Critical Review

Arrhythmia Models for Drug Research: Classification of Antiarrhythmic Drugs

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Abstract. The aim of this study was to classify antiarrhythmic drugs based on their effectiveness on 6 in vivo arrhythmia models, mainly using dogs. The models were produced by two-stage coronary ligation, digitalis, halothane-adrenaline, programmed electrical stimulation in old myocardial infarction dogs, coronary artery occlusion/reperfusion, or chronic atrioventricular block. Na⁺-channel-blocking drugs suppressed two-stage coronary ligation and digitalis arrhythmias. Ca²⁺-channel blockers and β-blockers suppressed halothane-adrenaline arrhythmia. Positive inotropic drugs aggravated halothane-adrenaline arrhythmia, but did not aggravate digitalis arrhythmia. K⁺-channel blockers suppressed programmed electrical stimulation induced arrhythmia, but induced torsades de pointes type arrhythmia in chronic atrioventricular block dogs and aggravated halothane-adrenaline arrhythmia. Na⁺/H⁺-exchange blockers suppressed coronary artery occlusion/reperfusion arrhythmias. This classification may be useful for predicting the clinical effectiveness in the preclinical stage of drug development.

Keywords: arrhythmia model, antiarrhythmic drug, proarrhythmic potential of drugs, ion channel, classification

1. Introduction

Currently, interest in arrhythmia treatment is turning towards new devices and ablation techniques; and it is, of course, certain that a failed sinus or Tawara’s (AV) node should be replaced by appropriate electronic pacemakers and that cardiac collapse by ventricular fibrillation (VF) or potentially lethal torsades de pointes (TdP) should be stopped by electrical external or implantable defibrillators. To implant pacemakers, only evidence of malfunction of pacemaking and atrioventricular conduction is necessary. To install a defibrillator, the genetic preponderance of the occurrence of these arrhythmias, or evidence of existence of ventricular arrhythmia types likely to develop into fatal sudden death or other precipitating factors should be known. Knowledge of pacemaking or precise mechanisms of generation of arrhythmias in cellular and molecular levels is no longer necessary. Also for applying the ablation technique to eliminate substrates of arrhythmias, only the proper area, automatic foci, or a part or the entire re-entry circuit need to be known. But what about pharmacological treatments? Is there no room for developing new antiarrhythmic drugs? As Christ and Ravens discussed in her review (1), the relative importance of antiarrhythmic drugs might have decreased, but they still are relied on for prevention of atrial fibrillation and sudden cardiac death by cardiologists world wide. More importantly, for developing antiarrhythmic drugs, it has been necessary to know the precise mechanisms of the generation of action potentials, the different forms of action potentials, and the abnormalities of normal action potentials that are responsible for development into arrhythmias, and so on. Thus introduction of newer drugs or understanding of drug actions developed in parallel with the advances in the knowledge about cardiac electrophysiology. Electrical mechanisms of the heart are generated by complex, multi-factorial phenomena simultaneously occurring with channel- or receptor-related events. Molecular research aims to elucidate the single function of a single molecule and the action of drugs on ion channels, transporters, or extracellular or intracellular receptors, but
how these drugs work on arrhythmias is only known from experimental or clinical arrhythmias. After the CAST study (2) revealed the possible proarrhythmic effects of Na\(^+\)-channel–blocking antiarrhythmic drugs, pharmacologists and cardiologists tried to make a new classification or guide for choosing proper drugs to treat clinical arrhythmias, namely the Sicilian Gambit, by defining drug actions on cardiac tissues based on newer molecular aspects of cardiac excitation and classifying clinical arrhythmias to correlate or match the drugs and the arrhythmias (3). However since the molecular mechanisms of arrhythmias are still not well known, and also comparative effectiveness of drugs on clinical arrhythmias are not fully known, we have been examining drugs on various experimental animal arrhythmias models. It is obvious that the present antiarrhythmic drugs are not ideal ones, and also for many different arrhythmias, many different types of drugs are necessary. Drugs are still used to maintain sinus rhythm after conversion from atrial defibrillation, to stop the transition to VF clinically, and to target pathophysiological alterations to prevent the occurrence of lethal arrhythmias. It may be worthwhile to review our studies and try to classify drugs by their effectiveness on arrhythmias of dogs, rats, guinea pigs, and so on. Our previous results on classical and investigational antiarrhythmic drugs and our recent results on cardioprotective drugs have been summarized here.

