Journal of Controlled Release 198 (2015) 62–70

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Multi-modal contributions to detoxification of acute pharmacotoxicity by a triglyceride micro-emulsion☆,☆☆,★

Michael R. Fettiplace [a,b], Kinga Lis [a,b], Richard Ripper [a,b], Katarzyna Kowal [a,b], Adrian Pichurko [a,b], Dominic Vitello [a], Israel Rubinstein [b,c], David Schwartz [a], Belinda S. Akpa [d,*], Guy Weinberg [a,b,*]

[a] Department of Anesthesiology, University of Illinois College of Medicine, 1740 West Taylor Street, Suite 3200 W, MC515, Chicago, IL 60612, United States
[b] Research & Development Service, Jesse Brown Veterans Affairs Medical Center, 820 S. Damen Avenue, 60612, United States
[c] Section of Pulmonary, Critical Care, Sleep and Allergy Medicine, Department of Medicine, University of Illinois College of Medicine, 840 South Wood Street (MC 719), Room 920-N CSB, Chicago, IL 60612, United States
[d] Department of Chemical Engineering, University of Illinois at Chicago, 810 S. Clinton Street, Chicago, IL 60607, United States

ARTICLE INFO

Article history:
Received 3 September 2014
Accepted 17 November 2014
Available online 4 December 2014

Keywords:
Lipid emulsion
Local anesthetic
Bupivacaine
Triglyceride
Antidote
Biodetoxification

ABSTRACT

Triglyceride micro-emulsions such as Intralipid® have been used to reverse cardiac toxicity induced by a number of drugs but reservations about their broad-spectrum applicability remain because of the poorly understood mechanism of action. Herein we report an integrated mechanism of reversal of bupivacaine toxicity that includes both transient drug scavenging and a cardiotonic effect that couple to accelerate movement of the toxin away from sites of toxicity. We thus propose a multi-modal therapeutic paradigm for colloidal bio-detoxification whereby a micro-emulsion both improves cardiac output and rapidly ferries the drug away from organs subject to toxicity. *In vivo* and *in silico* models of toxicity were combined to test the contribution of individual mechanisms and reveal the multi-modal role played by the cardiotonic and scavenging actions of the triglyceride suspension. These results suggest a method to predict which drug toxicities are most amenable to treatment and inform the design of next-generation therapeutics for drug overdose.

Published by Elsevier B.V.

1. Introduction

Micro- and nano-emulsions are frequently studied as drug delivery systems for therapeutic applications [1,2] with the aim of achieving more efficient or targeted delivery. The converse principle of biodetoxification by drug-scavenging-agents is of increasing interest to drug-delivery scientists based on the clinical utility of absorptive properties [3,4]. The need for efficient detoxification agents is clear as drug-overdoses account for thousands of deaths annually in the United-States [5] and around the world, most-often in a young and healthy population. Recently, micro-emulsions of triglycerides and phospholipids (most commonly Intralipid®) have been used clinically to treat pharmacotoxicity from tricyclic-antidepressants, atypical antipsychotics, serotonin antagonist and reuptake inhibitors, selective-serotonin reuptake blockers, calcium channel blockers, cocaine, local anesthetics and other agents [6,7]. However, without a clear understanding of their mechanism of action, some reservations remain as to their specific applicability [8–12]. The primary mechanism is often asserted to be an intravenous partitioning phenomenon, an effect which is supported by *in vitro* binding studies [13–15], isolated heart experiments [16], small animal experiments [17,18] and clinical reports [19]. This toxin-binding mechanism is both intuitive and relatively simple, and thus has stimulated efforts to optimize therapeutic formulations (*e.g.* stealth, anionic liposomal preparations) with optimized binding functionality [3,20–22]. Despite the promise of efficient drug scavenging, *in vivo* experiments demonstrate that tailored liposomes do not reverse cardiovascular toxicity as effectively as triglyceride-based emulsions during acute intoxication [23]. Further, trials in healthy volunteers dispute that a drug-scavenging effect occurs at all [24,25], and computational models assert that a scavenging effect alone cannot account for the rapid recovery from toxicity typically observed following emulsion administration [26]. A larger problem in

