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Charge is an important determinant of hemodynamic and adverse cardiovascular effects of cationic drugs

Michael K Pugsley[a,*], Simon Authier[a], Michael J Curtis[b]

[a] CiToxLAB Research Inc., 445 Armand Frappier, Laval, QC H7V 4B3 Canada
[b] Cardiovascular Division, King's College London, Rayne Institute, St Thomas' Hospital, London SE17EH, UK

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ABSTRACT

Cationic compounds are diverse and atypical therapeutic substances. In the present study we examined whether a prototypical class effect of cationic drugs in the cardiovascular system exists and whether this might be predictable on the basis of chemistry. The dose-dependent effects of cationic compounds of varying molecular weights and charge were examined on the blood pressure (BP), heart rate (HR) and the ECG in anesthetized rats. The compounds examined were protamine, hexadimethrine, tetraethylammonium (TEA), low molecular weight poly-*L*-lysine (LMW-PLL) and high molecular weight PLL (HMW-PLL). All of the compounds examined except TEA produced a dose-dependent reduction in BP. No changes occurred in HR even when high doses were administered. The ECG effects of these cationic compounds included a dose-dependent prolongation of the QT interval, especially at higher doses. All compounds transiently decreased the size of the P-wave after i.v. bolus administration whereas only protamine and hexadimethrine prolonged the PR and QRS intervals and only at the highest dose (32 mg/kg) administered. All cationic compounds, except TEA and saline, evoked ventricular premature beats (VPB), and protamine and HMW-PLL also evoked brief episodes of ventricular tachycardia (VT). The incidence and frequency of arrhythmias was not dose-dependent and no animals experienced protracted episodes of arrhythmia incidence. These dose dependent effects of the polycationic compounds tested suggest a collective mechanism of action that relates the effect of charge of the compound to the onset and persistence of observed cardiovascular toxicity, and adverse cardiovascular effect risk appears to be predictable on this basis.

1. Introduction

In the present study we evaluated the cardiovascular actions of a range of cationic compounds in order to explore the possible existence of class effects (potentially beneficial or adverse). Although many cationic compounds have been developed they have a disparate spectrum of use within medicine and no 'class effect' whether advantageous or adverse has yet been characterized. The most widely-used cationic compound is protamine, a highly basic, polycationic protein that neutralizes the acidic charge of heparin and thus reverses its anticoagulant effects [1,2,3]. Protamine also exhibits adverse effects on the cardiovascular system including hypotension, vascular leak, bradycardia [4] and reduced contractility [5,6,7]. Furthermore, in vivo studies show that protamine dose-dependently elicits hypotension, alters the ECG and may cause arrhythmias [8,9,10].

A number of cationic compounds have been developed as pharmacological tools or as marketed products. Tetraethylammonium (TEA) is a quaternary ammonium compound that was originally used as a ganglion blocker [11] and then as a pharmacological research tool to characterize the neuromuscular junction as well as function of sympathetic nerve terminals in autonomic pharmacology. Studies have shown that TEA blocks voltage-gated neuronal potassium channels [12,13] such as the delayed rectifier family of currents (Kv1.x) with a range of potencies [14]. Hexadimethrine (Polybrene) is a cationic polymer used by researchers to improve the efficiency of retroviral cellular infection rates in cell culture [15,16] and can also be used to transfect mammalian cells with DNA [17]. PLL is a highly basic, cationic homo-polypeptide where the charge depends upon the molecular weight (or number of repeating units of *L*-lysine). Like hexadimethrine, it can be used as an attachment factor for histochemical procedures. PLL can be used to augment the electrostatic interaction that develops between

* Corresponding author.
E-mail address: mkpugsley@yahoo.com (M.K. Pugsley).

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Table 1
Some physicochemical properties and molecular charge of cationic compounds.

	MW (g/mol)	Charge	Dose Range (mg/kg)	Reference(s)
TEA	130	+1	1–128	[13]
Hexadimethrine	374	+58	1–32	[73,74]
Protamine	5,500–13,000[a]	≅+21–65	1–32	[1,10,75]
LMW-PLL	5,500–15,000[a]	≅+25–70	1–32	[76,77]
HMW-PLL	>30,000[a]	≥+144	1–8	[18,77]

[a] Molecular weight is based on viscosity determinations by the manufacturer (Sigma–Aldrich, LLC) according to Yaron & Berger [78]. All polyamino acid molecular weights are an average molecular weight and not absolute. PLL charge is determined from literature sources and is also based upon the number of L-lysine repeating units as per Alamanda Polymers (http://www.alamanda-polymers.com/). TEA = tetraethylammonium; LMW-PLL = Low Molecular Weight Poly-*L*-Lysine; HMW-PLL = High Molecular Weight Poly-*L*-Lysine.

the negatively-charged cell membrane protein components and positively-charged cell culture surface [18]. Like most cationic proteins, PLL is polydisperse meaning that in aqueous solution it is a composition of multiple molecules in solution. There is some indication that cytotoxicity of cationic compounds is correlated with molecular size and charge [19,20] a phenomenon that is typically observed with common cytotoxic drugs [21]. However a class profile has yet to be elaborated.

