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Evidence for the hERG Liability of Antihistamines, Antipsychotics, and Anti-Infective Agents: A Systematic Literature Review From the ARITMO Project

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Abstract

A systematic review was performed to categorize the hERG (human ether-a-go-go–related gene) liability of antihistamines, antipsychotics, and anti-infectives and to compare it with current clinical risk of torsade de pointes (TdP). Eligible studies were hERG assays reporting half-minimal inhibitory concentrations (IC50). A "hERG safety margin" was calculated from the IC50 divided by the peak human plasma concentration (free C_{max}). A margin below 30 defined hERG liability. Each drug was assigned an "uncertainty score" based on volume, consistency, precision, and internal and external validity of evidence. The hERG liability was compared to existing knowledge on TdP risk (www.credibledrugs.org). Of 1828 studies, 82 were eligible, allowing calculation of safety margins for 61 drugs. Thirty-one drugs (51%) had evidence of hERG liability including 6 with no previous mention of TdP risk (eg, desloratadine, lopinavir). Conversely, 16 drugs (26%) had no evidence of hERG liability including 6 with known, or at least conditional or possible, TdP risk (eg, chlorpromazine, sulpiride). The main sources of uncertainty were the validity of the experimental conditions used (antihistamines and antipsychotics) and nonuse of reference compounds (anti-infectives). In summary, hERG liability was categorized for 3 widely used drug classes, incorporating a qualitative assessment of the strength of available evidence. Some concordance with TdP risk was observed, although several drugs had hERG liability without evidence of clinical risk and vice versa. This may be due to gaps in clinical evidence, limitations of hERG/C_{max} data, or other patient/drug-specific factors that contribute to real-life TdP risk.

Keywords

antipsychotic agents, histamine H1 antagonists, anti-infective agents, ether-a-go-go potassium channels, review

Of particular concern for the pharmaceutical industry, health regulatory agencies, and healthcare professionals is the potential for drugs to induce torsade de pointes (TdP). This is a ventricular tachycardia associated with delayed cardiac repolarization that can manifest as a prolonged QT interval on the electrocardiogram (ECG) and that may cause syncope, seizure, ventricular fibrillation, and sudden death.[1] In 2010 the ARITMO project (http://www.aritmo-project.org/), involving a pan-European multidisciplinary collaboration of researchers, was commissioned to investigate the arrhythmogenic potential of 3 drug classes: antihistamines, antipsychotics, and anti-infective drugs. The ultimate aim of the project was to integrate the evidence from a variety of sources in order to describe the potential for these agents to induce TdP. These sources included data from molecular in-silico modeling studies, animal studies, spontaneous reporting databases, and epidemiological studies in large healthcare databases.

Published clinical data on drug-induced TdP are relatively sparse and are usually limited to individual case reports. Formal early-phase clinical studies, known

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as thorough QT studies, focus on QT prolongation rather than TdP risk and have only been performed for drugs marketed after 2005. On the other hand, preclinical data on arrhythmogencity are widely published. Because there does not appear to be a single "gold standard" preclinical test that reliably predicts clinical events in routine drug use, regulatory agencies currently recommend that a number of different models be used in preclinical safety testing.[2] One of the core testing systems is the in vitro assay of the rapid component of the delayed rectifier potassium channel current (I_{Kr}), also known as the hERG assay because this channel is encoded by the human ether-a-go-go–related gene (hERG). In addition, in vitro action potential assays and in vivo QTc studies in laboratory animals (especially dog, monkey, swine, rabbit, ferret, and guinea pig) are used.[2] The merits and limitations of the different preclinical models are discussed elsewhere.[3–6]

Although assays for in vitro action potential and in vivo QTc studies provide important data, these often report a complex variety of electrophysiological parameters that are not well standardized across the literature. We chose, therefore, to focus our review on the hERG/I_{Kr} assay[7] because this is the target of virtually all torsadogenic drugs, and we considered that these data are more suitable for systematic review as they are reported in a relatively standardized format in the literature, and hERG/I_{Kr} blockade can be measured by the half-maximal inhibitory concentration (IC50). Thus, the aim of the present systematic review is to collect and assess (quantitatively and qualitatively) published hERG data; this will serve as a reference standard for future in silico studies and as the basis to compare the predicted hERG liability with apparent postmarketing risk identified in clinical and observational studies.

Methods

Selection Criteria

Intervention. Drugs were selected for inclusion in this review if at least 1 of the following 3 criteria were fulfilled:

1. Evidence about torsadogenic potential of the drug from any of the following types of evidence:
   - drug included in published lists of drugs linked to TdP or QT prolongation compiled by the Arizona Center for Education and Research on Therapeutics ("AZCERT") group (accessed June 1st, 2010, http://www.crediblemeds.org);
   - > 50 total items identified in a PubMed search for drug (some of which are related to QT/TdP/hERG) in the past 10 years;
   - specialist ECG studies (known as "thorough QT studies") identified for drug in PubMed.
2. Evidence of drug use in current clinical practice using databases enrolled in the ARITMO project:
   - Top-10 most frequently prescribed/dispensed drugs within each study drug class, based on analysis of prevalence of use in 2008 within 6 epidemiological databases; OR
   - Detectable hospital use (defined as a proportion of dispensing >0.1% of the drug class for at least 1 year), by checking 2 databases that include hospital records over the 2005-2008 period.
3. Newly marketed drugs (since 2006) through centrally authorized procedure (European Medicines Agency website).

The final list of drugs included 28 antihistamines, 37 antipsychotics, and 154 anti-infective agents (Online Supplementary Table S1).