☆ Meeting: Presented, in part at the 2013 Resuscitation Science Symposium (ReSS) Conference, Dallas, TX November 2013.
☆☆ Conflicts of interest: Guy Weinberg holds a US Patent related to lipid resuscitation. Guy Weinberg & Israel Rubinstein are co-founders of ResQ Pharma, LLC.
★ Author contributions: MRF, IR, BSA and GW conceived the experiments. MRF, KL, RR, KK, AP, GW performed physiological experiments, gathered data and performed analysis. BSA developed and executed the in silico model. MRF and AP performed biochemical analyses. MRF assembled figures and drafted the manuscript. All authors contributed to revisions.
* Corresponding authors.
E-mail addresses: akpa@uic.edu (B.S. Akpa), guyw@uic.edu (G. Weinberg).

http://dx.doi.org/10.1016/j.jconrel.2014.11.018
0168-3659/Published by Elsevier B.V.

the field is that the *in vivo* physiological effects of these delivery agents [27] and their influence on drug pharmacokinetics [28] are not well understood.

We recently demonstrated that a micro-emulsion of triglycerides produces an inotropic effect in the absence of toxicity [29], and further proposed that this effect coupled with the volume load is necessary for full recovery from cardiac pharmacotoxicity [30]. The prominent role of a non-scavenging effect in recovery and the ability of fatty-acid oxidase inhibitors to block emulsion-based recovery from pharmacotoxicity [31] call into question whether scavenging is relevant to recovery. In order to resolve these disparate results we combined *in vivo* pharmacokinetic experiments with a previously developed physiologically-based pharmacokinetic pharmacodynamic model (*in silico*) to assess the contributions of a cardiotonic effect, a scavenging effect and metabolic processing to the recovery from toxicity of a cardiotoxic agent with and without an adjuvant triglyceride micro-emulsion. Our results indicated the critical importance of a cardiotonic effect independent of cardiac drug concentration, the undisputable scavenging of drug by the lipid compartment and the acceleration of microsomal (*e.g.* liver) metabolism to increase processing and excretion of the drug. These long-postulated but previously unproven effects will help inform next-generation treatments for drug detoxification.

2. Materials and methods

2.1. Reagents

^{3}H-bupivacaine hydrochloride (97.4% pure, specific activity 2.87 Ci/mol) was purchased from Moravek biochemical & radiochemicals (Brea, CA). The intravenous lipid emulsion used was 30% Intralipid®, from Baxter Pharmaceuticals (Deerfield, IL). It is composed of 30% Soybean Oil, 1.2% Egg Yolk Phospholipids, 1.7% Glycerin, and water with pH adjusted to ~8 with sodium hydroxide. The oil is mostly triglycerides primarily composed of linoleic (44-62%) and oleic acid (19-30%) but also with palmitic (7–14%), linolenic (4–11%) and stearic acids (1.4–5.5%). Solvable and Ultima Gold Cocktail were purchased from PerkinElmer (Waltham, MA). NADPH colorimetric tests were purchased from Sigma Aldrich (St Louis, MO) and BioAssay Systems (Hayward, CA). Ketoconazole was also purchased from Sigma Aldrich (St. Louis, MO) and potassium chloride (KCl) was purchased from Fisher Scientific (Pittsburgh, PA). BSA protein assay was purchased from Thermo Scientific (Bradford, IL).

2.1.1. In vivo protocol

In brief, inhalational anesthesia was induced in a bell jar and rats were maintained on 1.2–1.75% isoflurane for the remainder of the experiment. Sixty-six animals (with two exclusions due to technical errors) received 10 mg/kg ^{3}H-bupivacaine hydrochloride into the left internal jugular over 20 s to produce a transient asystole followed 30 s later by either 10 mL/kg 30% intravenous lipid emulsion over 1 min or no intervention (Null). At pre-specified time-points (0.5, 1.5, 2.5, 4.5, 5.5, 8, 12, 60 min), animals were sacrificed by injecting 0.75 mL 1 M KCl solution into the right internal jugular vein. Four non-randomized animals received intraperitoneal injection of 25 mg/kg ketoconazole (dissolved in propylene glycol and 0.1 M HCl) 30-minutes prior to ^{3}H-bupivacaine challenge in order to inhibit cytochrome P450 3A4.

