Intralipid Infusion Ameliorates Propranolol-Induced Hypotension in Rabbits

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ABSTRACT

Objective: Recent case reports of successful amelioration of lipid-soluble drug toxidromes with Intralipid infusion have prompted interest in the scope of lipid emulsions as antidotal therapy. Propranolol is a highly lipid-soluble, nonselective beta-blocker with additional local-anaesthetic properties. We explored the hypothesis that propranolol toxicity may be similarly attenuated by Intralipid infusion in a rabbit model.

Methods: Twenty sedated, invasively monitored, and mechanically ventilated New Zealand White rabbits underwent propranolol infusion at 4.2 mg/min to a target mean arterial pressure (MAP) of 60% baseline MAP. Animals subsequently received 6 ml/kg 20% Intralipid, or 6 ml/kg 0.9% saline solution over a 4-minute period. Pulse rate and MAP were recorded at 2.5-minute intervals to 15 minutes.

Results: MAP was greatest in the Intralipid group (median 69 mmHg, interquartile range [IQR] 17.5 mmHg Intralipid vs. median 53 mmHg, IQR 12.75 mmHg saline; $p = 0.029$) at 15 minutes. No difference was observed in first derivative of MAP, or pulse rate between groups.

Conclusions: Propranolol-induced hypotension is ameliorated by Intralipid infusion in this intact rabbit model. The mechanism of action remains to be elucidated.

INTRODUCTION

Beta adrenoceptor blockers are among the most widely used cardiovascular drugs today. Propranolol is a highly lipophilic, non-selective beta-blocker with additional local anesthetic properties. In severe overdose bradycardia, seizures, and intractable cardiovascular collapse are common. While the amount of propranolol prescriptions issued has declined with the advent of more cardioselective beta-blockers, this agent remains a cause of mortality in human poisoning [1].

Propranolol's high lipid solubility and sodium channel blocking effect is thought to contribute to the observed increased

mortality when compared with other beta-blocker poisonings [1]. Conventional advanced cardiac life support in addition to glucagon administration [2], calcium infusion [3], phosphodiesterase III inhibition [4], high dose insulin infusion [5], intra-aortic balloon counter-pulsation [6], and extracorporeal circulation [7] have all been reported as potentially useful therapeutic modalities in overdose.

Case reports of successful resuscitation from cardiac arrest secondary to inadvertent intravascular local anesthetic [8,9] and intentional enteric bupropion/lamotrigine [10] overdose, have demonstrated clinical efficacy for lipid infusion in human lipophilic drug-induced cardiotoxicity. These follow animal

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studies by Weinberg et al. demonstrating amelioration of local anesthetic-induced cardiotoxicity with administration of lipid emulsions [11,12]. We have demonstrated attenuation of nonlocal-anesthetic lipophilic drug toxidromes with lipid infusion in animal models as well. To date, this includes amelioration of thiopentone-induced respiratory depression [13], increased survival and doubling of LD50 (median lethal dose) in rodent verapamil toxicity [14], and reversal of clomipramine-induced hypotension [15] with Intralipid infusion. Moreover, in a preliminary study we have demonstrated reduced QRS prolongation and trend toward amelioration of bradycardia with Intralipid infusion in a rat model of propranolol toxicity [16].

Two mechanisms of action have been proposed as the basis for the observed beneficial effects of lipid infusion in lipid-soluble drug toxidromes. Weinberg et al. have postulated a “lipid sink” whereby lipophilic drugs are sequestered in a newly created intravascular lipid compartment, thus reducing tissue binding [12,17]. Alternatively, a direct inotropic action may be effected via increased myocardial uptake and utilization of free fatty acids and phospholipids as greater than 70% of myocardial energy requirements are provided via lipid metabolism [18].

Propranolol’s high lipophilicity and sodium channel blocking effect suggest it may be an appropriate agent to further explore the potential benefit of lipid infusion in overdose. This study was therefore undertaken to investigate the hypothesis that propranolol toxicity may be ameliorated with infusion of a lipid emulsion in an intact animal model. Specifically, we compared Intralipid versus saline infusion in a rabbit model of propranolol-induced hypotension.

