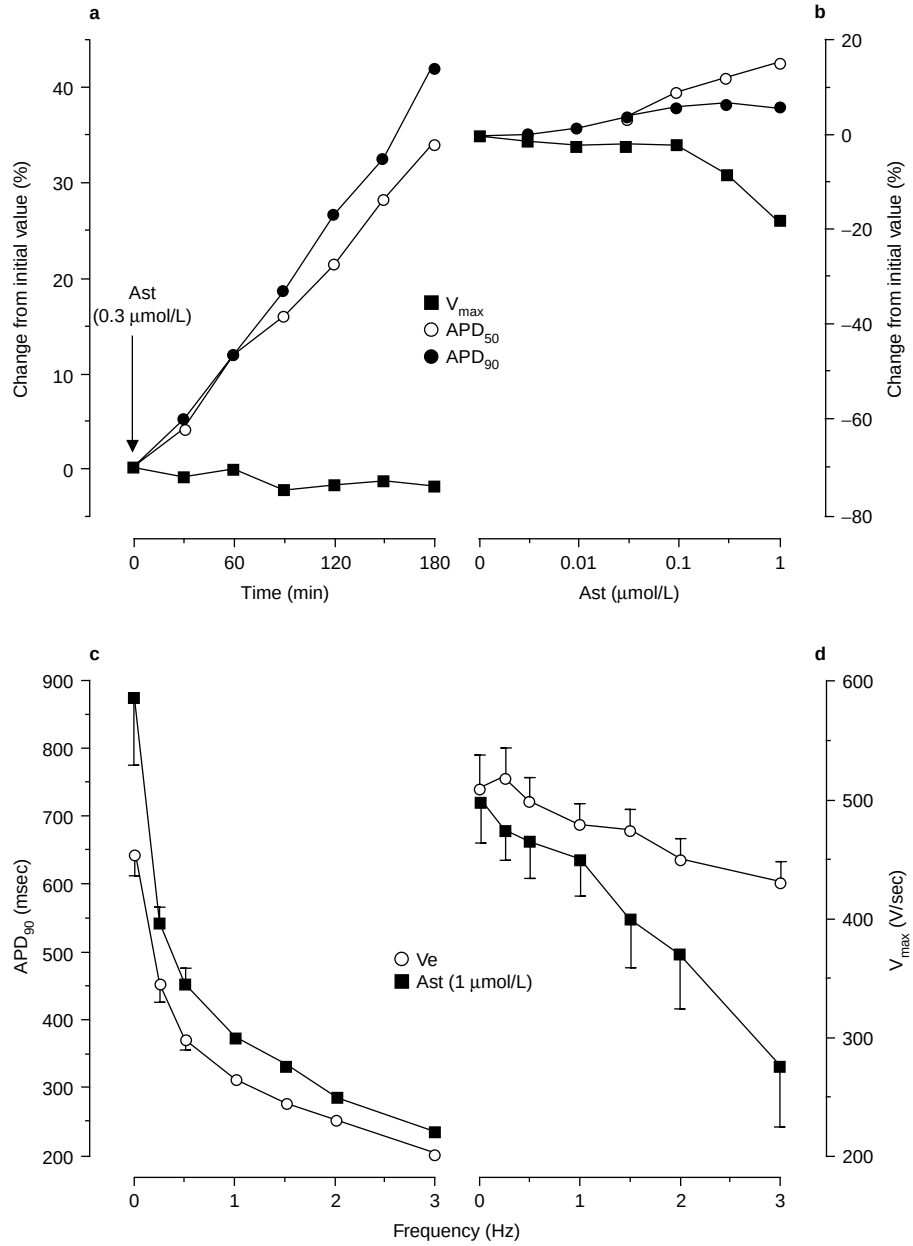

Fig. 3. (**a**) Time-course of the onset of the effects of 0.3 μmol/L astemizole (Ast) on the maximal rate of depolarisation (V_{max}) and the action potential duration measured at 50% (APD_{50}) and 90% (APD_{90}) of the repolarisation. (**b**) Changes in V_{max}, APD_{50} and APD_{90} measured at the end of 30 minutes' superfusion of each astemizole concentration investigated. These studies were carried out in rabbit Purkinje fibres paced at 1Hz (n = 7-8). The results shown are from preparations which did not respond with early afterpotentials to astemizole. (**c** and **d**) Pacing frequency-dependent effects of Ast or its vehicle (Ve) alone on V_{max}, APD_{50} and APD_{90} in rabbit Purkinje fibres (n = 11) [original data from Adamantidis et al.[17]].

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Fig. 4. Extracellular K^+ concentrations and pacing frequency-dependent effects of astemizole (Ast) and its vehicle (Ve) on the action potential duration measured at 90% of repolarisation (APD_{90}) in rabbit Purkinje fibres (n = 5) [adapted from Adamantidis et al.,[17] with permission].

not be always the case, since proteins regulating ionic movements in human heart cells are certainly similar, but not fully homologous, to corresponding ones in the hearts of experimental animals. A corollary to this observation is that a compound lacking electrophysiological activity in animal testing could, nonetheless, adversely affect the function of certain ionic pathways in the human heart. However, both these issues could be addressed in part by testing the compound under investigation on cloned human cardiac channels expressed preferentially in mammalian cells or, even better, on native channels present in samples of human heart tissues obtained during cardiac surgery.

