In 1997, the Committee for Proprietary Medicinal Products (CPMP) issued a document concerning the potential of non-cardiovascular drugs to cause prolongation of the QT interval of the electrocardiogram. This article reviews several aspects of this complex problem, including a preclinical strategy (*in vitro* electrophysiology in human cardiac cells and *in vivo* pharmacologically validated conscious dogs) to satisfy the expectations of the CPMP. In particular, the discussion stresses the danger of drugs prolonging the QT interval in patients with concurrent cardiac risk factors and the need for rigorous clinical testing to determine the risk of fatal cardiac events for drugs with the propensity to prolong QT.

William Crumb
Zenas Technologies L.L.C.
5896 Fleur de Lis Drive
New Orleans
LA 70124
USA
tel: +1 504 584 2416
fax: +1 504 837 5546

Icilio Cavero
Department of Lead Discovery
Rhône-Poulenc Rorer
Centre de Recherche de Vitry-Alfortville
13 Quai J. Guesde
B.P. 14
F-94400 Vitry sur Seine
France

▼ In December 1997, the European Agency for Evaluation of Medicinal Products of the Committee for Proprietary Medicinal Products (CPMP) issued a statement (Note CPMP/986/96) entitled '*Points to Consider: The Assessment of the Potential for QT Interval Prolongation by Non-cardiovascular Medicinal Products*'[1]. As explained in the following section, the QT interval of the electrocardiogram (ECG) is a widely used measure of the ventricular repolarization process and its prolongation may be associated with a risk of sudden death.

The foundations of the CPMP document are based on a substantial number of serious cardiac events produced by a wide range of non-cardiovascular therapeutic agents that are not expected on the basis of their mechanism of action to prolong QT. Such agents belong to different pharmacological classes, such as psychotropic drugs (tricyclic-amitriptiline and tetracyclic antidepressants, phenothiazine derivatives, haloperidol, pimozide, risperidone and sertindole), prokinetic (cisapride), antimalarial n
fantrine, quinine and chloroqui
belonging to several che
(azithromycin, erythromycin,
spiramycin, pentamidine, trin
famethoxazole and sparfloxac
agents (ketoconazole, fuconazol
zole), an agent for treating urina
(terodiline), and certain histam
antagonists (astemizole, terfenadi
hydramine). These drugs, in cert
stances, can trigger life-threateni
ventricular tachycardias, such as
often in the presence of additiona
ing, directly or indirectly, proarr
The relevant factors include co
quired long-QT syndrome, isch
ease, congestive heart failure, se
renal dysfunction, bradycardia,
balance (hypokalemia due to diu
hypomagnesemia, hypocalcemi
intracellular Ca^{++} loading), inte
dental overdose, and concomitan
ion channel blocking drugs or ag
the drug detoxification processes

The CPMP guideline should b
be a strong signal sent by
Authorities to drug developers th
of QT prolongation by non-cardi
is now very significant and, thus,
ful scrutiny and research effort
pound undergoing future develo

In an attempt to offer an overv
tiple aspects of this complex an
problem, this article will briefly
pects of cardiac electrophysiolo
erogeneity in ion channels interv
repolarization, and congenital c

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The cardiac action potential is the pattern of electrical activity associated with excitable heart cells. It is the result of numerous, distinct, successively activated currents generated by the passage of biologically important ions (Na^+, Ca^{++} and K^+) through specialized membrane structures such as ionic pumps and exchangers and, most importantly, voltage-gated ion channels. These currents are considered to be depolarizing when they carry extracellular positive charges into the cell and to be repolarizing when they carry positive charges to the cell exterior[5].

The cardiac action-potential recorded from either an atrial or a ventricular human myocyte can be dissected into five distinct phases (Fig. 1). The phase 0, or action potential upstroke, is generated by the rapid, transient influx of Na^+ into myocytes via Na^+ channels (inward current: I_{Na}). Phase 2, or plateau of the action potential, is essentially because of the entry of extracellular Ca^{++} into the cells through L-type Ca^{++} channels (inward current: I_{Ca}). Phases 1 and 3 describe, respectively, the early and late repolarization process and are mediated by the efflux of K^+ from the cell through the opening of several distinct K^+ channels. The transient outward current (I_{to}) contributes to the termination of the upstroke of the action potential by causing an early phase of rapid repolarization (Phase 1), whereas several