Study Type. Preclinical assays reporting a half-maximal inhibitory concentration (IC50) or percentage blockade of the hERG/I_{Kr} channel current were eligible. Only assays performed in mammalian transfected cell lines or cardiomyocytes were included. Studies in species that tend to underestimate hERG potency such as *Xenopus* oocyte[8] or in emerging but unproven technological models (eg, zebrafish) were excluded. Mammalian recombinant expression systems are preferred, as they resemble human cells, they can be studied at physiological temperature, and share electrophysiological features of the native I_{Kr} current. For this reason, they are recommended for evaluating the affinity of drug candidates for the hERG channel.[2]

Search Strategy, Study Selection, and Data Extraction

A systematic literature search was performed in PubMed, Embase, and Web of Science in December 2010 and updated in February 2014. The full search strategy is available in the Online Supplementary Table S2. Only English language articles were included, and data from conference proceedings or abstracts not already published in peer-reviewed journals were excluded.

Titles and abstracts were first screened and shortlisted, then full text was independently assessed by 2 reviewers (L.H. and E.R.). Data from eligible studies were extracted using a customized Microsoft Access database by 2 reviewers (L.H. and E.R.). The reported IC50 value expressed in millimoles per liter (mM), cell type, and selected experimental conditions (temperature, potassium concentration, voltage protocol, use of reference compounds) were extracted for each drug of interest. Disagreements in study selection or data extraction were resolved by consensus.

Data Synthesis

Study type and test conditions were summarized descriptively. Median, minimum, and maximum IC50 (mM) values were calculated from the values reported in eligible hERG/I_{Kr} studies for each drug. If more than one IC50 value was provided where different test conditions were applied in a study, these were regarded as separate experiments.

In line with a previous approach by Redfern et al,[9] a "hERG safety margin" was calculated from the ratio of the hERG IC50 value and the peak plasma concentration reported in humans using clinically relevant doses of the drug (C_{max}). Data on the C_{max} were provided to the ARITMO consortium by AstraZeneca. These data were compiled by extraction of pharmacokinetic parameters from 3 sources: the GVK BIO® drug database, the Prous Integrity® database, and Goodman and Gilman's textbook *The Pharmacological Basis of Therapeutics*.[10] These sources provide C_{max} data based mainly on oral administration in therapeutic use, although in some cases these data may have been derived from different routes of administration or dosing regimens or in different populations.

In the current study the hERG safety margin (IC50/C_{max}) was calculated using the average, minimum, and maximum values of IC50 and using the average and maximum values for free unbound C_{max} (ie, up to 6 possible values for the hERG margin for each drug depending on availability of data). Finally, 2 parameters were calculated for the hERG safety margin: (1) an average value (based on the ratio of average IC50 and average free C_{max} values) and (2) a range of possible values for the safety margin (based on the permutations providing the lowest and highest values).

Interpretation

Quantitative Interpretation. In order to assess and standardize hERG data, the safety margin range was used to interpret a drug's hERG liability. In keeping with the proposal by Redfern et al,[9] if the range was all below a threshold value of 30, then the drug was categorized as having evidence of hERG liability (red). If the margin range was all above 30, the drug was categorized as having no evidence of hERG liability (green). If the margin spanned 30, the evidence for hERG liability was categorized as inconclusive (orange). The hERG liability was compared to the perceived clinical risk by using the AZCERT list published on the CredibleMeds.org website (http://www.crediblemeds.org; accessed 1 June 2014), which classifies drugs according to whether there is known, possible, or conditional risk for TdP or if the drug should be avoided in congenital long QT syndrome. This classification is based primarily on expert review of data from spontaneous case reports submitted to the US adverse event monitoring system and from emerging evidence in the published literature. The hERG and AZCERT data were considered to have concordance when there was (1) evidence of hERG liability for drugs with known TdP risk on AZCERT or (2) no evidence of hERG liability for drugs and no mention on AZCERT. For drugs with inconclusive hERG liability or only possible or conditional TdP risk, concordance was not assessed.

Qualitative Interpretation. No quality assessment tool exists for hERG studies. Instead, the selection criteria used in this review were chosen so as to identify the highest quality studies from the outset. A number of parameters relating to experimental conditions were recorded to provide a means of identifying differences in results between hERG studies. In addition, it was noted whether the study had taken steps to validate the results by the use of positive and negative reference compounds.

Five "uncertainty factors" were identified that were considered to have a bearing on the interpretation of the results. The body of evidence for each drug was assessed according to each of these 5 factors and allocated 1 + for each that applied (equal weighting assumed). The following criteria were used to allocate uncertainty factors:

1. Volume of evidence: if only 1 study available
2. Precision of evidence: if range of hERG margin spanned 30, ie, not precise enough to dichotomize between red and green categories
3. Consistency of evidence: if available IC50 values or C_{max} varied by an order of magnitude or if there were clear outliers in the IC50 dataset
4. Validity of evidence: if most studies did not involve use of positive (eg, dofetilide) or negative (eg, amoxicillin, propranolol, nifedipine) control compounds
5. Representativeness of evidence: if studies appeared to use only 1, less useful test condition, eg, only high potassium levels or only at room temperature

Uncertainty assessments were made by 1 reviewer (L.H.) and checked by a second reviewer (E.R.). An overall uncertainty score was computed from the sum of these individual factors (up to +++++, maximal uncertainty) and presented alongside the quantitative results to assist interpretation. A low uncertainty score was perceived as less than or equal to +. The higher the overall uncertainty score, the lower the quality of the evidence from available published hERG data.