Antiarrhythmic drugs have been classified by Vaughan Williams mainly based on their effects on cardiac action potentials into classes I to IV and later correlated to their effects on Na\(^+\) channel, \(\beta\)-receptors, and K\(^+\) and Ca\(^{2+}\) channels (4). Here our animal experimental arrhythmia models, both the automaticity induced and re-entry induced arrhythmias are presented, which we used to quantitatively compare drug effects on the arrhythmias. There have been many studies of antiarrhythmic drug efficacies using animal arrhythmias, but most of them showed the effectiveness of a single drug or made comparisons of that drug with one or two standard drugs. To make a preclinical prediction of the usefulness of the drug, comparisons among many drugs were almost impossible. We used standard and reproducible models that can occur clinically and tried to study the antiarrhythmic drugs’ effects by comparing them quantitatively and qualitatively to find common pharmacological properties and predict antiarrhythmic mechanisms of action of drugs. We have already written several summary review papers (5–8), but in this review, we have added new data obtained in recent years to compare a wide range of drugs.

2. Drug effects on arrhythmia models

2-1. Canine two-stage coronary ligation arrhythmia

2-1-1. Characteristics

Ischemia, being a serious disorder of the myocardium, causes pump and electrical failure; thus it is used often to produce experimental arrhythmias. Ischemia is a time-dependent phenomenon and starts with reversible damage finally reaching the stage of irreversible damage or scar formation; thus it is no wonder that ischemia related arrhythmias also show a variety of electrical abnormalities. The two-stage coronary ligation technique was originally reported by Harris (9) and is characterized by consistent and stable subacute to chronic ischemic arrhythmias, showing severe ventricular tachycardia (VT), but never deteriorating to VF. This arrhythmia is thought to be an automaticity arrhythmia because overdriving the atria or ventricle, by electrical stimulation at rates higher than the rate of VT, suppressed the VT to conducted sinus rhythm (5).

2-1-2. Methods

“Two-stage” means that the first stage is the start of 30 min of ischemia, followed by the second stage of a permanent occlusion of the left anterior descending coronary artery (LAD) of the dog. In the first stage, isolated LAD soon after bifurcation with the left circumflex coronary artery (LCX) was surgically isolated from the neighboring coronary veins or connective tissues and then tied completely with one of double threads together with a needle of about 1 mm outer diameter, which was immediately withdrawn to make the needle size patency to produce a state of ischemia, but not result in complete occlusion. This usually produces ischemic changes in the electrocardiogram (ECG) and produces only premature ventricular contractions (PVC) lasting for a few min followed by a quiescent 30 min, and then another suture is used to completely occlude the LAD. After the final ligature, the thoracotomized chest is closed, and the animal is left to recover from the anesthesia for use 24 or 48 h after coronary ligation. Though cardiac surgery under anesthesia in a sterile environment is needed, and the technique of LAD isolation is not always easy, very stable arrhythmias, PVC and VT (defined by more than 3 continuous PVC), can be obtained which usually appear several hours later, and the VT becomes fulminating 24 h later in a conscious state, lasting up to 48 h; thus this arrhythmia can be used to evaluate and compare drug effects. The arrhythmias are continuous, and at the peak of about 24 h after ligation, the PVC consists of almost all the beats. To judge whether the beats are sinus beat or PVC, atrial electrodes were sutured on the epicardial surface of the right atrial appendage during
the operation, and ECG and atrial electrogram were simultaneously obtained. Preferably a His electrogram must be obtained to judge whether a beat is of a conducted sinus rhythm or not, but fixing intracardiac His electrodes usable in a conscious state is quite difficult and invasive, so we chose the alternate atrial electrogram recordings. We used the arrhythmic ratio for expressing the severity of ventricular arrhythmias by the number of PVC over a certain time, in our case usually 10 sec, divided by the total number of beats including the PVC and the normal sinus beats. This arrhythmic ratio cannot differentiate the most severe VF from severe VT, but as drugs are used usually before VF occurs, it is a good measure to express the severity of arrhythmia for quantitative comparison. We found that 24 h after LAD ligation, the arrhythmic ratio of the VT is almost 1.0 in the two-stage coronary ligation arrhythmia model, and we calculated the effective plasma concentration of a drug to decrease the control arrhythmic ratio to 50% from the arrhythmic ratio-drug plasma concentration curve (EC$_{50}$). For the drug plasma concentration determinations, intra-arterial or intravenous catheters were introduced, and the blood samples were drawn without the conscious animal being aware of the procedure. Another advantage of this arrhythmia model is that the drugs are administered in a conscious state, so that it is easy to evaluate the antiarrhythmic efficacy of drugs while simultaneously evaluating their central nervous system (CNS) side effects. Thus intravenous lidocaine was not effective without CNS stimulant effects, even though there has been a widespread belief that lidocaine is the most effective drug in the clinical setting of acute ischemic arrhythmias (5, 10). We gave drugs either by an intravenous or oral route. We tested many Na$^+$-channel–blocking drugs and other drugs. When a drug was effective, the time-dependent drug plasma concentration curve, either after a bolus intravenous injection or the curve at the rising phase after a constant infusion, gave almost the same effective plasma concentration values; namely, there was almost no hysteresis in the antiarrhythmic effect–EC$_{50}$ relationships (5).