MATERIALS AND METHODS

Study Design

This was a prospective, randomized, controlled animal experiment evaluating Intralipid infusion in a rabbit model of propranolol-induced hypotension, utilizing mean arterial pressure (MAP) as the primary outcome variable. The experiment was undertaken at the Small Animals’ laboratory facility of the Ruakura Animal Research Center, Hamilton, New Zealand. The study was approved by the Ruakura Animal Ethics Committee. Animal husbandry was in accordance with the Ruakura guidelines for ethical animal research.

Sample size estimation was powered for parametric (Student’s *t*-testing) comparison of MAP at 15 min, and based on data from our previous work in rabbits [15]. This suggested a 1.6 standard deviation difference in MAP (corresponding to 25 mmHg in humans and deemed to be clinically significant) would be demonstrated with 10 animals in each group with power set at 80%, at the 5% level of significance.

Study Protocol

Rabbits were raised in single-sex conditions, with no chance of pregnancy. All animals were housed with free access to food and water in a temperature-, humidity-, and light-controlled environment with standard 12-hour reverse day/night cycle.

On the day of utilization, animals were weighed to the nearest gram then cluster randomized according to age and sex to 0.9% saline solution, or Intralipid (isotonic emulsion of 20% soybean oil in water) treatment arms. Animals were sedated with ketamine at 50 mg/kg and xylazine at 4 mg/kg by intramuscular injection according to institutional protocol. After venous cannulation of the marginal ear vein, continuous intravenous anesthesia (ketamine 10 mg/ml, xylazine 1.5 mg/ml at 1.5 ml/kg/hour) was commenced and continued for the duration of the experiment. Additional 1-ml boluses of anesthetic solution were delivered manually on evidence of animal distress during invasive procedures. Three lead electrocardiogram (ECG) was sited for continuous monitoring of heart rate and rhythm.

Animals subsequently underwent arterial catheterization of the central artery of the contralateral ear (connected in standard fashion [Edwards Lifesciences pressure transducer, Irvine, CA] to Hewlett-Packard 78834A neonatal monitor), and formal tracheostomy with endotracheal intubation (truncated [50 mm] 3.5-mm internal diameter endotracheal tube) under direct vision. Nuffield series 200 pediatric ventilator (Penlon Ltd, Abington, England) provided an inspiratory flow rate of 0.25 L/min 100% oxygen at 12 breaths per minute with 5 cm H₂O positive end expiratory pressure. Ventilatory adequacy was assessed in all animals via continuous color-change capnography (Pedi-Cap end tidal CO₂ detector, Nellcor Puritan Bennett Inc, Pleasanton, CA). Vecuronium 0.1 mg/kg was administered following the establishment of mechanical ventilation and repeated as necessary to maintain paralysis.

Following a period of stabilization (range 3–5 minutes), propranolol infusion (12.5 mg/ml at 20 ml/hour) was commenced and continued to target MAP of 60% baseline MAP. At this endpoint propranolol infusion was discontinued and 6 ml/kg 0.9% saline solution, or 6 ml/kg 20% Intralipid was infused over a 4-minute period. Six mL/kg was chosen in line with the clinical recommendation of Weinberg in local anesthetic induced arrest [19].

Hemodynamic parameters (pulse rate, MAP) were collected at baseline (time zero); target MAP (nominal time one minute); midway and at the completion of saline/Intralipid infusion (3 and 5 minutes, respectively); and at 2.5-minute intervals to 15 minutes. At completion all animals were killed by an intravenous bolus administration of 3 mL (300 mg/mL) pentobarbitone according to institutional protocol.

Outcome measures/Analysis

Comparison of MAP served as the primary outcome measure. Pulse rate and first derivative of MAP were analyzed as variables of interest. Statistical analysis of all variables was performed using SPSS for Windows (version 10.0, SPSS, Chicago, IL). The Shapiro-Wilk test was used to assess for normality. Paired Student’s *t*-testing and Mann-Whitney analysis were utilized as appropriate to evaluate differences in continuous variables. Chi-squared testing was used to evaluate dichotomous outcomes. A *p* value of less than 0.05 was deemed significant.

RESULTS

Ten animals were used in each of the saline and Intralipid arms of the study protocol. Baseline animal characteristics and sedation dosing are presented in Figure 1. Propranolol dosing to toxicity was 8.63 ± 2.60 mg/kg and 8.42 ± 2.12 mg/kg in the saline and Intralipid groups, respectively.

