citoxlab
Safety and Health Research

TOXICOLOGY SERVICES

- General toxicology in all species
- Special toxicology
  - infusion
  - Inhalation
  - Dermal
  - Ocular
- Immunotoxicology
- Regenerative medicine
- Reproductive toxicology including minipigs and NHPs
- Carcinogenicity studies also in rasH2 and p53+/- mice
- Genetic toxicology: ICH compliant package
- *In vitro* toxicology: BCOP, h-CLAT, KeratinoSens™, DPRA, Photo 3T3-NRU, Episkin™, chicken eye test
- Agrochemical / chemical / REACH
- QSAR
- Physico-chemical testing
- Ecotoxicology: wide range of test species

SAFETY PHARMACOLOGY

- Integrated safety pharmacology in toxicology studies
  - CV (JET), BP
  - Respiratory (JET), plethysmography
  - CNS (FOB) and JET-EEG
- Safety pharmacology core battery
- *In vitro* assays
  - GLP compliant ion channel testing panel (hERG +5)
  - CNS *ex vivo* models for seizure liability screening
- Screening and follow-up models
  - Rodent and non-rodent LVP telemetry
  - Anesthetized models
  - Polysomnography
  - Gastrointestinal motility

DMPK, BIOANALYSIS, BIOMARKERS

- ^{14}C and ^{3}H ADME studies in all species
- *In vitro* metabolic clearance, metabolite ID and profiling, DDI package (metabolism and transporters)
- Bioanalysis: LC-MS/MS, GC-MS/MS, LC-ICP/MS, LC-Radiodetection, ELISA, RIA
- Toxicogenomics, miRNA: Affymetrix™ accredited service provider, next generation sequencing (Illumina®)
- Immunology: 10-color flow cytometer, Luminex, Meso Scale

MEDICAL DEVICE

- Biocompatibility testing
- Cardiovascular stents, electrophysiology and structural heart studies
- Long-bone defects and craniomaxillofacial/dental models
- Spinal fusion models
- Joint and cartilage repair models
- Regenerative medicine (growth factors, biomaterials, cell and gene therapy)

SPECIALIZED EXPERTISE

- Juvenile studies including minipigs
- Ototoxicity in rats
- Fertility studies in rodents and NHPs
- Radiation safety and efficacy studies
- Drug transporter studies and Drug-Drug Interactions
- Tissue Cross Reactivity (TCR): human and animal tissue banks
- Gene therapy vector biodistribution via qPCR
- ES cell testing: devTOX™ and cardioTOX™ (with Stemina)
- Lead optimization and predictive toxicology services: Leadscreen™

aaalac INTERNATIONAL GLP CERTIFIED

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Safety and Health Research

HESI

J-Tp and Tp-e as Biomarkers of Proarrhythmic Risk in Nonclinical Models: Historical Data Evaluation by the HESI Consortium