This two-stage coronary ligation arrhythmia was stable and the success rate to obtain arrhythmias for drug evaluation is high, nearly 90%, in dogs. Pigs are prone to develop VF after coronary ischemia, and smaller rats and mice do not show such stable VT. Guinea pigs never develop ischemia by in vivo coronary ligation (11). It may still be the one of the best models for a new Na$^+$-channel–blocking antiarrhythmic drug to be tested preclinically for further development.

2-1-3. Antiarrhythmic drugs

Drug effects can be summarized as follows: Na$^+$-channel–blocking drugs suppress this two-stage coronary ligation arrhythmias and the effective plasma concentrations are closely related to the intraarterial doses to suppress intraventricular conduction (12), suggesting that they suppress arrhythmia through a mechanism involving Na$^+$-channel block (5, 10, 12 – 40). Figure 1 shows the correlation between the reported Na$^+$-channel–blocking concentration in in vitro experiments mainly obtained in the isolated canine cardiac ventricular muscle preparations and their effective plasma concentrations (EC$_{50}$) for suppressing the two-stage coronary ligation arrhythmia models of the effective 29 drugs; earlier data (before 1991) of Na$^+$-channel–blocking concentration were those listed in Hashimoto et al. (5), those after 1991 are from the respective paper (12, 34, 35), and data on disopyramide isomers were those of Vanhoutte et al. (36). These canine effective plasma concentrations thus determined were also shown to be very close to their clinical effective concentrations (5, 12, 34 – 37), thus from this series of experiments, we could predict clinically effective drug plasma concentration levels before these drugs went to clinical trials. Though Na$^+$-channel blockers are now classified according to their mode and kinetics of Na$^+$-channel inhibition, our conclusion was that any drug that blocks Na$^+$ channels can suppress this two-stage coronary ligation arrhythmia, but by using this arrhythmia model, we could demonstrate the interaction between two Na$^+$-channel blockers with different binding affinities (41) used in combination in an in vitro preparation (42). In the study, the actions of mexiletine and disopyramide were additive, but those of mexiletine and aprindine were not. As shown in Table 1, TJN-505 is a chemically aventadine-like drug acting on Na$^+$ channels and was effective on the two-stage coronary ligation arrhythmia, but there is no data on this drug as a Na$^+$-channel–blocking drug in the in vitro cardiac tissue (38). HNS-32 is also such a drug with Na$^+$-channel–blocking activity and was shown to be effective on this arrhythmia (39). Unlike most of the Ca$^{2+}$-channel blockers, AH-1058, suppressed two-stage coronary ligation arrhythmia possibly by inhibiting Ca$^{2+}$ overload (43).

2-1-4. Ineffective and proarrhythmic drugs

Two-stage coronary ligation arrhythmias differ from acute coronary occlusion/reperfusion arrhythmias, described later, in that they almost never turn into VF or result in animal deaths, and there were no drugs aggravating the two-stage coronary ligation VT to VF. Among classical antiarrhythmic drugs, the Ca$^{2+}$-channel–blocking drugs verapamil (10) and gallopamil (44) did not suppress these arrhythmias and also β-blockers were not effective unless high doses to produce Na$^+$-channel–
blocking effects were given (10, 13, 14, 17, 19, 45). Pure
K\(^+\)-channel–blocking drugs that we tested did not
suppress these arrhythmias (6, 46 – 49), but amiodarone
given intravenously suppressed the two-stage coronary
ligation arrhythmia, but the plasma concentration was
not determined in this experiment (50). Since there
was no prolongation of QT interval by intravenous
amiodarone injection, this antiarrhythmic effect was
thought to be due to amiodarone’s Na\(^+\)-channel–block-
ing action (51). Positive inotropic drugs, catechola-
mines, phosphodiesterase (PDE) inhibitors (52 – 54), the
forskolin derivative, colforsin daropate (55) or the Ca
\(^{2+}\)-sensitizer, MCI-154 (56), neither showed antiarrhythmic
effects nor aggravated the arrhythmia, unless used in
extreme doses. Zatebrazine, an I\(_f\)-blocking specific
negative chronotropic agent, did not suppress this
arrhythmia (57). As stated above, this arrhythmia was
suppressed by overdriving, so isoproterenol given at
1 – 3 µg/kg produced sinus tachycardia and transiently
suppressed this VT (our unpublished observation). A
cardioselective M\(_2\)-receptor blocker, AF-DX 116, was
also examined on this arrhythmia, but it had no effect
(58).