Furthermore, for extrapolation to humans, it is generally accepted that undesirable effects in *in vitro* cardiac preparations should occur at clinically relevant concentrations of the drug. These concen-

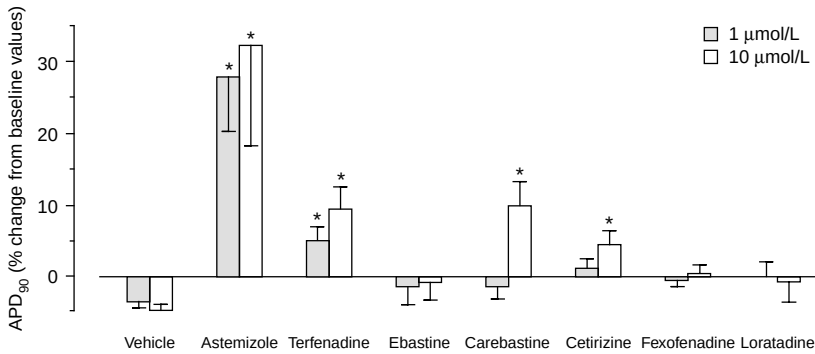

Fig. 5. Effects of vehicle [1% dimethylsulfoxide (DMSO)], astemizole, terfenadine, ebastine, carebastine, cetirizine, fexofenadine and loratadine on action potential duration at 90% of the repolarisation (APD_{90}) measured at the end of 30 minutes' superfusion of each concentration (1 and 10 μmol/L) in rabbit Purkinje fibres paced at 1Hz and bathed in a salt solution (Tyrode) containing a standard concentration of K^+. $*$ $p < 0.05$ *vs* vehicle, unpaired t-test.

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trations should be similar to those occurring in human plasma, and more correctly in the human myocardium, after therapeutic doses (taken alone or in combination with other drugs by patients with or without diseases known to impair drug clearance or metabolism) or, in exceptional cases, supratherapeutic doses taken in suicide attempts.[16,17] Thus, for drugs with unwanted electrophysiological activity, it is essential to know whether they, as well as their major metabolites, accumulate in the myocardium. The study of the time-course effects of astemizole (fig. 3) clearly indicates that heart tissue gradually becomes impregnated with this drug, since a steady-state increase in APD_{90} was not achieved even after 3 hours of superfusion of rabbit Purkinje fibres with a salt solution containing 0.3 μmol/L of astemizole. This observation was directly confirmed in the Langendorff perfused rabbit heart, in which the ratio of drug concentration in left ventricle tissue to that in the perfusion medium (containing 1 μmol/L of astemizole) reached a value of 450 after a 3-hour period of astemizole perfusion (Llenas et al., personal communication). In concurrent experiments, the ratio for terfenadine was found to be approximately 250.

In dogs, the heart concentration of astemizole was reported to be 300-fold higher than that in plasma[19] This implies that human heart tissue concentrations of astemizole and terfenadine will be many times higher than those measured in human plasma. Thus, great caution should be exercised when preclinical electrophysiological effects determined in rabbit Purkinje fibres or in any other experimental animal heart preparation are extrapolated to the human myocardium by considering only human plasma concentrations of these drugs.

Additionally, preclinical electrophysiological studies should be carried out by using drug incubation times sufficiently long to achieve steady-state concentrations in the biophase. However, this may often not be possible because of experimental difficulties (a very long incubation time can affect the viability of the preparation). This is of particular importance when drugs with substantially different physicochemical properties are compared. For example, cetirizine and carebastine (each tested at 10 μmol/L on rabbit Purkinje fibres) slightly prolonged the cardiac action potential but did not significantly accumulate in the rabbit heart (Llenas et al., personal communication). In the latter respect, these agents contrast with astemizole and terfenadine, as in humans their cardiac tissue and plasma concentrations are expected to be similar. Thus, cetirizine and carebastine should not be expected to significantly affect QT interval duration in humans, since their maximal human plasma concentrations, even in the most unfavourable clinical situations, are substantially lower than 10 μmol/L.

According to a recent letter to the *Lancet*[2] comparing the cardiac adverse effects of nonsedating antihistamines on the basis of spontaneous adverse reactions reported to WHO, cetirizine is also clearly much better tolerated by the heart than terfenadine, since complications in patients with cetirizine included cardiac rhythm disorders, only two of which were fatal versus 98 for terfenadine.[2] However, in this report the spontaneous rate of cardiac events in a matched population not taking the drug was not measured concurrently.[2] Furthermore, up to now, no case of torsade de pointes has been causally related to the intake of cetirizine[20] or ebastine,[21] the parent compound of carebastine.