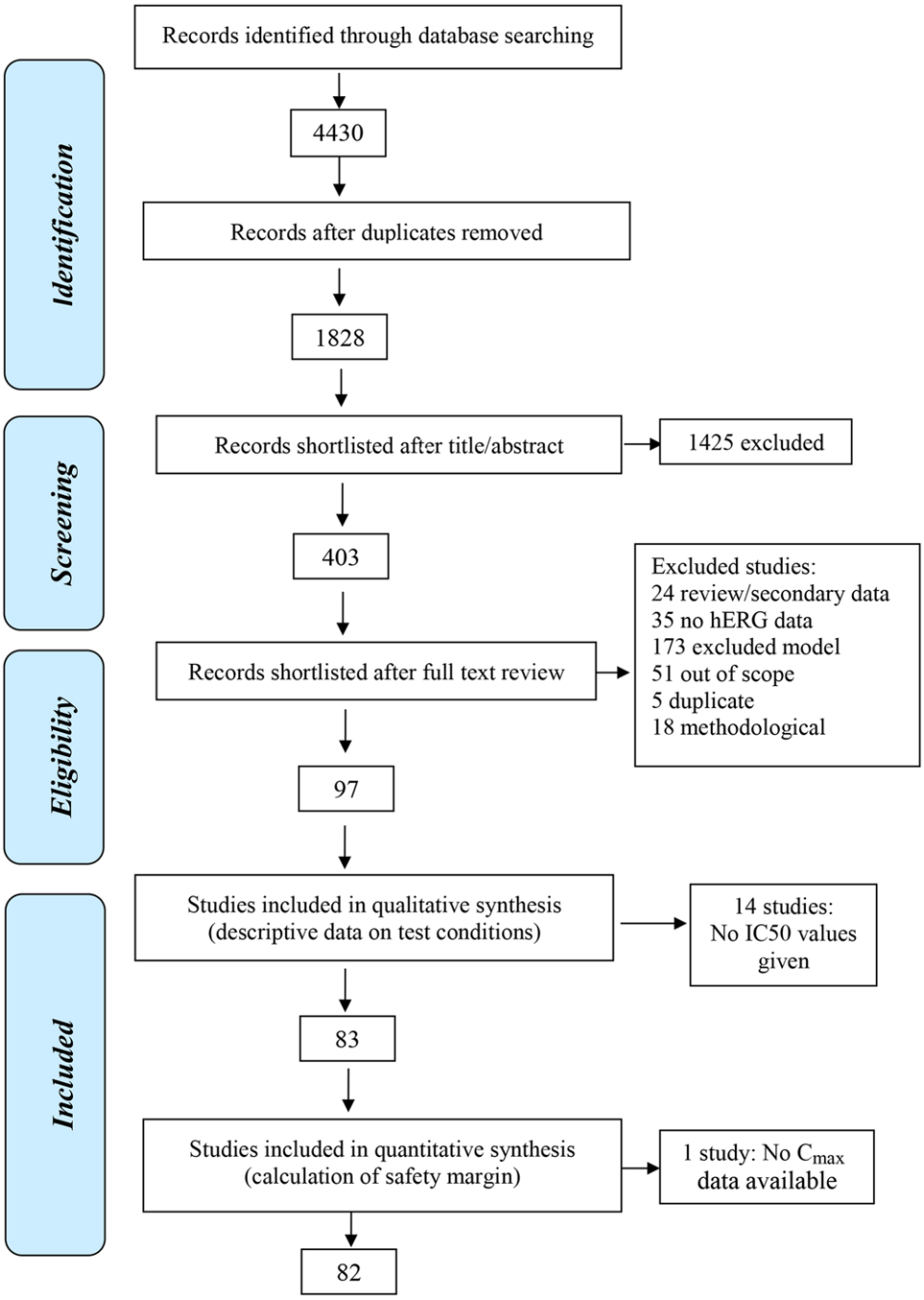

Figure 1. Flow chart of search results and eligibility assessment.

Results

In total, 1828 studies were retrieved from the literature search, of which, 97 (5%) were deemed eligible hERG/I_{Kr} assays (Figure 1). The characteristics of the eligible studies are detailed in Online Supplementary Table S3.[11–107] Eighty-three (86%) of these studies provided IC50 values for at least 1 drug of interest.

Overall, 263 IC50 values were provided for 69 drugs (Table 1). Most IC50 values (252, 96%) originated from studies using transfected mammalian cells such as Chinese hamster ovary cells and human embryonic kidney (HEK-293) cells. The majority of experiments used a normal physiological potassium concentration (212, 81%) and a step-step pulse voltage protocol (167, 63%). The temperature used was more variable across the studies, with only a third of IC50 estimates originating from assays performed at physiological temperature. A positive reference compound was used in 40 studies (171

Table 1. Study Characteristics of Eligible hERG/I_{Kr} Assays

Characteristic	Antihistamines	Antipsychotics	Anti-Infectives
No. of hERG/I_{Kr} studies	36	42	46
No. of IC50 values provided	70	110	83
Model, No. of IC50 values (%)			
Transfected mammalian cells	64 (91)	105 (95)	83 (100)
Cardiac myocytes	6 (9)	5 (5)	0
Temperature, No. of IC50 values (%)			
Room	30 (43)	63 (57)	47 (57)
Physiological	25 (36)	37 (34)	26 (31)
Unspecified	15 (21)	10 (9)	10 (12)
Potassium, No. of IC50 values (%)			
Normal (4-5 mM)	45 (64)	96 (87)	71 (86)
High (10 mM)	1 (1)	0	0
Low (2.67 mM)	3 (4)	3 (3)	0
Unspecified	21 (30)	11 (10)	12 (14)
Protocol, No. of IC50 values (%)			
Step-step	38 (54)	74 (67)	55 (66)
Step-ramp	8 (11)	9 (8)	12 (14)
Automated	20 (29)	27(25)	6 (7)
VAP	0	0	2 (2)
Unspecified	4 (6)	0	8 (10)
Reference Compounds			
Positive control used	58 (83)	75 (68)	38 (46)
Negative control used	31 (44)	38 (35)	12 (14)

VAP, ventricular action potential.