2-2. Digitalis-induced ventricular arrhythmias
2-2-1. Characteristics
The cardiotonic steroid digitalis is known to be
arrhythmogenic and its effect can be explained by intra-
cellular Ca\(^{2+}\) overload due 1) to intracellular Na\(^+\)
accumulation by Na\(^+\)-K\(^+\)-ATPase inhibition, followed by
2) intracellular Ca\(^{2+}\) accumulation by enhanced Na\(^+\)/Ca\(^{2+}\)
exchange (5, 59, 60). Ca\(^{2+}\) overload, thus produced,
can induce abnormal automaticity or delayed after-
depolarization-induced arrhythmia or cause re-entry
arrhythmia and conduction block by uncoupling the cell-
to-cell connections and/or stimulating vagal influence
on the AV nodal conduction (60). However the precise
mechanism of Ca\(^{2+}\) overload by the Na\(^+\)/Ca\(^{2+}\) exchanger
and the resultant increase in the inward current, leading
to the development of delayed afterdepolarization is
still not known, and emergence of new chemicals to
selectively inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger may solve the
problem and may also be useful for clinical digitalis
toxicity arrhythmias. The automaticity mechanism can
be demonstrated by the overdrive suppression of this
arrhythmia (5).
Two-stage coronary ligation arrhythmia

I: A-2545, AFD-19, AFD-21 (NS-2), AHR 10718, AN-132, aprindine, AP-792, bidisomide, bisaramil, cibenzoline, disopyramide, l-disopyramide, etacizin, E-0747, flecainide, HNS-32, KT-362, mexiletine, nicainoprol, OPC88117, penticainide, phenytoin, pirmenol, propafonene, SD-3212, tocaainde, NS-2, procainamid, pilcicainide, TJN-505, TYB-3823, YUTAC
II: pindolol, N-696
III: amiodarone (i.v.)
M: dilazep

Digitalis arrhythmia

I: A-2545, AFD-19, AFD-21 (NS-2), AHR 10718, AN-132, APR-792, aprindine, bidisomide, bisaramil, cibenzoline, disopyramide, E-0747, etacizin, flecainide, HNS-32, KT-362, mexiletine, nicainoprol, OPC88117, penticainide, phenytoin, pirmenol, propafonene, SD-3212, tocaainde, NS-2, procainamid, pilcicainide, TJN-505, TYB-3823, YUTAC
II: atenolol, carvedilol, pindolol, N-696
III: amiodarone (i.v.)
M: dilazep

Halothane-adrenaline arrhythmia

II: atenolol, betaxolol, Ko 1400, oxprenolol, pindolol, propranolol
IV: AH-1058, AP-792, diltiazem, gallopamil, nifedipine, nisoldipine, verapamil
III: amiodarone (i.v.)
I: AFD-19, aprindine, cibenzoline, E-0747, flecainide, KT-362, mexiletine, nicainoprol, OPC88117, penticainide, phenytoin, pirmenol, propafonene, SD-3212, TJN-505, TYB-3823, YUTAC
M: dilazep, NCO-700, nitroglycerin, trapidil, trimetazidine, l-carnitine

PES-induced arrhythmia

III: azimilide, dofetilide, KCB-328, nifekalant, sematilide, tocaainde
I: A-2545, aprindine, disopyramide

Occlusion/reperfusion arrhythmia

Halothane-anesthetized dogs
III: E-4031, nifekalant
Pentobarbital-anesthetized dogs
III: KCB-328, nifekalant, d-sotalol
M: cariporide, KB-R9032
Rats
M: cariporide, FR 186666, KB-R9032, DIDS, pravastatin

Halothane-anesthetized dogs
III: amiodarone (i.v., p.o.), dofetilide, KCB-328, sematilide, d-sotalol
Pentobarbital-anesthetized dogs
III: dofetilide, sematilide
M: KB-R7943, SEA0400, pravastatin
Rats
M: SITS, fluvastatin

M: pacing-induced heart failure

Chronic AV block dog (Tdp induction)
I: Na\(^+\)-channel–blocking drugs; NIK-244 = etacizin, ME3202 (CM7857) = penticainide, SUN 1165 = pilcicainide. II: \(\beta\)-blocking drugs. III: K\(^+\)-channel–blocking drugs; MS-551 = nifekalant. IV: Ca\(^2+\)-channel–blocking drugs. M: Miscellaneous drugs with different mechanisms of action; AR-L115 = sulmazole, OPC8212 = vesnarinone, HOE642 = cariporide, NKI477 = colforsin daropate.

2-2-2. Methods

Digitalis can induce toxicity characterized by VT, VF, and death both by intermittent intravenous bolus injection or continuous intravenous infusion in various animals except in rats, which are known to need thousands of times higher doses of digitalis than man and other animals (61). Test drugs are usually administered before digitalis administration by intravenous bolus
injection, and a simple test to record the occurrence of PVC, VT, and VF using small animals, especially the guinea pig, is often used for screening possible antiarrhythmic effects of drugs, but precise quantitative data are difficult to obtain from these experiments. We used dogs, and they were anesthetized with intravenous pentobarbital, and then intravenous catheter atrial electrodes were introduced from the femoral vein (15, 62). Cumulative administration of digitalis, initially 40 µg/kg intravenous ouabain followed by a 10 µg/kg every 20 min were administered to produce continuously occurring VT, lasting more than 1 h, and then the test drugs were administered and the blood to determine drug plasma concentrations were drawn. As the drug concentration decreased, VT reappeared after effective drug administration, thus we could determine the canine effective plasma concentrations of drugs (5, 15).