The Shapiro-Wilk statistic for MAP in the Intralipid-treated group was 0.843 ($p=0.052$) and 0.866 ($p=0.094$) at 5 and 15 minutes, respectively. Subsequent comparison of MAP data was conducted using Mann-Whitney testing. MAP was greater in the Intralipid-treated group at 15 minutes (median 69 mmHg, interquartile range [IQR] 17.5 mmHg Intralipid vs. median 53 mmHg, IQR 12.75 mmHg saline; $p=0.029$). A trend toward increased MAP in the Intralipid group was observed at 12.5 minutes (median 68 mmHg, IQR 15.25 mmHg Intralipid vs. median 55 mmHg, IQR 9.75 mmHg saline; $p=0.052$, Figure 1). No statistically significant difference in first derivative of MAP was observed between groups.

Pulse rate at toxicity was 206 ± 30 bpm and 206 ± 11 bpm in the saline and Intralipid groups, respectively. No statistically significant difference in pulse rate was observed between groups (197 ± 28 bpm saline vs. 172 ± 35 bpm Intralipid [$p=0.095$] at 5 minutes, 192 ± 28 bpm saline vs. 185 ± 44 bpm Intralipid [$p=0.677$] at 15 minutes).

LIMITATIONS

Our study has a number of limitations. The principal investigators were unblinded to the study intervention, therefore potential for observer or information bias exists. Collated endpoints were objective, however, and transcribed directly from monitoring equipment in a systematic fashion.

The inability of this study to demonstrate greater differences in hemodynamic parameters between groups may be secondary in part to deficiencies in the model of toxicity utilized. First, administration of intravenous propranolol over a short period (mean 5.4 minutes) denotes rapidly progressive toxicity. In this instance, initial drug redistribution following abrupt cessation of infusion may have contributed significantly to the observed improvements in MAP irrespective of treatment employed. Second, the endpoint chosen to define toxicity may have been insufficiently injurious to demonstrate greater treatment effect. Previous experimental series demonstrating rescue from cardiovascular collapse have utilized animal models of pharmacological cardiac arrest [12], and ongoing drug infusion [14]. Moreover, case reports citing successful resuscitation outcome with lipid infusion have occurred after human cardiac arrest secondary to inadvertent local anesthetic toxicity [8,9]. In addition, we have demonstrated the efficacy of lipid infusion in rabbits following infusion of clomipramine to 50% baseline MAP, and immediately

Figure 1: Boxplot of MAP vs. time (O, Outlier)

prior to arrest [15]. In the present study, however, we elected to use a lesser toxic endpoint following initial dose ranging experiments ($n = 4$) that demonstrated precipitous cardiovascular collapse with associated animal demise at MAP reduction of 50% or below. Third, improvements in hemodynamic performance may have been augmented by the known sympathomimetic effect of ketamine, utilized as sedation. We nonetheless elected to use this agent in line with both institutional protocol and our previous experience denoting minimal observed effect with ketamine infusion in a similar model of tricyclic antidepressant toxicity [15].

Sample size estimation in the present study was based on a greater expected differential in MAP between the control and treatment groups. This assumption was based on comparable solubility profiles of propranolol and the agents with demonstrated efficacy for lipid treatment, in addition to our recently published work examining hemodynamic performance following clomipramine-induced hypotension in rabbits. Our reported results, however, indicate inadequate power to detect lesser differences in MAP earlier in the study protocol. Nonetheless, these data may be gainfully employed to power future studies investigating the role of lipid infusion in propranolol-induced cardiotoxicity.

The observed beneficial effect of lipid infusion has been documented to 15 minutes only in this experiment. While this interval is of some relevance in the clinical scenario of human poisoning, the potential for delayed toxicity secondary to later drug redistribution has not been explored. Additionally, the potential for lipid-induced adverse events has not been investigated in this study. Notably, lipid emulsions have been shown to precipitate decreased cardiac output and increased pulmonary capillary wedge pressure when infused after coronary bypass surgery in humans [20]. Recent case publications following human application report no such sequelae, however [8,9].

Finally, the present study is by nature solely demonstrative, and thereby cannot elucidate the beneficial mechanism of action of lipid infusion in this model.

DISCUSSION

Intralipid administration resulted in greater improvements in blood pressure compared with saline solution in this rabbit model of propranolol-induced hypotension. This finding is consistent with published reports of amelioration of lipophilic drug cardiotoxicity with infusion of lipid emulsions [8–15]. Moreover, these findings are in accord with our previously reported amelioration of propranolol-induced QRS prolongation and bradycardia in rats [16].