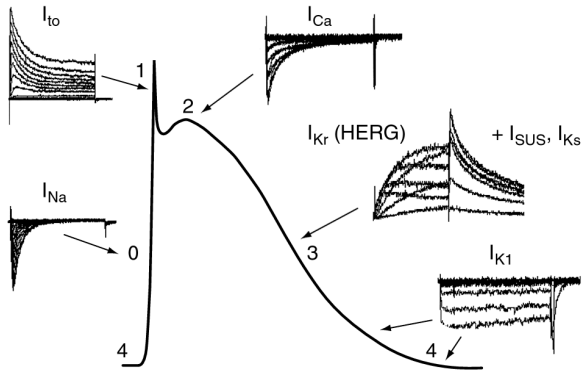

Figure 1. Example of an action potential recorded from a myocyte of human atrium and Na^+ (I_{Na}), Ca^{++} (I_{Ca}), K^+ (I_{Kr}, I_{sus}, I_{Ks} and I_{K1}) ion channel currents, which underlie each of its phases (0, 1, 2, 3 and 4).

Figure 2. Examples of action potentials recorded
pig, rat and human atrial myocytes. Note the dra
morphology and duration. Action potentials were
1°C using the whole-cell patch clamp technique.
were elicited by a 4 ms current pulse of 1.5–2 tir
level. The solution bathing the cell consisted of (i
NaCl, 4 KCl, 1 $MgCl^2$, 1.8 $CaCl^2$, 11 Glucose, 10 H
pH of 7.4 with NaOH. Glass pipettes were filled v
solution that consisted of (in mmol L^{-1}): 120 K-a
4 Na-ATP, 5 EGTA, 5 HEPES; adjusted to a pH of 7

distinct K^+ channels contribute to Phase 3
Human Ether-a-go-go Related Gene (HEI
I_{Ks}] by opposing Ca^{++} influx during the pl

Finally, the inward rectifier (I_{K1}), thoug
for maintaining resting potential (Phase 4
nent role in the final repolarization proc
although to a lesser extent in the human h
cardiac myocyte at its resting potential (Fi

All of the currents described in humar
been shown to be present in human atrium
tribution and amplitudes are a tissue-speci
sults imply that human atrial myocytes, a
section, could be used for determining the
ological safety of novel drug candidates b
tissue can be obtained from virtually norm

The shape and duration of the cardiac act
tures specific to each animal species (Fig. 2
differences in type, structure, cellular dist
contribution to the generation of the cardia
the transmembrane-current through the va

Species heterogeneity in cardiac ion chann

K^+ channels represent the class of chann
species-dependent heterogeneity. Differe
identity and pharmacology of cardiac ion c
the results obtained from tissue derived fro
mals may not adequately predict drug effect
cardium. Extrapolation of such data to hu
great caution and may not always be valid

Loratadine	Antihistamine	No change K^+ currents (Ref. 33)	?	No change K^+ currents (Refs 33, 35, 36)	Decreased HERG current (Ref. 34)	Arrl
Dofetilide	Antiarrythmic	Decrease I_{Kr} (Ref. 37), increase APD (Ref. 38)		No change APD (Ref. 39)	Decreased HERG current (Ref. 15)	Arrl

Transient outward current

This current (I_{to}) responsible for Phase 1 of the action potential is present in cardiac myocytes of several species, including rat, dog, cat and man. However, this current is not present in guinea pig myocytes. In addition to species differences in the expression of I_{to}, there are also differences in the molecular identity of I_{to} in those species that do possess it. For example, rabbit heart I_{to} is most likely the protein product of the Kv1.4 gene, whereas that of the rat heart appears to be encoded by the Kv4.2 gene and possibly Kv4.3 gene[6–9]. In contrast, human heart I_{to} is believed to predominantly be the product of the Kv4.3 gene[8].

The importance of I_{to} in the normal electrical activity of the heart is illustrated by the fact that the blockade of this channel by tedisamil, an I_{to} blocker, can result in changes in cardiac-action potential duration[10]. Furthermore, in a canine model of ventricular arrhythmias, a reduction in I_{to} amplitude is believed to be an underlying arrythmogenic factor[11].