IC50 values), and 12 studies used a negative reference compound (81 IC50 values).

Because C_{max} data were not available for 8 drugs, a hERG safety margin could be calculated for 61 drugs including 13 of the 28 (46%) target antihistamines, 21 of the 37 (55%) target antipsychotics, and 27 of the 154 (18%) anti-infective agents.

Antihistamines

Drugs categorized as having evidence of hERG liability included astemizole and terfenadine with low levels of uncertainty and desloratadine and mizolastine with higher uncertainty. Cetirizine, clemastine, ebastine, fexofenadine, and oxatomide were categorized as having no evidence of hERG liability with low to moderate uncertainty. For the other drugs in this class, evidence of hERG liability was inconclusive (Table 2, Figure 2). The main source of uncertainty across the hERG data for this class was from issues concerning the external validity of the experimental methods (applicable for 7 of the 13 drugs). Concordance between hERG data and the AZCERT classification was seen for 7 of 9 drugs assessed for concordance.

Antipsychotics

Several antipsychotics were categorized as having evidence of hERG liability including aripiprazole, clozapine, droperidol, mesoridazine, olanzapine, perphenazine, risperidone, and thioridazine. Pipamperone was also included in this category but with a higher level of uncertainty in the available hERG data (Table 3, Figure 3). Drugs classified as having no evidence of hERG liability included amisulpride, chlorpromazine, sulpiride, flupentixol, fluphenazine, prochloperazine and ziprasidone with varying levels of uncertainty. Evidence of hERG liability was inconclusive for older drugs such as haloperidol, sertindole, pimozide and quetiapine but also for one of the newer drugs (post 2000), paliperidone, with a high level uncertainty in the latter case. The main source of uncertainty within the hERG evidence for the antipsychotics related to issues over external validity of experimental methods (9 of the 21 drugs). Concordance between hERG data and the AZCERT classification was seen for 6 of 9 drugs assessed for concordance.

Anti-Infective Agents

Most anti-infectives (18 of 27 drugs) were categorized as having evidence of hERG liability with varying levels of uncertainty (Table 4, Figure 4). Amoxicillin, ciprofloxacin, and gemifloxacin were categorized as having no evidence of hERG liability, also with varying levels of uncertainty. For the remaining drugs in this class (amantadine, erythromycin, mefloquine, grepafloxacin, moxifloxacin, and telithromycin) the hERG evidence was inconclusive. The main source of

Table 2. Safety Margin Data for Antihistamines

	ARITMO Review of hERG						
		Safety Margin					
Drug Studied	No. of IC50 Values	Average	Range	Evidence of hERG Liability	Uncertainty Score	Risk of TdP on AZCERT List	Concordance Between hERG and AZCERT
Astemizole	12	<1	<1-2	Yes	+	Known	Yes
Desloratadine	1	0.2	<1	Yes	+++	No	No
Mizolastine	1	14	2-14	Yes	+++	No	No
Terfenadine	27	1.1	<1-28	Yes	+	Known	Yes
Chlorphenamine	4	51	21-231	Inconclusive	++	No	Not assessed
Diphenhydramine	7	84	14-445	Inconclusive	++	Conditional	Not assessed
Loratadine	8	193	<1-536	Inconclusive	++	No	Not assessed
Promethazine	1	196	22-196	Inconclusive	+++	Possible	Not assessed
Cetirizine	3	2350	1200-5224	No	+	No	Yes
Clemastine	1	159	103-159	No	++	No	Yes
Ebastine	2	1070	164-1580	No	+	No	Yes
Fexofenadine	2	774	30-1371	No	+	No	Yes
Oxatomide	1	60	46-60	No	++	No	Yes