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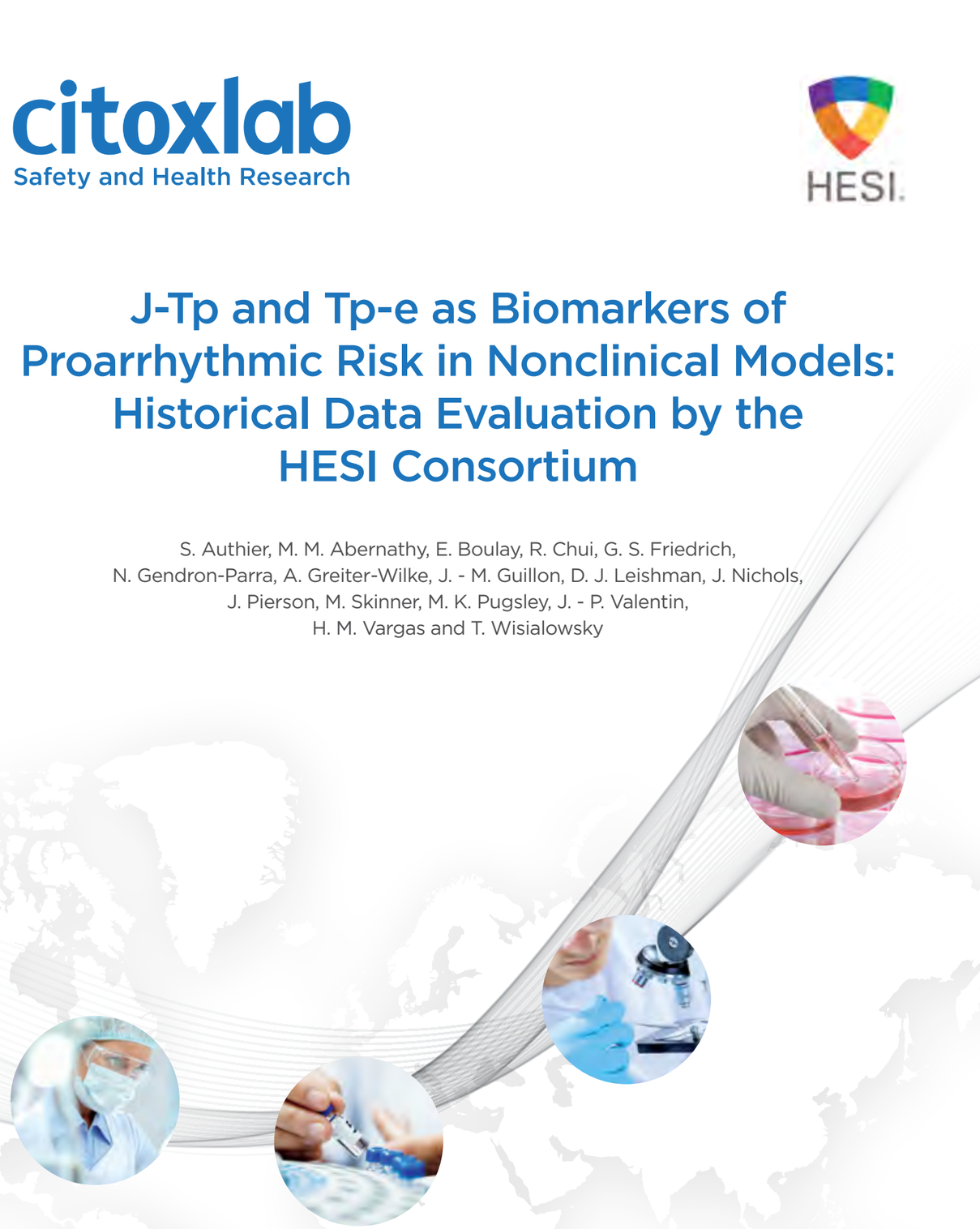

J-Tp and Tp-e as Biomarkers of Proarrhythmic Risk in Nonclinical Models: Historical Data Evaluation by the HESI Consortium

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INTRODUCTION

The QT interval remains the most important biomarker that can be used by safety pharmacologists to evaluate drug-induced proarrhythmic risk. However, emerging clinical data have suggested that comprehensive T-wave morphology analysis (Vincente et al., 2015) provides additional information on proarrhythmic risk. This study assessed the use of the interval from the J-point of the QRS interval to the T-wave peak (J-Tp) (early repolarization) and the duration from the peak to the end of the T wave (Tp-e) (late repolarization) as potential additional complementary ECG proarrhythmia biomarkers in non-clinical species to drug-induced changes in the QT interval.

METHODOLOGY

ECG telemetry data derived from Beagle dogs and non-human primates (NHP) from studies conducted by members of the HESI consortium were analyzed using pattern recognition programs using a wide range of software platforms (EMKA ECGauto, DSI Ponemah and Notocordhem). J-Tp and Tp-e interval durations were quantified in experimental groups receiving vehicle or drugs with known pharmacological mechanisms of action such as cisapride, verapamil, medetomidine, moxifloxacin, sotalol and thioridazine. Individual heart rate correction was conducted using the analysis of covariance method of QT correction, i.e., the Spence et al. (1998) method and applied to the QT, J-Tp and Tp-e intervals to calculate QTca, J-Tpca and Tp-Teca.