2.1.2. Tissue collection and processing

In animals treated for 60 min, 50 μL of arterial blood were collected at 5, 10, 15, 30, 45 & 60 min for subsequent analysis. Arterial blood was taken prior to sacrifice and organs were harvested. Blood was separated into clear plasma, red blood cell and lipid-plasma-component by ultracentrifugation. Organ (10–30 mg) and fluid (5–50 μL) samples were dissolved overnight in Solvable in scintillation vials at 55 °C including heart (apex), lung (left anterior lobes), liver samples (right lateral lobe & left lateral lobe), kidney (apical pole), spleen (anterior edge), brain (frontal lobe), cerebellum (whole), fat (omentum), skeletal muscle (adductor longus), lipid-free plasma, red blood cells, lipid-plasma component, whole blood, and urine. Dissolved tissues were suspended with Ultima Gold Cocktail and radioactivity was quantified (Tri-Carb 1600 TR Liquid Scintillation Analyzer, Packard, Meriden, CT) and reconciled to ^{3}H bupivacaine standards. Tissue bupivacaine content was normalized to tissue weight or fluid volume.

2.1.3. Pharmacokinetic analysis

Bupivacaine concentration was calculated in Excel (Microsoft, Redmond WA) and transferred to Prism 6.0 (Graph Pad, La Jolla, CA). Organ data were fit to a one-phase exponential decay with the plateau set to zero and a shared intercept. Fluid data was set to a two-phase exponential decay to account for redistribution and elimination. The x-axis of fluid data-fits is plotted on $\log_2$ scale to increase readability. Blood:organ plots were fit using a Boltzmann-sigmoidal function setting the lower limit to zero and setting a shared upper limit. Ratio of drug partitioned into tissue relative to blood was calculated for the V-50 point on the Boltzmann curves.

2.1.4. Statistical analysis

For inter-group comparisons, time points were grouped into 2, 5, & 10 minute time points (after confirming no differences between grouped time-points). Data were checked for normality using a D'Agostino & Pearson omnibus normality test, and for equal variances using Bartlett's test. Groups exhibiting normal distribution and equal variations were analyzed with a 1-way ANOVA and post-hoc Bonferroni multiple-comparison tests. For unequal variances, a Welches ANOVA with post-hoc Bonferroni multiple-comparison tests were used (in SAS). For non-normal distributions, groups were compared with a Kruskal–Wallis non-parametric ANOVA and post-hoc Dunn's multiple comparison tests. Total organ content and distribution were analyzed by the same metric.

2.1.5. Continuous analysis of physiological data

Continuous data at 1000 Hz were transferred to MatLab for analysis of the first 8-minutes ($n = 12$ per group). Data were down-sampled to 1 Hz and transferred to Prism 6.0 for plotting. To ascertain the cardiac bupivacaine concentration to flow relationship, one-phase exponential decay fit-curve parameters were identified and time of whole-number concentrations were identified (39–300 nmol/g). Using those time-points, corresponding carotid flow values were identified from both null (time = 0–20 min, $n = 4$), and ILE treated (0–12 min, $n = 7$). Subsequently, differences between groups were calculated using a continuous two-sided Mann–Whitney U-test.

2.1.6. In silico

The *in silico* approach is a computational model of the *in vivo* system that includes representations of the physiological (cardiovascular) and pharmacological (toxin & therapeutic) components. Physiological distribution and metabolism (pharmacokinetics) are coupled with a mathematical model of the toxin and therapeutic's effects on cardiac function (pharmacodynamics). Solution of the resulting differential equations predicts the time course of cardiac failure and lipid-mediated resuscitation as a function of hypothesized mechanism of therapeutic action. The results reported herein were obtained using an augmented version of the model described in reference [30]. Modifications made include: (i) adjustment of the lipid delivery timeline to correspond with the *in vivo* experiments; and (ii) incorporation of a non-competitive inhibition model for the interplay between bupivacaine toxicity and the cardiotonic effect of the lipid. The consequence of this latter modification is a decrease in the maximal effect constant for lipid inotropy by a factor of $(1 + C_{bup}/K_I)$ where the inhibition constant K_I is taken to be equal to the ion-channel

Download English Version:

https://daneshyari.com/en/article/1423846

Download Persian Version:

https://daneshyari.com/article/1423846

Daneshyari.com