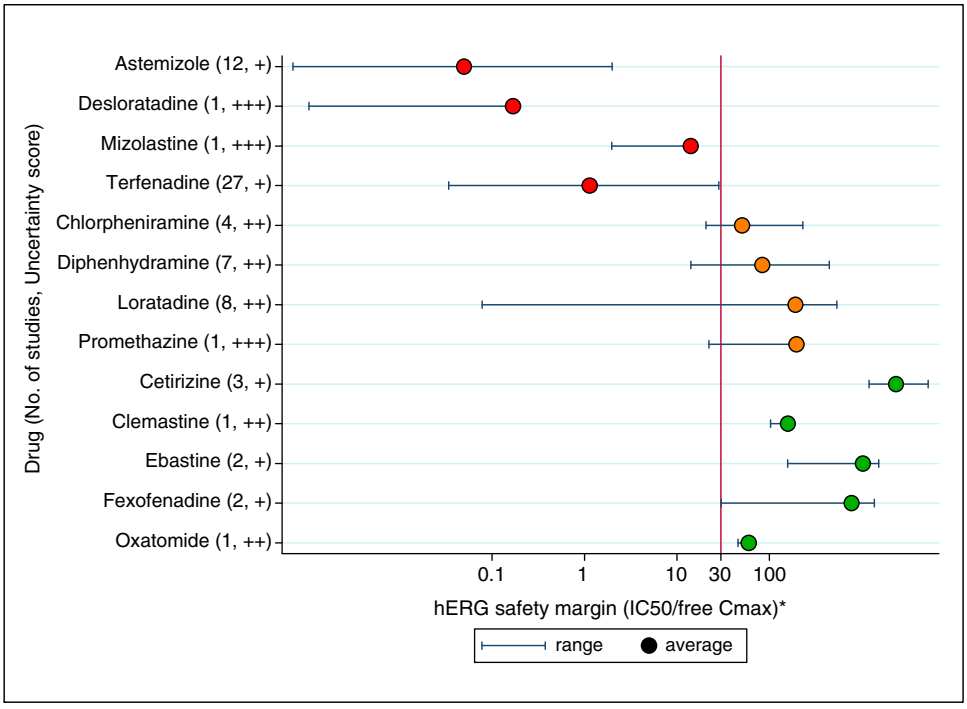

Figure 2. hERG safety margin range for antihistamines. IC50, half-maximal inhibitory concentration; C_{max}, peak plasma concentration reported in humans using clinically relevant doses of the drug. *hERG liability classified as Yes (red circle), No (green circle), or Inconclusive (orange circle) on the basis of whether the range of possible values for hERG margin is all below, all above, or spans the threshold value of 30, respectively.

uncertainty within the hERG evidence for the anti-infectives was the lack of use of reference compounds (19 of the 27 drugs). Concordance with the AZCERT classification was seen for 7 of 10 drugs assessed for concordance.

Overall, 31 (51%) of the 61 drugs studied had evidence of hERG liability. Of these, 25 (81%) were classified by AZCERT as having at least conditional, possible, or known TdP risk; 6 drugs had no mention with respect to clinical risk in the AZCERT lists including 2 antihistamines (desloratadine and mizolastine), 1 antipsychotic (perphenazine), and 3 anti-infectives (miconazole, lopinavir, and primaquine). Conversely, 16 drugs (26%) of the 61 drugs were classified as having no evidence of hERG liability. Of these, 2 drugs, according to the AZCERT classification, had

Table 3. Safety Margin Data for Antipsychotics

	ARITMO Review of hERG						
		Safety Margin					
Drug Studied	No. of IC50 Values	Average	Range	Evidence of hERG Liability	Uncertainty Score	Risk of TdP on AZCERT List	Concordance Between hERG and AZCERT
Aripiprazole	2	<1	<1	Yes	+	Possible	Not assessed
Clozapine	4	13	<1-21	Yes	+	Possible	Not assessed
Droperidol	6	<1	<1-2.2	Yes	++	Known	Yes
Mesoridazine	2	<1	<1-1.1	Yes	+	Known	Yes
Olanzapine	5	<1	<1	Yes	+	Possible	Not assessed
Perphenazine	2	5	<1-6	Yes	+	No	No
Pipamperone[a]	1	<1	<1	Yes	+++	Possible	Not assessed
Risperidone	7	<1	<1-1.2	Yes	++	Possible	Not assessed
Thioridazine	18	<1	<1	Yes	+	Known	Yes
Haloperidol	13	11	2-114	Inconclusive	++	Known	Not assessed
Paliperidone	1	48	8-48	Inconclusive	++++	Possible	Not assessed
Pimozide	15	66	3-2524	Inconclusive	++	Known	Not assessed
Quetiapine	2	31.5	8-44	Inconclusive	++	Possible	Not assessed
Sertindole	9	148	<1.6-1296	Inconclusive	++	Possible	Not assessed
Amisulpride	1	135	49-135	No	++	Conditional	Not assessed
Chlorpromazine	2	174	59-201	No	None	Known	No
Flupentixol[a]	1	69	69	No	+++	No	Yes
Fluphenazine	2	4352	597-7895	No	None	No	Yes
Prochlorperazine	1	4311	2094-4311	No	++	No	Yes
Sulpiride	1	6301+	4297-6301	No	++	Known	No
Ziprasidone	7	271	84-1340	No	++	Possible	Not assessed

[a]Based on total C_{max}.

known TdP risk (sulpiride and chlorpromazine), and 5 drugs had at least conditional or possible TdP risk (amisulpiride, ziprasidone, amantadine, ciprofloxacin, and gemifloxacin). Concordance between hERG and AZCERT was assessed for 28 drugs, of which 20 (71.4%) were concordant.

A breakdown of the assignment of the uncertainty factors is available in the Online Supplementary Table S4.

Discussion

In examining cardiac safety, an important assessment is the extent to which a drug may block the hERG cardiac potassium channel, a well-known mechanism involved in drug-induced arrhythmia.[7] The purpose of this review was to categorize systematically the hERG liability of 3 widely used drug classes. This evidence is intended to complement data on in vivo models[108] and other ion channels that may contribute to the arrhythmogenicity. Our approach has 2 main strengths. First, we have systematically categorized hERG liability considering, qualitatively, the strength of the published evidence of hERG liability for each drug. Assessment of study quality is routinely performed in the context of systematic reviews of clinical trials.[109,110] In animal studies, quality assessment is performed in around half of reviews[111,112] and in only 20% of reviews of bench studies (such as hERG assays).[112] The other key strength is that this review highlights gaps and uncertainties in existing knowledge in which additional experimental studies on hERG liability may still have an important role.