In the present study, wide variation in the rapidity of MAP recovery was observed in the Intralipid-treated group. In 5 animals, MAP returned to >80% baseline at 5 minutes. Conversely, 5 animals demonstrated minimal improvement or deterioration during this period, with 4 of these demonstrating later recovery to MAP >90% baseline. These early MAP findings are not as uniformly striking as those reported for similarly

lipophilic agents. Studies demonstrating resuscitation from local anesthetic-induced cardiac arrest in animals [11,12], and similar case reports in humans [8,9] indicate extremely rapid response following infusion of lipid emulsion. Additionally, in rabbits rendered hypotensive secondary to clomipramine infusion, blood pressure restoration proved greatest in the first 5 minutes following Intralipid infusion [15].

Previous investigators have postulated sequestration of lipid-soluble drugs into a newly created intravascular “lipid sink” as the beneficial mechanism of action of lipid infusion. This hypothesis is supported by the high lipid solubility of agents with documented efficacy for lipid administration (bupivacaine log pK octanol:water 3.4 [21]; clomipramine log pK octanol:water 4.71 [22]; verapamil octanol:water partition coefficient 67.8 [23]) and the recent demonstration of increased bupivacaine removal from isolated rabbit hearts with Intralipid perfusion by Weinberg and co-workers [24]. Additionally, reduction in QRS interval prolongation with lipid infusion in clomipramine [15] cardiotoxicity, further suggest reduced drug-receptor binding attributed by the authors to drug-lipid sequestration. Propranolol is highly lipid soluble (log pK octanol:water 3.65 [25]) and as such the positive results observed in this study are consistent with this lipid sink hypothesis.

In our study, however, Intralipid infusion resulted in resolution of hypotension in the absence of significant deviation in pulse rate. This is counterintuitive to what might be predicted with an indirect effect of lipid, in which the expected consequence of propranolol-Intralipid sequestration would be attenuation of beta blockade-induced bradycardia. As such, an additional intrinsic inotropic effect of Intralipid is suggested.

A direct metabolic effect of lipid emulsions has been forwarded by previous investigators. Van de Velde et al. [26] have demonstrated improved recovery from myocardial stunning in conscious dogs following infusion of 20% Intralipid. The same authors further report increased contractile function in isolated rabbit hearts following myocardial stunning when Intralipid was administered during reperfusion. The beneficial observed effect correlated with increased high-energy phosphate content in the globally stunned myocardium and was attributed to augmentation of free fatty acid and phospholipid metabolism [27].

Furthermore, Intralipid infusion—both into the bypass perfusate and intravenously following return of coronary blood flow—has been shown to improve myocardial contractility, mean aortic pressure, and cardiac output in dogs undergoing cardiopulmonary bypass following 30 minutes of ischemic cardiac arrest [28]. A strong preference for, and aerobic use of, fatty acids as substrate has been demonstrated in isolated working swine hearts undergoing reperfusion post-stunning [29]. Myocardial substrate preference under conditions of increased lactate concentration is known to shift toward greater oxidation of free fatty acids [30]. Notably the majority of animal models, and to a lesser degree the present study, demonstrating hemodynamic improvement with lipid infusion follow significant hypotension and/or cardiac arrest wherein myocardial hypoperfusion is overtly manifest or may be inferred.

Additional investigators have observed a significant positive inotropic effect with lipid application in bupivacaine-induced cardiac depression in isolated rat hearts [31]. These authors attributed improved cardiac performance to a direct, albeit unidentified, effect of lipid perfusion. An indirect plasma-binding effect of lipids was not excluded, however. Similarly, the results of the current study may be due to more than one complementary mechanism of action of Intralipid.

The results of this experiment represent encouraging preliminary evidence in support of lipid infusion in propranolol cardiotoxicity. Currently available evidence, however, is insufficiently compelling to advocate clinical application in any but the most severe of human poisonings, in which all available alternative treatment modalities have been exhausted. Future studies to determine efficacy of lipid infusion in models of propranolol-induced cardiac arrest, in addition to the beneficial mechanism of action of lipid emulsions, are indicated.

CONCLUSION

Infusion of lipid emulsion attenuates propranolol induced hypotension in an intact rabbit model. These findings are consistent with re-establishment of plasma propranolol equilibrium favouring sequestration into a lipid sink. An additional intrinsic positive inotropic effect of Intralipid is not excluded.

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