Sustained current

This current is referred to as the sustained (I_{SUS}) or pedestal current. In the rat heart, I_{SUS} is entirely due to $I_{Kv1.5}$, a current highly sensitive to blockade by 4-aminopyridine[12]. However, this 4-aminopyridine-sensitive current has not yet been described in guinea pig or dog heart and, therefore, it cannot account for the I_{SUS} observed in these species. $I_{Kv1.5}$ partly mediates the human atrium I_{SUS}, with the remaining portion being due to a novel, specific, non-selective cation channel[13]. This channel also appears to be responsible for I_{SUS} in the human ventricle, where no $K_{v1.5}$-like current can be recorded[14].

Delayed rectifier

The rapid component of the delayed rectifier K^+ current (I_{Kr}) has been the topic of much research, because of its involvement in both congenital and acquired forms of long (LQTS). Electrophysiological studies performe protein product of the gene believed to be respo human heart indicate dramatic interspecies di channel. For instance, the Class III antiarrhythi 100-times more potent in blocking HERG th Bovine Ether-A-go-Go (BEAG), the channel be sponsible for I_{Kr} in bovine[15]. This remarkable dif sult of a single-point mutation occurring in the channel. Thus, very subtle changes in the protei stituting a channel can dramatically affect ionic-c cology. Mutations in both of the proteins (KVL that are believed to co-assemble and form the slo the delayed rectifier, I_{Ks}, have also been reported role in congenital and acquired forms of L However, a recent study questions whether IKs p role in the repolarization of the human cardiac-a

Possible cardiac adverse-effects of drugs modu cardiac ion channels

Drugs that modify the normal flux of ions th may modify certain aspects of the action pote affect cardiac function. Therefore, blockers of N duce the rate of rise of the action potential (produce disturbances in cardiac conduction, v may be life-threatening. Drugs that decrease the rent inactivation and increase residual Na-curr the duration of the action potential (ADP), pr val and thus may trigger *torsades de pointes* arrhyt of Ca^{++} channels decrease ADP, reduce the rate tion and produce cardiac depression, whereas activators prolong ADP and may cause arrhythi channel-blockers prolong ADP and QT (Fig. 3)

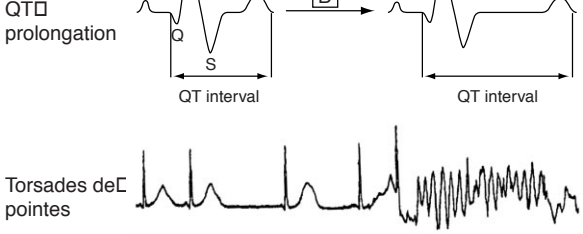

Figure 3. Simplified diagram showing the effect produced by a drug blocking cardiac K^+ channel (B) on the duration of the action potential duration and on electrocardiogram (ECG). This drug can produce prolongation of the unicellular action potential followed by early post-depolarization, QT interval prolongation and *torsades de pointes*.

arrhythmias, whereas K^+ channel-activators shorten ADP and can also trigger arrhythmia. It should be noted that Na^+, K^+ and Ca^{++} channel-blockers can also be useful antiarrhythmics in patients with existing arrhythmias.

QT-interval of the electrocardiogram

The electrical activity of the whole heart is reflected in the ECG. The wave sequence comprising the ECG-trace during a normal cardiac cycle results from the sum of the elementary electrical activities of each excitable cell in the heart chambers (Fig. 3).

QT-interval duration represents the sum of both ventricular depolarization (QRS interval) and ventricular repolarization (QT minus QRS). However, QT prolongations very rarely result from widening of the QRS complex. The QT segment of the ECG itself or its heart rate-corrected form (QTc), according to the formulae of Bazzett ($QTcB = QT/\sqrt{RR}$) or Fridericia ($QTcF = QT/\sqrt[3]{RR}$), are clinically used indices of the cardiac repolarization process. Its value is influenced by several factors, such as heart rate (the correction formula by Bazzett is relatively inaccurate because it under- or over-estimates the true duration of repolarization at low and high rates, respectively), extent of the sympathetic and parasympathetic drive to the heart, the ECG-lead selected to measure this parameter and even the person performing the manual measurement of the QT. For this reason, the section of the CPMP QT guideline dealing with

Long QT syndrome, a genetic disease

Long QT syndrome is a clinically heterogen
ders of cardiac repolarization, which may r
a drug or from a pathological condition w
genetic basis. The essential electophysiolog
derlying this condition is a reduction in th
outward-current responsible for the repola
can result from either delayed inactivation
current or a decrease in the current carried
channels (gain and loss of function mutati
congenital LQTS patients). These patholo
channel function lead to a delay in the rep
which can trigger the development of early
larizations (particularly at the level of the
system) followed by episodes of *torsades de po*