JTp : Interval between the J point and the T peak - TpTe : Interval between the T peak and T end

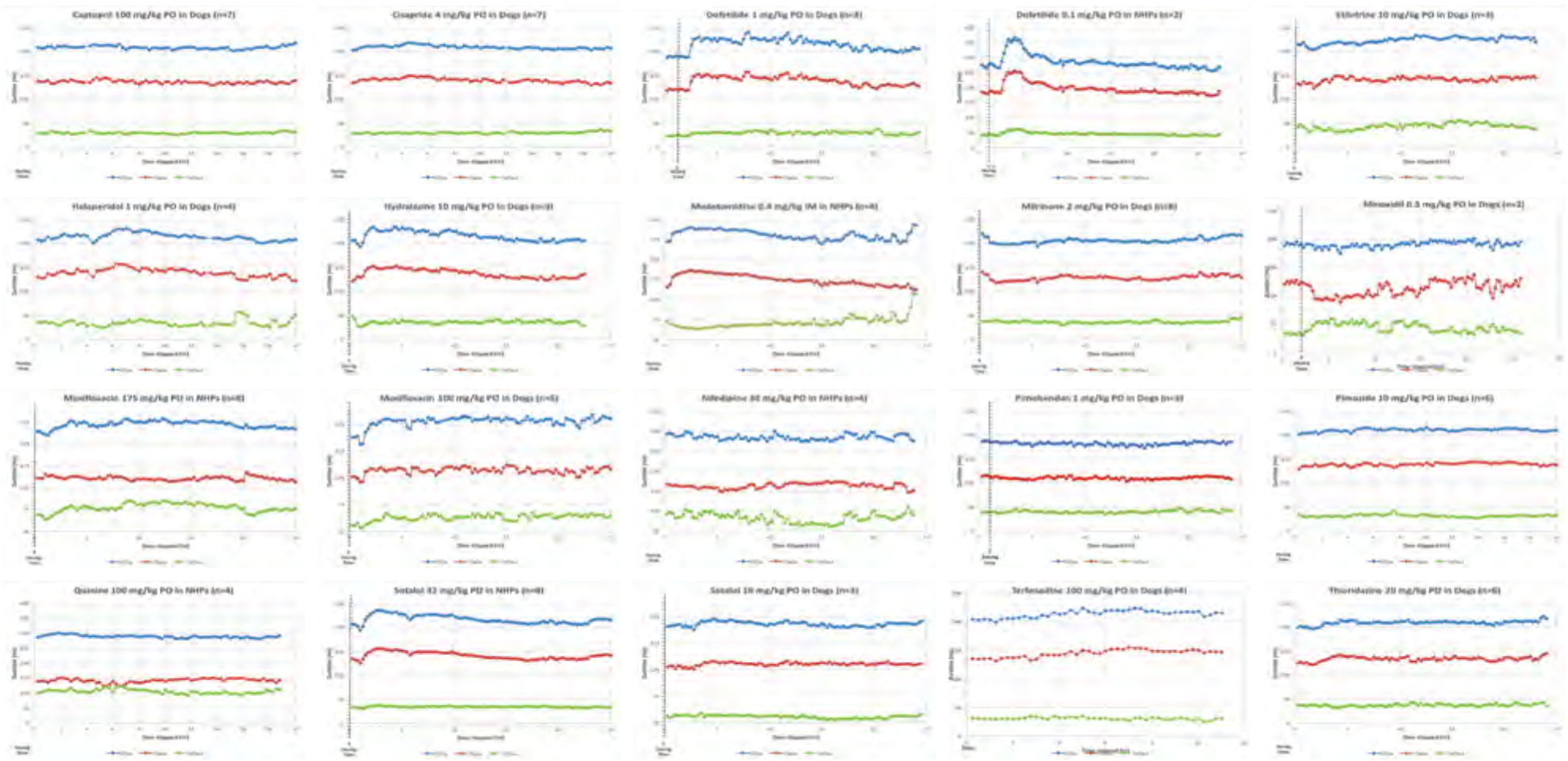

Table 1: Magnitude of Drug Response for Corrected QTca, J-Tpca and Tp-Teca Measures