In general, there was reasonable concordance between the hERG categorization in the current study and the classification of clinical TdP risk according to the AZCERT group (the only available reference standard), particularly when considering only the drugs for which data on hERG liability and TdP risk were well established (~70% concordance). For the antihistamine class, we classified 4 drugs as having hERG liability. Two of these drugs, terfenadine and astemizole, are known to have TdP risk and were withdrawn from the market in several countries in the 1990s for this reason.[113,114] Desloratadine and mizolastine were also classified as having hERG liability but have not been associated with TdP in clinical studies, although the product label for mizolastine refers to a "weak potential to prolong the QT interval."[115] Uncertainty scores were high for these drugs, however, because only one IC50 was available for desloratadine with considerable variation in available C_{max} data. Similarly, the only study found for mizolastine reportedly used a higher extracellular potassium concentration (10 mM) than most other

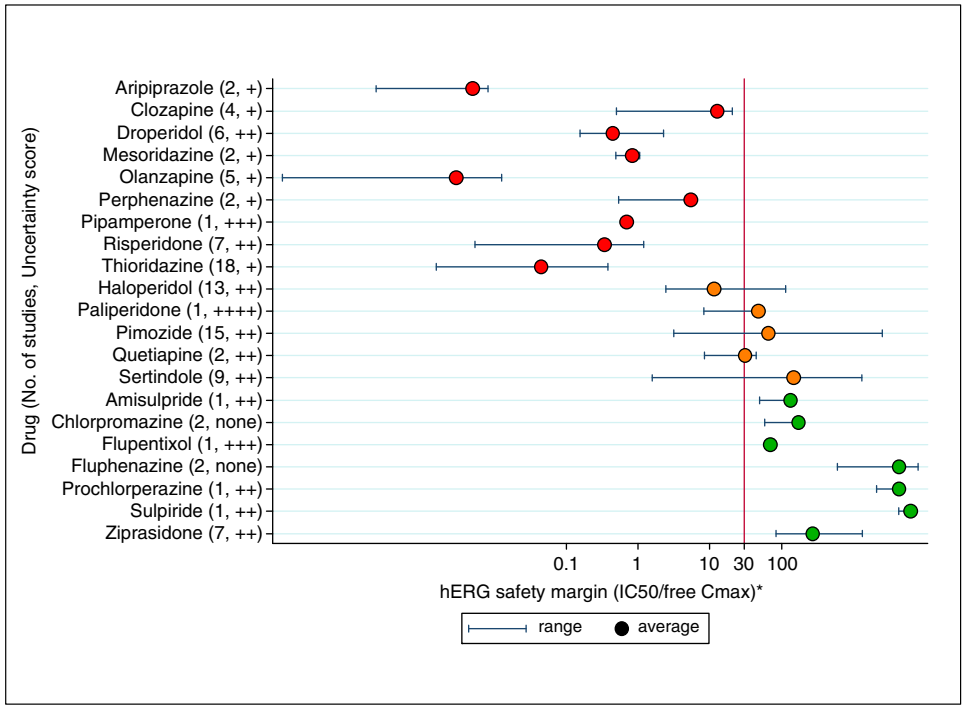

Figure 3. hERG safety margin range for antipsychotics. IC50, half-maximal inhibitory concentration; C_{max}, peak plasma concentration reported in humans using clinically relevant doses of the drug. *hERG liability classified as Yes (red circle), No (green circle), or Inconclusive (orange circle) on the basis of whether the range of possible values for hERG margin is all below, all above, or spans the threshold value of 30, respectively.

hERG studies, which has been shown to reduce hERG block for some drugs but not others.[116,117]

For the antipsychotics, there was reasonable agreement between hERG liability and AZCERT classification. Exceptions were for amisulpride and ziprasidone, drugs associated with TdP in overdose as well as chlorpromazine and sulpiride, drugs classified by AZCERT as known to cause TdP.[118,119] We found no evidence of hERG liability for these 4 drugs, but the available hERG data had moderate levels of uncertainty. Drugs such as perphenazine, fluphenazine, flupenthixol, and prochloperazine, 3 of which we classified as having no evidence of hERG liability, were not mentioned on the AZCERT list. QT prolongation and ventricular arrhythmia are, however, listed as possible undesirable effects within these products' labels.

Most of the anti-infective drugs had hERG liability with the majority classified by AZCERT as having some degree of clinical risk. This may reflect publication bias because hERG studies tend to be performed and published for drugs where TdP risk has been questioned. Amoxicillin, as expected, had no evidence of hERG liability with a wide margin of safety. For some drugs (eg, erythromycin and moxifloxacin), hERG evidence was inconclusive or intermediate. This concurs with the dose dependency seen with erythromycin; TdP risk is mainly seen with high-dose intravenous administration of erythromycin or in combination with drugs that increase drug levels.[120] Moxifloxacin is used routinely as a positive control in clinical cardiac safety studies due to its relatively moderate effect on the QTc interval.[2]

Several reasons may explain the discordance between hERG data and perceived clinical risk. First, there may be gaps in the clinical knowledge compiled by AZCERT because much of the published literature on TdP risk is based on case reports, which vary in context and completeness of information. There are relatively few formal controlled studies, and these focus on a drug's potential to prolong the QT interval as a surrogate marker for TdP, whereas larger-scale observational studies tend to measure broader outcomes such as sudden cardiac death.[121] Second, there may be gaps in the available hERG data because this is not always available in the public domain. Third, there is a varying degree of uncertainty within the published hERG data. Moderate or high levels of uncertainty (++ or higher) in the evidence were identified for 45 of the 61 drugs studied. This was mainly due to lack of use of reference compounds or to the use of less optimal experimental conditions (eg, conducted only at room temperature). Experimental variations and limitations such as adsorption of test substances in the perfusion systems of patch-clamp experiments can introduce error in the IC50 values reported.[45] Current regulatory guidelines do not prescribe a specific gold standard methodology for hERG assays.[2] In addition,