Studies utilizing genetic analysis and m
techniques have identified mutations in gen
that form ion channels in individuals affli
LQTS. Recently, three ion channel encodi
HERG and SCN5A) have been found to be
mutations, which produce unfavourable cha
of the encoded channel protein[16,18]. Hen
with such a mutated α-subunit (SCN5A)
enhanced time-dependent residual curren
equivalent current carried by the wild-t
responsible for the prolongation in action p

Several mutations in the *HERG* gene that
unit of the K^+ channel carrying the rapi
delayed rectifier (I_{Kr}) have also been descri
also been identified in the KvLQT1 α-subu
with the minK β-subunit to form the K^+
slow component of the delayed rectifier
tations have also been reported in the mi
mutations are associated either with a red
tude of a repolarizing K^+ current or with
ing or non-functional channels. The phen
of such alterations is generally a prolongatio
duration accompanied by a particular susc

cular safety pharmacology studies are required to be rigorous, and to use a range of escalating doses. Furthermore, they should include measurements (typically in the dog) of heart rate, blood pressure and ECG analysis. In addition, before first-use in humans it is also recommended that an *in vitro* electrophysiological study be performed using a suitable cardiac preparation and physiologically relevant conditions. In fact, *in vitro* Purkinje fibers or papillary muscles taken from the myocardium of an established laboratory animal species (such as rabbit, guinea pig, dog or pig) are considered suitable, because it is believed the major ionic currents underlying their action potentials resemble those contributing to the repolarization process of the human heart.

In addition, these studies should be extended to inspection for a reverse rate-dependency phenomenon if the compound under study is found to prolong the action potential duration. The concentrations of the drug to be tested are expected to cover and well exceed (in our opinion, 10–30-fold) the anticipated maximal therapeutic plasma concentrations of the drug candidate. Furthermore, these studies should take into consideration certain aspects of the drug's pharmacokinetics, such as the existence of major active metabolites. The effect of the drug on the action potential prolongation at 90% of repolarization (ADP_{90}), on possible early post-depolarization events and subsequent triggered activities are considered of primary relevance in the context of a proarrhythmic potential accompanying the prolongation of QT interval. Additional parameters to be measured are ADP_{30}, ADP_{60}, membrane resting potential, action potential amplitude and upstroke velocity (Vmax), because they can provide additional information on the cardiac electrophyisological safety of the compound.

If the results of all these studies indicate that the novel agent does not prolong the QT interval in an unacceptable manner, then the drug candidate can be cleared for safety assessment studies in healthy volunteers provided all other normal safety requirements are met[1].

The preclinical *in vitro* electrophysiologic approach proposed by the CPMP guideline and as outlined above is a classical one. However, it may not be the best one available, because

electrophysiological effects of a drug candid

The *in vitro* electrophysiologic profile of candida
be performed whenever possible on ion chan
the electrical activity of the human heart myoc
I_{sus}, I_{K1} and I_{kr} (HERG)]. If recording a particula
tive human heart cells (I_{Kr}) is difficult, then th
channel (HERG) expressed stably in a human c
HEK cells) is a suitable alternative. In addition,
tal conditions (temperature, holding potential
tions) for these studies should be as close as p
existing on a physiological level. The concen
should cover a 2–3 log unit range, with the l
tration studied being at least 10- to 30-fold
anticipated plasma or tissue concentration n
therapeutic activity. Results obtained for the co
study using this assay should then be compar
compounds known to block a particular io
which are clinically associated with arrhythmia
if a concentration of the drug under study has
ion channel, it is essential to determine the po
of a rate-dependency relationship for this effec

The proposed departure from the classical stu
potential profile on ventricular preparations fr
experimental animals is supported by the fact t
are ultimately responsible for any drug-induce
action potential pattern. Although ion pumps
do contribute to the morphology of the action
ally every drug that has been associated with ar
in man has been found to affect one or more
ion channels. Finally, the extent of the effects
compound on the whole action potential prof
on the morphology of the action potential at
study, making interpretation of such experimen

The main objection against any novel approa
safety is whether the proposed tests are suffic
to reveal, at least as well as established method
verse effect of a compound. Figure 5 illustrate
the ion channel-blocking effects of three clinic
terfenadine, haloperidol, and cisapride, wh

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