2-2-3. Antiarrhythmic drugs

Drugs effective on our digitalis arrhythmias are seen in Figure 2 with plasma EC50 values and in vitro Na+-channel–blocking concentrations. The pattern of drug effects are similar to that obtained using the two-stage coronary ligation arrhythmias, namely, that the Na+-channel–blocking drugs suppressed these digitalis arrhythmias and the effective plasma concentrations are closely related to their in vitro Na+-channel–blocking concentrations (5, 15, 16, 18 – 40). Zatebradine did not suppress the two-stage coronary ligation arrhythmia, but digitalis arrhythmia was suppressed by a high dose of intravenous 1.5 mg/kg bolus injection (57). The mechanism of this effect might be either due to Ic block or other channel effects, but there was no supporting data of plasma concentration determination and also there were no other Ic blockers available for our study. Other drugs effective on digitalis arrhythmias without Na+-channel–blocking effect, but for which the exact mechanisms of action are unknown, are intravenous magnesium sulfate (63), a possible Ca2+-channel blocker, and the Na+/Ca2+-exchange inhibitor SEA0400, reported to be relatively selective when suppressing inward Ca2+ movement (reverse mode) (64). Also positive inotropic drugs with prominent tachycardic action, amrinone, colforsin daropate, and sulmazole, suppressed this arrhythmia probably by overdriving the increased automaticity (53, 55).

2-2-4. Ineffective and proarrhythmic drugs

Ca2+-channel–blocking drugs did not suppress this arrhythmia (44, 65 – 67) even though the mechanism of generation is thought to be increased intracellular Ca2+ concentration. The exception was magnesium sulfate,
which acts as a Ca\textsuperscript{2+}-channel blocker in vivo, and which suppressed digitalis arrhythmia (63). As for the ineffectiveness of Ca\textsuperscript{2+}-channel blockers, its hypotensive effect might have made it impossible to raise the doses high enough to decrease influx of Ca\textsuperscript{2+} to the myocardium. We therefore tried intracoronary administration of a high-dose verapamil in digitalis-induced VT, and as a result, rapid pacing-induced and probably DAD-induced PVC was suppressed by verapamil (68). In this model, Na\textsuperscript+-channel blockers were tested, including bisaramil, disopyramide, lidocaine, and flecaïnide, which also suppressed this DAD-induced PVC. β-Blockers were not effective unless high doses to suppress Na\textsuperscript{+} channels were given (17, 19, 45, 69). K\textsuperscript+-channel–blocking drugs did not suppress this arrhythmia (6, 46 – 49), but intravenous amiodarone, probably by its Na\textsuperscript{+}-channel–blocking effect, suppressed this digitalis arrhythmia (50). Also non-Ca\textsuperscript{2+}-channel–blocking coronary vasodilators such as bepridil, nicorandil, nitroglycerin, trimetazidine (65 – 67), the Na\textsuperscript{+}/H\textsuperscript{+}-exchange inhibitor cariporide (70), and the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchange inhibitor KB-R7943, which has multichannel effects and is not selective concerning reverse mode Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange as compared to SEA0400 (71), were not effective on the digitalis arrhythmia.

As for proarrhythmic drugs, adding more digitalis changed the VT to lethal VF, so catecholamines must theoretically aggravate digitalis-induced arrhythmia because they add intracellular Ca\textsuperscript{2+} by opening Ca\textsuperscript{2+} channels via β-receptor stimulation; thus, we did not systematically examine catecholamines. However we examined PDE-inhibiting inotropic drugs in order to obtain safety pharmacology data taking into consideration the possible combined use of different types of positive inotropic drugs. Milrinone, and vesnarinone increased intracellular cyclic AMP concentration and increased intracellular Ca\textsuperscript{2+} concentration, but actually had no aggravating effects (53). As compared to amrinone and sulmazole, milrinone and vesnarinone may induce less tachycardia and might not have reached a level of atrial rate sufficient to overdrive digitalis-induced ventricular automaticity. Also MCI-154, a positive inotropic drug having Ca\textsuperscript{2+} sensitizing and PDE inhibiting action, did not aggravate digitalis arrhythmia (56).