Table 4. Safety Margin Data for Anti-Infectives

	ARITMO Review of hERG						
		Safety Margin					
Drug Studied	No. of IC50 Values	Average	Range	Evidence of hERG Liability	Uncertainty Score	Risk of TdP on AZCERT List	Concordance Between hERG and AZCERT
Azithromycin	1	24	1-24	Yes	++++	Known	Yes
Chloroquine	2	<1	<1	Yes	+	Known	Yes
Clarithromycin	2	2.2	<1-2.7	Yes	++	Known	Yes
Fluconazole	1	2.8	<1-2.8	Yes	+++	Conditional	Not assessed
Gatifloxacin	1	16	3-16	Yes	+++	Possible	Not assessed
Halofantrine	4	<1	<1	Yes	++	Known	Yes
Ketoconazole	5	8	2-19	Yes	None	Conditional	Not assessed
Levofloxacin	4	<1	<1-1.8	Yes	++	Possible	Not assessed
Lopinavir	1	16	<1-16	Yes	++++	No	No
Miconazole	1	2	<1-2.1	Yes	+++	No	No
Nelfinavir	1	1.1	<1-1.1	Yes	++++	Conditional	Not assessed
Ofloxacin	1	6	<1-6	Yes	++++	Possible	Not assessed
Pentamidine	1	<1	<1	Yes	++	Known	Yes
Primaquine	1	<1	<1	Yes	+++	No	No
Ritonavir	1	6	<1-6	Yes	++++	Conditional	Not assessed
Roxithromycin	2	4.5	<1-5.5	Yes	++	Possible	Not assessed
Saquinavir	1	19	<1-19	Yes	++++	Possible	Not assessed
Sparfloxacin	7	19	<1-27	Yes	+++	Known	Yes
Erythromycin	11	190	22-2649	Inconclusive	++	Known	Not assessed
Grepafloxacin	2	54	19-70	Inconclusive	++++	No[a]	Not assessed
Mefloquine	5	14	1.4-77	Inconclusive	+	No	Not assessed
Moxifloxacin	18	24	<1-65	Inconclusive	++	Known	Not assessed
Telithromycin	3	68	2-121	Inconclusive	+++	Possible	Not assessed
Amantadine	1	49	39-49	No	+++	Conditional	Not assessed
Amoxicillin	1	1092	207-1092	No	++	No	Yes
Ciprofloxacin	1	250	160-250	No	+++	Conditional	Not assessed
Gemifloxacin	1	149	149-222	No	+++	Possible	Not assessed

[a]Drug not listed in AZCERT but no longer marketed; withdrawn due to arrhythmia concerns.

for some drugs only one study was available. Nevertheless, among the 16 drugs studied with the lowest level of uncertainty (+ or less), only 2 appeared to completely disagree with the AZCERT lists (chlorpromazine and perphenazine). Fourth, the hERG safety margin, even with perfect data, does not provide the whole story with respect to a drug's clinical risk for TdP. Other drug-specific pharmacodynamic and pharmacokinetic factors may be involved. There are also patient-specific factors that may modulate TdP risk including congenital long QT syndrome, heart failure, bradycardia, electrolyte imbalance, and sex.[1] We addressed only hERG as a possible target implicated in TdP; however, other ion channels (eg, sodium and calcium) may have roles to play.[122] Although the hERG safety margin attempts to consider some of a drug's pharmacokinetics by quantifying the hERG blockade in the context of plasma concentration, the latter may not represent the tissue concentration achieved at the myocardial membrane cells.[123] Indeed, other important properties such as bioavailability, steady-state concentration, and volume of distribution should be considered. The route of administration may be particularly important for some drugs. This may explain, in part, the results for erythromycin where inconclusive evidence of hERG liability was observed. Use of C_{max} values based only on intravenous administration may have resulted in a much narrower margin of safety.[124]

Our choice of metric, the ratio of IC50/C_{max}, has been used by several authors to stratify arrhythmogenic risk. This was first used by Redfern et al, who compared hERG data for 100 drugs that were classified according to their perceived clinical risk.[9] From this study, a 30-fold hERG safety margin was proposed as a guide for decision making in identifying high-risk drugs. DeBruin et al found that a similar metric (effective therapeutic plasma concentration/IC50) was positively correlated with case reports of serious arrhythmias in an international adverse drug-monitoring system.[125] More recently, Lin et al attempted to identify thresholds for a range of hERG metrics that may help in prediction of TdP risk.[126] They compared percentage hERG inhibition (at normal plasma concentrations), IC50 for hERG, and the IC50/C_{max} ratio between 9

