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Research Article

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The Novel Development of an Experimental Model of Dihydropyridine Calcium Channel Blocker Poisoning using Intravenous Amlodipine

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Abstract

Background: Cardiovascular drug poisoning remains a leading cause of fatality. Within this class, calcium channel blockers (CCBs) account for the majority of deaths. CCBs are typically categorized as dihydropyridines (i.e. amlodipine or nifedipine) versus the non-dihydropyridine (i.e. verapamil and diltiazem) which are the most potent and once considered the CCB type responsible for all CCB-related deaths. Most recently, dihydropyridine deaths have increased. While there are established models of nondihydropyridine poisoning there currently are no established experimental models of dihydropyridine poisoning.

Methods: Electrocardiogram electrodes and intravenous lines were placed in anesthetized Spraque-Dawley rats. Various doses of amlodipine were administrated as a constant infusion to mimic continued gastrointestinal absorption. Intravenous amlodipine dosing was determined by the Dixon "up-and-down" method. Animals were observed for a total of two hours and death or survival was recorded.

Results: Various solvents were used such as tween and ethanol. Amlodipine was successfully dissolved in 20% DMSO. The maximum likelihood estimate for LD50 was 8.65 mg/kg (SE, +/- 2.67 mg/kg). Conclusions: A reliable experimental model of dihydropyridine poisoning using intravenous amlodipine is presented which will allow future studies concerning pathophysiology of shock from dihydropyridine poisoning and treatment.

Keywords

Cardiovascular drug; $LD_{50;}$ Dihydropyridine

Background

Cardiovascular drug poisoning remains a leading cause of fatality with Calcium Channel Blockers (CCBs) making up the vast majority [1]. CCB toxicity is typically due to the combination of negative inotropy and loss of vasomotor tone. CCBs are often categorized as dihydropyridines (i.e. amlodipine and nifedipine) versus the non-dihydropyridines (i.e. verapamil and diltiazem). The non-dihydropyridines are the most potent and were once considered the CCB type responsible for all CCB-related deaths [2,3]. Dihydropyridines such as amlodipine exert their primary action on calcium channels located in vascular smooth muscles demonstrating less toxicity on cardiac tissue than do verapamil and diltiazem and often used as peripheral vasodilators [4]. Many view dihydropyridines as significantly less toxic when compared to the non-dihydropyridines. However, severe dihydropyridine toxicity often mimics distributive shock similar to sepsis and anaphylaxis with significant toxicity and reported deaths despite maximal supportive care [5-11].

At this time there are no established experimental models of intravenous dihydropyridine poisoning. We designed a novel experiment to establish a first approximation for the dose required to kill 50% of the animals (LD_{50}) with intravenous amlodipine using the Dixon up-and-down method.

Methods

Experimental model

We performed this study using Sprague-Dawley rats. The rat model was chosen because it has previously been used to study calcium channel blocker toxicity with various experimental treatments such as high dose insulin-euglycemic therapy and intravenous fat emulsion therapy. The animal care and use committee of the institution approved this protocol, and the care and handling of the animals are in accordance with National Institutes of Health guidelines. All rats are cared for and handled according to the National Institute of Health guidelines.

Healthy Sprague Dawley rats weighting between 300-600 grams were anesthetized in an induction chamber with 5% isoflurane. A tracheostomy was also performed using a 14-gauge catheter under anesthesia with oxygen provided through a nose cone until the tracheostomy was performed and rat was attached to an Ohio V5A anesthesia ventilator and Ohio Modulus anesthesia machine (Ohmeda Corp., Helsinki, Finland). The neck was dissected and a catheter (22-gauge) was placed into the right carotid artery under direct visualization. This catheter was used for continuous blood pressure monitoring. A femoral venous catheter (24-gauge) was performed under direct visualization using a cut-down technique. The femoral vein was used for the amlodipine infusion using McGaw infusion pumps (Model 360 infuser, B. Braun Medical Inc., Bethlehem, PA). A three-lead ECG and arterial blood pressure tracings was recorded using a Power Lab 4/20 ML840 (ADI Instruments, Houston, TX). Prior to the start of the dosing portion of the study, various solvents were used to dissolve amlodipine (amlodipine besylate, 99.9% purity). Normal saline, tween, and DMSO solvents were used to attempt to dissolve amlodipine. DMSO was successfully used to dissolve amlodipine at 20% concentration.

Fifteen minutes after achieving venous access, rats were administrated intravenous amlodipine in the dosing regimen described below. The end-point for this study was either death or until the end of the 2-hour protocol where surviving rats was euthanized with Euthasol.

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Dixon's up-and-down method of estimating LD_{50}

LD_{50} was estimated using the up-and-down method as described by Dixon, which uses an iterative dose-selection algorithm. Starting with an initial exposure of 25 mg/kg, each subsequent dosage was raised or lowered based on the survival of the preceding animal. The maximum likelihood estimate for LD_{50} with SE was established using the following equation:

LD_{50} = average *(Xi)* + *d* / *N x (A + C)*, where average *(Xi)* is the average test level (in mg/kg) for the last *n* trials, *N* is the nominal number of samples or total number of samples, minus 1 less than the number of identical samples at the beginning of the trial, *A* and *C* values are acquired from Dixon's tables after the series of experiments are performed, and *d* is the distance between data points [11-14].

Results

Various vehicles were tested to dissolve amlodipine which was ethanol, tween, normal saline and DMSO. We were able to successfully dissolved amlodipine in 20% DMSO. Prior to the start of out experiment we tested various concentrations of DMSO ranging from 10% to 60% for the duration of our two hour protocol and found no changes in hemodynamics.

LD_{50}

The Dixon up-and-down method for N > 6 gave a maximum likelihood estimate for the LD_{50} of 8.65 mg/kg (SE, +/- 2.67 mg/kg). The distance, d, between data points was 5 mg/kg. The total number of samples was 10. The nominal number of samples, *N*, is the total number of samples, minus 1 less than the number of identical samples at the beginning of the trial.

Discussion

The usual method to obtain a LD_{50} is to perform a bio-assay experiment where a prescribed number of animals are tested at each of several fixed doses. This typically results in the use of a large amount of subjects which may be both time consuming and cost-prohibtive when a LD_{50} is not known. The up-and-down method described by Dixon, dose levels are determined in a sequential manner and in many cases reduces the amount of subjects needed as well as time to obtain an accurate LD_{50}.

At this time there are many experimental models of non-dihydropyridine poisoning used in various animals such as canine and rats. Our findings represent one of the first experimental models of dihydropyridine poisoning using intravenous amlodipine. There are currently no reliable experimental models of dihydropyridine poisoning. The applications are wide and can be used to study various treatment options such as high-insulin-euglyemic therapy.

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