2-3. Halothane-adrenaline arrhythmia

2-3-1. Characteristics

Another automaticity arrhythmia is the classical catecholamine-induced arrhythmia. Normal pacemaker activity of the heart is under the control of sympathetic and vagal influences and sympathetic stimulation and sympathomimetic drugs are known to increase pace-
2-3-4. Ineffective and proarrhythmic drugs

Some of the Na\(^{+}\)-channel blockers (5), such as AHR 10718, AN-132, and tocainide, required higher concentrations than needed to suppress two-stage coronary ligation and digitalis arrhythmias; and since those concentrations are not thought to be attainable in clinical use, those drugs could be said to be ineffective. Furthermore, some Na\(^{+}\)-channel blockers even aggravated the adrenaline-induced VT to VF, namely, worsened this arrhythmia. They include commonly used Na\(^{+}\)-channel blockers (5), such as disopyramide, etacizin, pilscaine, and procainamide (29). K\(^{-}\)-channel blockers, azimilide (75), dofetilide (48), E-4031 (46), KCB-328 (49), nifekalant (76), and sematilide (47), did not suppress, but instead aggregated this arrhythmia (6, 76). To demonstrate K\(^{-}\)-channel blockers' proarrhythmic effect, we added another adrenaline arrhythmia experiment (77, 78). We constructed a dose-response relationship by administering bolus intra-arrhythmia experiment (77, 78). We constructed a dose-response relationship by administering bolus intra-

2-4. Programmed electrical stimulation induced re-entry arrhythmia in old myocardial infarction dogs

2-4-1. Characteristics

This arrhythmia is produced in dogs given surgery to produce myocardial infarction by two-stage coronary ligation more than a week before, when there was no spontaneous PVC (47). Since arrhythmias were induced only by applying premature extrastimuli, the mechanism of generation is thought to be re-entry around the scar tissue of the old infarction. Drug effects can be demonstrated by the change of the severity of induced arrhythmias before and after the drug administration. The rank of arrhythmias was used to evaluate drug effects, and the order by their severity was VF as the most severe, followed by sustained VT, non-sustained VT, PVC, and no arrhythmia.

2-4-2. Methods

Dogs with old myocardial infarction were anesthetized with pentobarbital and after reopening the thoracic incision, epicardial stimulating bipolar electrodes were sutured on the non-infarcted left ventricle, and up to trains of 3 premature extrastimuli were applied to induce non-sustained VT (lasting less than 30 s), sustained VT (lasting more than 30 s), or VF (47). If sustained VT or VF occurred, epicardial defibrillation was performed to stop them. After 2 control runs to reproduce re-entry arrhythmias, an intravenous bolus injection of a drug was given and then the same protocol of arrhythmia induction was applied.

2-4-3. Antiarrhythmic drugs

Consistent effectiveness was observed for K\(^{-}\)-channel blockers, azimilide (75), dofetilide (48), KCB-328 (49), nifekalant (80), and sematilide (47), with simultaneous QT prolongation in ECG (6). Usually the control arrhythmias were set up as severe ones by applying 3 premature extrastimuli. K\(^{-}\)-channel blockers decreased the severity of arrhythmias to PVC or not inducible (6). For this arrhythmia, the procedure to induce arrhythmias took some time, 2 – 3 min, so it was difficult to determine the effective plasma concentration for each drug so only quantitative analysis was made by comparing the intravenous doses. Besides K\(^{-}\)-channel blockers, several Na\(^{-}\)-channel blockers, A-2545 (37), aprindine, and disopyramide (81), have also been reported to be effective.

2-4-4. Ineffective and proarrhythmic drugs

After demonstration by CAST of proarrhythmic potentials of Na\(^{-}\)-channel blockers (2), this arrhythmia model was used to demonstrate the potentials of these side effects. The worst reputed drug flecainide has been.

adrenaline arrhythmia (65). Many other drugs also suppressed adrenaline arrhythmia by undetermined mechanisms whether due to additional Ca\(^{2+}\)-channel– or \(\beta\)-receptor–blocking action or due to other mechanisms. These include the Na\(^{+}\)-channel blockers (5), AFD-19, aprindine, cibenzoline, E-0747, flecainide, KT-362, mexiletine, nicainoprol, OPC88117, penticainide, phenytoin, pirmenol, propafenone, SD3212, TJN-505, TYB-3823, and YUTAC, and other miscellaneous drugs, such as l-carnitine (66) and colforsin daropate (55).
shown to aggravate EPS induced arrhythmias when the control arrhythmias were set up as mild arrhythmias, such as PVC (37, 82); however it is difficult to judge whether this arrhythmia model is an appropriate and sufficient one to differentiate or select dangerous Na\textsuperscript{-}-channel blockers.