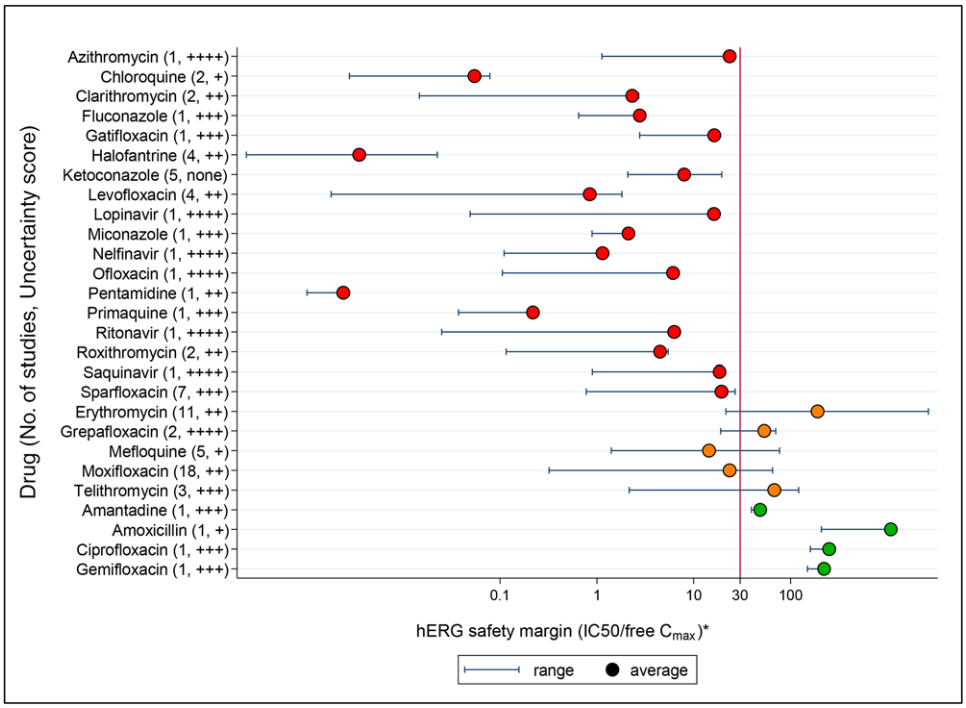

Figure 4. hERG safety margin range for anti-infectives. IC50, half-maximal inhibitory concentration; C_{max}, peak plasma concentration reported in humans using clinically relevant doses of the drug. *hERG liability classified as Yes (red circle), No (green circle), Inconclusive (orange circle) on the basis of whether the range of possible values for hERG margin is all below, all above, or spans the threshold value of 30, respectively.

drugs strongly associated with TdP and 11 drugs with less evidence for an association. They found that >30% hERG inhibition provided reasonable sensitivity and specificity for distinguishing between the 2 groups. The IC50 and the IC50/C_{max} ratio, however, discriminated less well, possibly due to the limited number of drugs included in the analysis. Gintant suggested an optimal threshold value of 45 for the safety margin following a comparison of hERG data with the degree of QTc prolongation reported in clinical thorough QTc studies for 39 drugs.[127] This threshold would not have changed the categorization in the present study with the exception of 1 drug (quetiapine) that would have been classified as having hERG liability rather than "inconclusive."

Some of the results of the current study are in agreement with other studies that have report a hERG safety margin for the drugs of interest in the ARITMO project, although some differ in the category to which they are classified. This may be explained by the different approaches used. Some studies have used a more conservative approach by using the lowest IC50 and largest C_{max},[9] whereas other authors have chosen a best estimate for hERG block in which multiple data were available from different species or different test conditions.[126] We adopted a more inclusive approach, calculating the range of possible values.

Many developmental drugs have been eliminated from early screening due to hERG liability, which may or may not translate to a real-life TdP risk. Our analysis supports the view that drug manufacturers should not rely only on hERG data when making decisions regarding the viability of development of new drugs. For regulatory authorities, the emphasis is on assessing the absence of torsadogenic potential of a drug. We found that 7 drugs with at least possible risk of TdP did not have hERG liability. These findings may be due to limitations of the available hERG data itself or interpreted as false negatives because of different pharmacological properties (eg, sodium channel blockade), actions on additional potassium channels (eg, the transient outward current I_{to}), or interference with hERG trafficking rather than its inhibition. Therefore, recommending a standardized method for the hERG assay (which was not the aim of the present work) is unlikely to overcome the limitations of hERG as a predictive tool. The recent Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative incorporating analysis of multiple ion channels, in silico modeling, and use of human stem cell technology has shown promise in this respect.[128,129]

In conclusion, we have categorized the hERG liability for 3 widely used drug classes. In keeping with systematic reviews of clinical studies, we have also incorporated a qualitative assessment of the strength of the available evidence. Overall, we, as did other authors, observed a reasonable correlation between drugs with hERG liability and existing knowledge on torsadogenic risk. It is clear, however, that there remain

gaps and inconsistencies in the existing evidence, and a more comprehensive integration of other important data is necessary to fully understand this issue. In particular, a comparison of the predicted risk on the basis of preclinical or molecular modeling for TdP liability with real-life risk estimates of symptomatic QTc prolongation, TdP, ventricular fibrillation/ventricular tachycardia, and sudden death is required.

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Author Contributions

All authors have contributed to the conception and design of this study, interpretation of data, have revised the article critically, and provided final approval of the version to be published. Lorna Hazell and Emanuel Raschi have also contributed to the acquisition, analysis, and interpretation of data, drafting of article, and investigation of the accuracy and integrity of parts of the work. Ernst Ahlberg Helgee and Scott Boyer provided pharmacokinetic data parameters. All authors had full access to all of the data and give agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Lorna Hazell and Professor Saad Shakir, as guarantors, affirm that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) the authors have received no support from any commercial company for the submitted work; (2) L.H. and S.S. work for the DSRU, an organization that has received unconditional research grants from drug companies, although none is related to this study. M.S. has had specified relationships on other matters with the following drug companies that might have an interest in the submitted work: Pfizer, Eli Lilly, AstraZeneca, Boehringer, and Novartis. E.A.H. is an employee of AstraZeneca. E.R., F.D.P., F.S., S.T., and S.B. declare no relationships with companies that might have an interest in the submitted work in the previous 3 years; (3) all authors declare their spouses, partners, or children have no financial relationships that may be relevant to the submitted work and (4) have no nonfinancial interests that may be relevant to the submitted work.

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