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Cardioprotective Effects Of Calcium Gluconate And Dopamine On Acute Propranolol Toxicity In Mice

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Abstract

Acute beta-adrenergic blocker toxicity is an uncommon complication of acute and chronic cardiac failure treatment. Most acute cardiac toxicity studies use large animals. These studies use rats as animal model by giving propranolol intravenously. This assessed the utility of mice model in acute propranolol toxicity without intravenous cannulation and if calcium gluconate and dopamine can provide cardioprotection. Fifteen male Balb/c mice were assigned to NSS Group (NG; n=5), Calcium Group (CG; n=5) and Dopamine Group (DG; n=5). The NG, CG and DG received normal saline solution, calcium gluconate and dopamine intraperitoneally (*i.p.*) respectively. Thirty minutes later, propranolol 10 mg/kg BW was given *i.p.* All mice were observed and the time until demise was noted. Survival rates were presented. Statistical analysis was done using chi-square. Statistical significance was set at $p < 0.05$. All mice in CG group survived with 100% survival rate. The DG mice had 25% survival rate while the NG mice had 33% survival rate. This study showed that calcium gluconate and dopamine given intraperitoneally provided cardioprotection from acute propranolol toxicity. This model of giving propranolol intraperitoneally in mice is a simple method to follow in screening for cardioprotective properties of various natural products.

Keywords: Acute propranolol toxicity, calcium gluconate, dopamine, and mice model

1. Introduction

Cardiovascular diseases (CVD) are the number one cause of death globally according to the World Health Organization. This premise portrays that more people die from CVD than from any other cause annually.

As one of its pharmacological management, a class of pharmaceutical compound developed for CVD is beta-adrenergic receptor antagonists or beta-blockers. Beta-blockers, also known as beta-antagonists, or beta-adrenergic antagonists, are drugs that are prescribed to treat several different types of conditions, including hypertension (high blood pressure), angina, some abnormal heart rhythms, and myocardial infarction (heart attack). This drug targets beta-receptors found on the cells of the heart muscles and other smooth muscles stimulated by epinephrine. This causes weakening the effect of stress hormones. They slow down heartbeat, decrease the force of contractions of heart muscles, and reduce blood vessel contraction in the heart, brain, and as well as the rest of the body.

The first clinically useful beta-adrenergic receptor antagonist is propranolol, and has been used for nearly 50

years. In addition to their traditional role in treating hypertension and other cardiovascular disorders, beta-blockers are also used for additional purposes such as migraine headaches, hyperthyroidism, glaucoma, anxiety, and various other disorders (NHS Choices 2012). Because of their expanded use, the incidence of overdose with these agents has also increased. Beta-adrenergic antagonist toxicity can produce clinical manifestations including bradycardia, hypotension, arrhythmias, hypothermia, hypoglycemia, and seizures. Severe beta-blocker toxicity consists of bradycardia with associated hypotension and shock (BP <80mmHg and HR <60 bpm). Intermediate toxicity results in a moderate drop in blood pressure (BP <80mmHg) and heart rate (HR <60 bpm). The first critical signs of overdose can appear 20 minutes post ingestion but are more commonly observed within 1-2 hours after ingestion. All clinically significant beta-blocker overdoses develop symptoms within 6 hours. While the half-life of these compounds is usually short (2-12 hours), half-life in the overdosed patient may be prolonged because of a depressed cardiac output reducing blood flow in the liver and kidneys or because of the formation of active metabolites (Sharma 2013).

To counter this type of toxicity, certain substances provide cardioprotective effects such as calcium gluconate and dopamine. Calcium gluconate provides cardioprotection by stabilizing cardiac cell membranes against depolarization. On the other hand, dopamine produces positive chronotropic and inotropic effects on the myocardium, resulting to increased heart rate and contractility of the heart by exerting an agonist action on beta-adrenergic receptors (Kerns 2007).

In this study, intraperitoneal method of infusion of drugs was used, where substances were injected into the peritoneum (body cavity). In general, it is preferred that large amounts of blood replacement fluids are needed, or when low blood pressure or other problems prevent the use of a suitable blood vessel for intravenous injection. It also is used predominantly in veterinary medicine and animal testing for the administration of systemic drugs and fluids due to the ease of administration. However, the animal model used is rat, which is bigger than mice. It allows IV infusion in rats to continuously induce the effects of various agents for experimentation and allows wash-over period. However, this set-up is very tedious and costly.

In our parallel efforts to provide an alternative murine model like mice and avoid the more elaborate and expensive experimental methodology to perform drug discovery with cardioprotective potential using natural products in acute propranolol toxicity, this study aimed to assess the cardioprotective effect of calcium gluconate and dopamine on acute propranolol toxicity in mice using a simple methodology of introducing the median lethal dose of propranolol intraperitoneally.

2. Materials and Methods

2.1 Experimental Animal Use and Care

Five male Balb/c mice were used in the acute toxicity testing part of this study to determine the minimal dose to induce mortality within 30 minutes adapting the Organisation of Economic Co-operation and Development (OCED) Acute Toxicity Test guidelines with modifications (OECD 2000, FDA 2014) and fifteen male Balb/c mice were used in the main experiment part of the study. They were procured from the National Institutes of Health, University of the Philippines-Manila.

The male Balb/c mice were acclimatized in the laboratory under 18 0C and 70 % humidity for one week. The mice were caged individually. Standard food pellets and water were given ad libitum. They were kept in a 12-hour light and dark cycle. Cages were changed every other day with 1-2 inches layer of unscented autoclaved wood shavings as bed litters.