2-5. Coronary artery occlusion/reperfusion arrhythmia

2-5-1. Characteristics

This arrhythmia model mimics myocardial ischemia related arrhythmias that are frequently observed in the clinic. Coronary occlusion can be done by thread ligation of the isolated coronary artery of the anesthetized animal under thoracotomy, by inflating a balloon occluder or ligature of a prepositioned thread around the coronary artery in a conscious state, or by intracoronary balloon inflation. For this model, various animals could be used, such as dogs, cats, pigs, rabbits, rats, and so on, with the exception of the guinea pig whose coronary artery collateral circulation is too well developed (11). It is known that there are predominant time zones for the occurrence of acute coronary occlusion arrhythmias (83). In the case of dogs, the first 5 and 30 min after occlusion are those time zones (46, 84). The generation mechanism of this arrhythmia is thought to be automaticity induced ectopic beats and subsequent re-entry movement of the excitation. The severity of arrhythmias can be categorized by the type of arrhythmias, like PVC, VT, or VF, or by the number of ectopic beats over time, if VF does not occur. Reperfusion, after complete coronary occlusion, often induced VF immediately. This spontaneous arrhythmia occurs in almost 60\% – 70\% in dogs and nearly 100\% in pigs and rats, when the main trunk of the LAD is occluded (83 – 86). The VF results in death in dogs and pigs, but small animals like rats, VF very often reverts to sinus rhythm (83 – 86).

2-5-2. Methods

Our canine coronary occlusion/reperfusion arrhythmias were produced in halothane- or pentobarbital-anesthetized animals (46). After the left thoracotomy, the LAD was isolated and a string was placed for tying the artery for usually 30 min, but we also used 20-min occlusion in some studies (48). After stabilization following the surgical procedures, a drug was given by an intravenous bolus or by an infusion and then coronary occlusion followed by reperfusion was performed. In some experiments, chronic oral administration of drugs for days or weeks before the experiment was performed (87, 88). The occurrence of PVC or VF was monitored by continuous ECG recordings. Normally, drug plasma concentration determinations were not done because of the normal variability of the time of occurrence of the arrhythmias. In our rat model, LAD was not isolated, instead a silk thread with a curved needle was placed under the myocardium together with the coronary artery and vein and both the artery and vein were tied for coronary occlusion under intraperitoneal pentobarbital anesthesia; in this method, the ischemia period ranged from 5 – 120 min for occlusion arrhythmia studies and usually 5 min for reperfusion studies (85, 86). In the case of rat experiments, the number of VF occurrence was counted along with the number of PVC.

2-5-3. Antiarrhythmic drugs

Some Na\textsuperscript{-}-channel and K\textsuperscript{+}-channel blockers have been reported to be effective in suppressing coronary occlusion/reperfusion arrhythmias, but in the canine model, the control occurrence of reperfusion VF was variable and differed by anesthesia, about 60\% in halothane anesthetized dogs (our unpublished data of 43/76 dogs) and about 70\% in pentobarbital anesthetized dogs (our data of 146/217 dogs); we always compared drug effects with the vehicle-treated control group using the same number of dogs (6). We examined several K\textsuperscript{+}-channel blockers (6, 46 – 50, 87) because ischemia must activate the ATP-dependent K\textsuperscript{-} channel and shorten action potential duration, so the K\textsuperscript{+}-channel blocker with the opposite effect to prolong action potential duration should be effective. Actually E-4031 (46) and nifekalant (84) suppressed coronary occlusion/reperfusion arrhythmia in halothane-anesthetized dogs, and KCB-328 (49), nifekalant (84) and d-sotalol (84) suppressed those in pentobarbital anesthetized dogs. Also ischemia stops the action of Na\textsuperscript{+}/K\textsuperscript{-} ATPase followed by increase in the intracellular Na\textsuperscript{-} concentration by Na\textsuperscript{+}/H\textsuperscript{+} exchange, which is then followed by Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange, inevitably resulting in intracellular Ca\textsuperscript{2+} overload (7). Thus the two important exchange system blockers also are expected to overcome this intracellular Ca\textsuperscript{2+} overload. Among these drugs, only the Na\textsuperscript{+}/H\textsuperscript{+}-exchange inhibitors cariporide (70) and KB-R9032 (89) showed a consistent antifibrillatory effect in pentobarbital anesthetized dogs (7) and cariporide (70, 90 – 92), FR 186666 (93), and KB-R9032 (94), in rats. These Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitors have been reported to be protective in various animal models of coronary occlusion/reperfusion injuries, and the antiarrhythmic effects are one of the common effects (7). Other effective drugs in rat models were DIDS (95) and long-term pravastatin administration (88), but whether the effects were due to Cl/HCO\textsubscript{3} exchange inhibition or HMG-CoA reductase inhibition is unknown. Our recent unpublished data on halothane anesthetized dogs, short or long term pravastatin administration did not demon-
Ineffective and proarrhythmic drugs