In the present study we characterized the dose-dependent effects of several cationic compounds of varying MW and molecular charge on BP, HR and the ECG of anesthetized rats. The compounds examined were protamine, hexadimethrine, TEA, LMW-PLL (5.5–15 kDa) and HMW-PLL (>30 kDa).

2. Materials

Male Sprague-Dawley rats (Charles River Labs, Hollister, CA) weighing 200–300 g were used for studies. All studies conducted were performed according to the guidelines established by the Institutional Animal Care & Use Committee (IACUC), the American Association of Laboratory Animal Sciences (AALAS) [22] and the EU Directive 2010/63/EU. The study design and animal ethics conform with ARRIVE [23] and more recent guidance on experimental design and analysis [24]

3. Animal preparation

In vivo dose-response curves were constructed (Table 1). Male Sprague-Dawley rats were randomly selected weighed and administered an intraperitoneal injection of anesthetic (65.0 mg/kg sodium pentobarbital at dose volume of 1.0 mL/kg). The trachea was cannulated for artificial ventilation (at a stroke volume of 12 mL/kg and rate of 60 strokes/min) and polyethylene catheters were implanted into the right jugular vein and left carotid artery of each animal. Cannulation of the right jugular vein allowed for administration of test articles while cannulation of the left carotid artery allowed for recording of BP and whole blood collection. The ECG was recorded in a Lead II configuration [25]. Silver wire needle electrodes were placed along the anatomical axis (right atrium to apex) of the heart as determined by palpation. All animals were monitored post-surgically prior to administration of test articles, for approximately 10 min. Animal body temperature was maintained between 35 and 38 °C using a heating pad and rectal thermistor (Digital Long Probe Thermometer, Nasco, Fort Atkinson, WI)

4. Dose administration

Animals randomly received either an intravenous (i.v.) injection of 0.9% phosphate buffered saline (PBS) or cationic test article administered as bolus doses through the jugular vein cannula. Test article dosing began 5 min after blood samples were obtained for determination of the pre-dose (i.e., time 0) packed red cell volume. Cumulative dose-response curves were constructed for each compound until a maximum tolerated dose was achieved (Table 1). The maximum tolerated dose achieved was defined as that resulting in a mean BP reduction to 25 mmHg or marked conduction difficulties in the heart resulting in arrhythmias. If neither effect occurred the dosing was terminated once the required dose volume of a single dose of injected test article exceeded 1.5 mL. ECG changes, BP and HR were monitored for 10 min after each bolus dose, whereupon the next dose was administered. At the end of dosing, a second approximate 0.5 mL sample of whole blood was collected for determination of packed red cell volume.

5. Packed red cell volume (Hematocrit)

The packed red blood cell volume was determined before test article administration and upon completion of the dosing interval [10]. Approximately 50 μL of whole blood was collected using non-heparinized glass microcapillary (hematocrit) tubes. The tubes were placed in Seal-Ease®, a microhematocrit tube sealer and holder (Becton Dickinson, Franklin Lakes, NJ) at room temperature for 15 min before being microcentrifuged using an IEC MB microcentrifuge (Damon/IEC, Needham Heights, MA). The packed red blood cell volume of the sample was determined from the 50 μL sample of whole blood collected using a CRITOCAPS™ microhematocrit capillary tube reader (Oxford Labs, St. Louis, MO).

6. Arrhythmia analysis

Arrhythmias were categorized according to guidelines established by the Lambeth conventions [26,27]. VPBs were defined as single QRS complexes which occurred before any identifiable P wave. Doublets (bigeminal) or salvo (runs of 2 or 3 VPBs) variations in the single complex were not classed as distinct arrhythmias but rather were summed for each group [26]. VPB occurrence (number of VPBs per animal) was $\log_{10}$ transformed to a normal distribution [28]. VT was defined as 4 or more consecutive VPBs and not sub-classified according to rate. VT incidence was classified by characteristic changes in ECG morphology, typically accompanied by step function elevation in HR and fall in mean BP. Ventricular fibrillation (VF) was defined as a sequence of at least 4 consecutive ventricular complexes without intervening diastolic pauses, in which the intrinsic shape, peak–peak interval and height vary, and the variation between each is non-progressive [26].

7. Drugs

Tetraethylammonium (PubChem CID: 5413), Protamine (CAS Number: 9007-31-2), hexadimethrine (1,3-dibromopropane;N,N,N′,N′-tetramethylhexane-1,6-diamine; PubChem CID: 24769), LMW-PLL (Poly[imino](2S)-2-amino-1-oxo-1,6-hexanediyl; PubChem CID: 53628747) and HMW-PLL (Poly[imino](2S)-2-amino-1-oxo-1,6-hexanediyl; CAS Number 26124-78-7) were purchased from Sigma–Aldrich Co. LLC (St. Louis, MO). Note that the PLL molecules used in these studies are positively charged amino acid polymers with approximately one HBr or HCl per lysine residue. The HBr or HCl allows the PLL to be in a crystalline form soluble in phosphate buffered saline (PBS). All drugs were dissolved in PBS immediately prior to administration. Table 1 provides details on some physicochemical and charge properties of the cationic compounds selected for evaluation. These cationic compounds have only minimal pharmacology literature available (Table 1) but were easily purchased for use.