DRUG [Dose]	SPECIES	Proarrhythmic drug (Y/N)	QTca Variation (Peak vs Baseline)	JTpca Variation (Peak vs Baseline)	TpTeca Variation (Peak vs Baseline)
ATENOLOL [10mg/kg] Novartis	DOG	N	-4%	4%	-10%
CAPTOPRIL [100mg/kg] Citoxlab	DOG	N	3%	5%	27%
ETILEFRINE [10mg/kg] Covance	DOG	N	9%	12%	32%
MEDETOMIDINE [0.4mg/kg] Citoxlab	NHP	N	14%	27%	-20%
MINOXIDIL [0.5mg/kg] Novartis	DOG	N	2%	-27%	79%
NIFEDIPINE [30mg/kg] Citoxlab	NHP	N	-6%	-12%	-29%
PIMOBENDAN [1mg/kg] Novartis	DOG	N	-3%	-6%	13%
VERAPAMIL [15mg/kg] HESI	DOG	N	10%	16%	72%
VERAPAMIL [20mg/kg] Citoxlab	NHP	N	0%	-12%	20%
VERAPAMIL [30mg/kg] Covance	DOG	N	-6%	-15%	-31%
RANOLAZINE [150mg/kg] Citoxlab	NHP	N	9%	12%	17%
HYDRALAZINE [10mg/kg] Covance	DOG	N	14%	27%	32%
MILRINONE [2mg/kg] Covance	DOG	N	-9%	-13%	0%
L-NAME [20mg/kg] Covance	DOG	UNKNOWN	-8%	-12%	0%
CISAPRIDE [4mg/kg] HESI	DOG	Y	7%	11%	7%
DOFETILIDE [0.1mg/kg] Amgen	NHP	Y	34%	39%	51%
DOFETILIDE [0.3mg/kg] Roche	DOG	Y	26%	38%	19%
DOFETILIDE [1mg/kg] Amgen	DOG	Y	28%	33%	42%
HALOPERIDOL [1mg/kg] HESI	DOG	Y	11%	21%	-29%
MOXIFLOXACIN [100mg/kg] Covance	DOG	Y	19%	14%	61%
MOXIFLOXACIN [175mg/kg] Covance	NHP	Y	13%	5%	48%
PIMOZIDE [10mg/kg] HESI	DOG	Y	7%	10%	6%
SOTALOL [10mg/kg] Covance	DOG	Y	7%	7%	16%
SOTALOL [32mg/kg] Citoxlab	NHP	Y	9%	25%	-28%
SOTALOL [32mg/kg] Covance	NHP	Y	14%	15%	12%
TERFENADINE [100mg/kg] HESI	DOG	Y	10%	15%	0%
THIORIDAZINE [20mg/kg] Roche	DOG	Y	8%	14%	0%
QUININE [100mg/kg] Citoxlab	NHP	Y	5%	9%	16%

DISCUSSION AND CONCLUSION

All designated proarrhythmic drugs were associated with a QTca interval prolongation ranging between 5-34%. Seven out of 11 (64%) non-proarrhythmic drugs evaluated resulted in a QTca increase (defined as any increase in QTca, irrespective of statistical analysis outcome; range 2-14%). Five out of 7 (71%) non-proarrhythmic drugs resulted in a percent increase in Tp-Te that was superior to the percent increase found with the J-Tp measure. Medetomidine is an anesthetic drug that causes hypothermia which physiologically is expected to increase the QTca interval. If medetomidine is excluded from the analysis, 83% of non-proarrhythmic drugs associated with a positive change in the QTca interval presented with a higher percent increase in Tp-Te when compared to J-Tp. In comparison, four out of 9 (44%) proarrhythmic drugs presented with a percent increase in Tp-Te that was superior to the percent increase in J-Tp. These preliminary results suggest that non-proarrhythmic QTca prolongation in dog and nhp is associated with relatively larger effects on Tp-Te, wheras proarrhythmic QTca prolongation is associated with relatively larger effects on J-Tp. In addition, these results are generally consistent with the clinical data described by Vicente et al. 2015 and Johannesen et al., 2014. False positive results from non-clinical cardiovascular studies (i.e., drugs which prolong QTca, but cause a proportionally greater increase in TpTe compared to J-Tp) may result in discontinued development of potentially useful drugs due to possible safety concerns, and furthermore represents an economic burden to the pharmaceutical industry as additional resources and time are likely spent to identify suitable backup compounds. The usefulness of either J-Tp and/or Tp-Te as potential proarrhythmia biomarkers in non-clinical studies will need to be further evaluated with a larger drug database. In light of the variability observed when the same drugs were repeatedly evaluated (e.g., verapamil, moxifloxacin, sotalol, dofetilide) albeit from studies conducted at different labs using different recording methods/procedures, a comprehensive assessment using a standardized safety pharmacology model is recommended. The study should evaluate ECG monitoring methodologies (e.g., a standard Lead II vs. a cardiovectogram) along with drug exposure.

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