As stated above, some K⁺-channel blockers were effective, but others, amiodarone (50, 87), dofetilide (48), KCB-328 (49), sematilide (47) and, d-sotalol (84), were not effective in halothane-anesthetized dogs; and dofetilide (48) and sematilide (47) were not effective in pentobarbital-anesthetized dogs (6). Also in the halothane anesthetized low heart rate open chest dogs, some of the K⁺-channel blockers, amiodarone (50), dofetilide (48), E-4031 (46), KCB-328 (49), nifekalant (84), and sematilide (47), showed a proarrhythmic effect by spontaneously inducing PVC and VT before applying coronary occlusion, but these arrhythmias before applying coronary occlusion were not observed in high heart rate pentobarbital-anesthetized dogs by dofetilide (48), KCB-328 (49), nifekalant (84), sematilide (47), and d-sotalol (84). Not like Na⁺/H⁺-exchange inhibitors, the Na⁺/Ca²⁺-exchange inhibitors KB-R7943 (71) and SEA0400 (64) had no antiarrhythmic effect on the coronary occlusion/reperfusion arrhythmia in pentobarbital-anesthetized dogs. It may be that the Na⁺ overload by inhibition of the Na⁺/H⁺ exchange may be more arrhythmogenic than Ca²⁺ overload by inhibition of the Na⁺/Ca²⁺ exchange, but there is no proof for the in vivo heart to support this idea. Other ineffective drugs were SITS, a Cl⁻/HCO₃⁻ inhibitor (95), and long-term fluvastatin, a lipid soluble HMG-CoA reductase inhibitor (88), administration in rat models. Because of the well known fact that this arrhythmia is severe and often fatal, it is difficult to evaluate or reveal proarrhythmic effects of drugs using this model, except in the pacing-induced heart-failure dogs, where all halothane-anesthetized dogs fibrillated just after reperfusion (96).

Animal models to reveal proarrhythmic potentials of drugs for safety pharmacological studies

Arrhythmia models are not only useful and essential to find a means to suppress arrhythmias, but recent demonstration of proarrhythmic effects of drugs, such as a CAST study showing more deaths in patients receiving Na⁺-channel blockers (2) and in patients receiving QT-prolonging cardiovascular drugs, highlighted needs to demonstrate proarrhythmic effects of drugs (97). Most of the lethal QT-prolonging drugs produce tachyarrhythmias, thus put simply, animals at slower basal heart rates help in the detection of proarrhythmia. So anesthetized with bradycardic agents, such as halothane (6, 98), unanesthetized animals (99), sinus node-crushed, or AV node-crushed animals (100 – 102) have been used. However, it was difficult to observe TdP arrhythmias with K⁺-channel blockers in normal animals anesthetized with halothane or in a conscious state and also in the old myocardial infarction dogs in a conscious state (99). For finding the proarrhythmic effects of QT-prolonging K⁺-channel blockers, many laboratories demonstrated AV block dogs are suitable (100 – 102) and probably other animals such as monkeys seem to be useful. Already it has been demonstrated that in chronic AV block dogs, bradycardia plus myocardial remodeling increased the baseline QT prolongation and QT-prolonging cardiac and non-cardiac drugs induced TdP andVF in high incidences (100 – 107). Since the occurrence of K⁺-channel blockers’ induced TdP and death occur rarely in man, may be one in 10,000 or more, whether this very high incidence of TdP in chronic AV block dogs really mimics the human situation is still open for discussion.

Other models already mentioned are halothane (not pentobarbital) anesthetized open chest dogs, and sensitization in halothane-adrenaline arrhythmia models (6, 46 – 49, 75 – 77). Since QT prolongation per se is beneficial in increasing contractile force and prevention from re-entry arrhythmias, to identify high risk patients among the QT-prolonging drug users, the human gene analysis for the long QT syndrome using inquiry of hereditary channelopathy may be an alternative approach to prevent cardiac accidents by drugs with properties to alter cardiac excitation and contraction. This problem is still a central issue for developing new drugs, and the International Conference on Harmonization (ICH) has been working successfully to establish guidelines for finding QT-prolonging properties of drugs in preclinical and early clinical experiments (108). Future efforts are needed to differentiate good or non-lethal QT-prolonging drugs from possible dangerous ones and also identifying precipitating factors making safe QT prolongation to TdP induction, such as sex hormones, electrolyte imbalance such as hypopotassemia, hereditary abnormalities, and so on.

3. Limitations

The present review mainly summarizes our own experimental results on drugs using various animal arrhythmia models. Already, there have been many excellent reviews on antiarrhythmic drugs that made pharmacologically and pharmacokinetically sound use of the presently available antiarrhythmic drugs, but there have been few experiments to compare drugs using several different animal models and applying the same protocol for quantitative comparisons. Presently, the pharmacological and non-pharmacological treatments for arrhythmias have reached a mature state, where
dramatically different new drugs may not appear and effective electronic devices are being refined, downsized, and becoming less expensive. Additionally the resistance to use whole animals in medical research is making arrhythmia model studies difficult and expensive, so summarizing our previous results and others may help decrease the useless repetition of experiments and sacrifice of precious animals and hopefully promote the refining of antiarrhythmic drug research and as a result promote the emergence of some dramatically different new drugs in the future.

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