An important issue is whether the extent of the prolongation of the action potential repolarisation phase in preclinical cardiac preparations is predictive of the magnitude of QT interval prolongation and its consequences in humans. This is almost certainly not the case, since astemizole is more potent than terfenadine in prolonging the action potential in rabbit Purkinje fibres but clinically it appears to cause fewer cardiac adverse effects than terfenadine.[2] It is possible that the site of action of these compounds or their affinity for this site on rabbit Purkinje fibre K^+ channels is not the same as that in the human heart channels. Moreover, terfenadine may have additional sites of action on ion transporting mechanisms in the human heart cells.

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The prolongation of the QT interval by a drug seems to have poor predictive value for mortality and morbidity in individuals without cardiac problems, who are generally studied in the early clinical development phases of a drug and constitute the great majority of patients using histamine H_1 receptor antagonists. However, there appears to be better predictive value for prolonged QT for patients with preexisting cardiac disorders.[22,23] Thus, drugs that lengthen the action potential in *in vitro* heart preparations, and particularly in the rabbit Purkinje fibres, should be investigated for their cardiac tolerability, particularly in patients with concurrent cardiac problems. This conclusion has important consequences for the development and postmarketing surveys of drugs that, in preclinical and possibly early clinical studies, are found to delay the repolarisation process. An appropriate assessment of their cardiac tolerability requires studying populations with known cardiac risks including, whenever ethically possible, patients with congenitally mutated cardiac ion channels, and particularly those phenotypes not showing a QT prolongation.[10] Indeed, some fatal terfenadine-related adverse events have been linked to patients with LQTS.[3] Recently, it was reported that a patient developed a very long QT prolongation (to 700 msec from baseline value of 440 to 480 msec) after receiving cisapride, which blocks cardiac repolarisation channels. This patient was subsequently demonstrated to have an underlying congenital LQTS.[24] These results indicate that, in some individuals, LQTS may remain a silent proarrhythmogenic substrate until revealed by a trigger (e.g. a drug blocking repolarisation K^+ channels). Thus, it is tempting to support the proposal of Napolitano et al.[24] that patients who experience drug-induced torsade de pointes, whenever appropriate, be screened with the goal of identifying whether they carry LQTS genes. This would help physicians to take preventive measures for these patients, as well as their affected relatives, when they need drug treatment that carries potential cardiac risk.

It is important to stress that prolongation of QT interval, in a reasonable range, is not itself necessarily followed by torsade de pointes. However, any increase in QT interval should be considered as a risk factor for torsade de pointes, since this electrocardiographic abnormality often precedes the appearance of torsade de pointes. Although the 2 phenomena do not appear to be causally, but are circumstantially, related, the establishment of such a possible relationship for a given drug should be studied by using therapeutic as well as, if possible, supratherapeutic doses of the drug in normal patients and in patients with identified cardiac risks predisposing to arrhythmias.

In conclusion, prolongation of repolarisation by a noncardiovascular drug in preclinical heart preparations should be simply taken as an indication that the drug has the potential to cause cardiac morbidity and mortality, particularly in individuals with cardiac risk factors. However, the translation of such a preclinical observation into the clinical reality requires great caution, even when all experimental measures have been taken to satisfy a few basic pharmacological assumptions such as confirmation in human cardiac preparations of the site of action of the drug demonstrated in animals. Additionally, the possible myocardial tissue concentrations of the drug producing the adverse effects in animals should also be indirectly (from animal or human body volume distribution data) estimated in order to avoid the exclusive use of plasma concentrations in the extrapolation of preclinical results to humans. Although the latter point may be critical, the appearance and magnitude of a drug effect on cardiac repolarisation in preclinical preparations depend also on the experimental conditions adopted. Thus, as a general rule, investigators should also select, whenever possible, experimental conditions mimicking as closely as possible the most unfavourable clinical conditions for drug use. This is because cardiac rhythm disorders associated with drugs prolonging QT interval occur principally in individuals with additional cardiac risk factors and abnormally altered biological functions or parameters.

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Clearly, the translation of animal findings to clinical situations is one of the most difficult problems faced by pharmaceutical research. However, for a noncardiovascular drug with the potential to induce QT prolongation it may be possible to obtain a reliable point estimate of the risk of adverse cardiac reactions if the drug is carefully investigated in sufficiently large populations of carefully selected patients with known cardiovascular risks and for a sufficiently long period. Furthermore, physicians should avoid, whenever possible, prescribing drugs with the potential to adversely modify cardiac repolarisation to patients who have suspected or identified cardiac disorders. However, if such a therapy becomes necessary, the patient should be informed of the possible undesirable and serious effects of the medication. Finally, and perhaps most importantly, the decision to institute any type of drug therapy should always obey the classical golden rule of drug clinical development, prescription, and use – that the expected therapeutic benefit from a treatment must always outweigh the expected potential risk for an individual patient and, in general, for all members of population segments who may be exposed to the drug.

Acknowledgements

The authors wish to thank Drs William Crumb, Dominique Thuringer and Roger Small for their suggestions for improving the manuscript and Mme Evelyne Pasquet for the preparation of the manuscript.

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