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2.2 Determination of Lethal Dose (LD_{50}) of Propranolol

A different set of mice was used for this part of the experiment. The concentration of propranolol solution for the acute toxicity study was determined by intraperitoneally injecting several mice with different concentration of propranolol (i.e. 2 mg/kg body weight, 4 mg/kg body weight, 6 mg/kg body weight and so on) and observing the mortality rate within thirty minutes. The lowest dose that resulted in mortality after intraperitoneal injection was considered the minimum lethal dose to be used in the subsequent main experiment. This was modified from the Acute Toxicity Testing advocated by the OECD.

2.3 Experimental Groupings and Treatments

The mice were randomly assigned to NSS Group (NG; n=5), Calcium Group (CG; n=5) and Dopamine Group (DG; n=5). They received NSS, calcium gluconate 10% or 100mg/ml given at 60 mg/kg body weight (Tianjin Jinyao, China) and dopamine 40 mg/ml given at 3.2 mg/kg body weight (Harman Pharmaceutical Laboratory, Lahore, Pakistan) intraperitoneally respectively. Thirty minutes later, a solution of propanolol HCL 10 mg/tablet given at 10 mg/kg body weight (Inderal™, AstraZeneca, Cambridge, England) was given intraperitoneally. All mice were observed and the time until demise was noted.

Those mice that survived were sacrificed via cervical dislocation. At the end of the study, the animal carcasses were disposed according to the protocol approved. The survival rates were computed.

2.4 Statistical Analysis

Survival rate was presented in percentage. Statistical analysis was done using chi-square. Statistical significance was set at $p < 0.05$.

3. Results

Ten mice were used in the determination of the dose to be used in acute toxicity testing. A dose of 10 mg/kg BW was determined to be the LD_{50} dose necessary to cause mortality of the mouse in 30 minutes in half of the population.

In the NSS group, only one mouse survived and the other four died after eight minutes (2 mice) and after twenty-six minutes (2 mice). This group has a 20% survival rate after administration of propranolol *i.p* . In the calcium gluconate group, all mice survived after the propranolol injection *i.p*. This group has 100% survival rate. While four of the mice survived in the dopamine group which has a survival rate of 80%. The observation time was 30 minutes.

The calcium gluconate and dopamine groups showed significant higher survival as compared with the NSS group ($p<0.05$). While the difference noted between the calcium gluconate and dopamine groups was not significant ($p>0.05$). The result is summarized in Table 1.

Table 1. Survival Rates of all the different groups

Groups	Percent Survival
NSS Group	0
Calcium Gluconate Group	100
Dopamine Group	80

NG vs. CG ($p<0.05$); NG vs. DG ($P<0.05$); CG vs. DG ($p>0.05$)

4. Discussion

Several researches have shown the toxic effects of administering increased amount of beta-blocker agents particularly propranolol (Reith et al. 1996, Cave et al. 2006). In addition to the lipophilicity of propranolol, the sodium channel blocking effect of propranolol when compared with other beta-blockers is considered contributory to the increased mortality (Cave et al. 2006). Propranolol is a nonselective beta-blocker, demonstrating

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equal affinity for both beta1 and beta 2 receptors. Beta 1 receptor blockade reduces heart rate, blood pressure, myocardial contractility, and myocardial oxygen consumption. While beta 2 receptor blockade inhibits relaxation of smooth muscle in blood vessels, bronchi, the gastrointestinal system, and the genitourinary tract. In addition, beta-adrenergic receptor antagonism inhibits both glycogenolysis and gluconeogenesis, which may result in hypoglycaemia (Sharma et al. 1994). The present research investigated on the utility of mice model in an acute cardiac toxicity study through intraperitoneal administration of propranolol. The concentration of propranolol solution for the acute toxicity study was determined by intraperitoneally injecting several mice with different amount of propranolol (i.e. 2 mg/kg body weight, 4 mg/kg body weight, 6 mg/kg body weight and so on) and observing the survival rate within thirty minutes. The propranolol concentration of 10 mg/kg body weight was observed to induce death among mice within a thirty-minute observation time. The information obtained from this procedure is useful in choosing doses for repeated-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Also, this dose was used for the succeeding part of the experiment.

A similar study done in rats has shown that rat hearts exposed to high Ca^{++} medium showed a lesser decline in the myocardial contractility after the beta-blocker administration as compared to those rat hearts exposed to normal Ca^{++} medium. In terms of the heart rate and the AV conduction time, the results were similar between the two preparations. This experiment concluded that Ca^{++} concentration therefore affects the contractility of the rat hearts (Langemeijer et al. 2013). In a study using canine model, CaCl improved the subjects hemodynamic particularly the hearts inotropic ability as compared to those subjects that received saline solution (Love et al. 2013). Calcium gluconate increases the Ca^{++} level necessary to counter the effect of propranolol. The propranolol blocks the Ca^{++} channels leading to the decrease release of Ca^{++} from the sarcoplasmic reticulum, which causes the actin myosin contraction (Su & Weiselberg 2010). Based on results of this study, calcium gluconate, having 100 % survival rate, once given intraperitoneally. This agent can provide a significant cardioprotective effect on mice suffering from a propranolol-induced cardiac toxicity.

Dopamine is an alpha-adrenergic agonist as well as a beta 1 selective adrenergic agonist. It acts directly on sympathetic nerve terminals and causes the release of norepinephrine. It mediates vasoconstriction in blood vessels and through the beta1 -receptor. It potentiates positive inotropy in the heart. Small scales studies on the use of intermittent dopamine infusions and oral levodopa provided initial evidence for symptomatic benefits in patients with severe heart failure. (Rajfer et al. 1987) In another study however, the combination of dopamine and glucagon resulted in decreased survival in propranolol poisoned rabbits and rats, despite a temporary improvement in heart rate and mean arterial blood pressure (Toet et al. 1996). It was evident in this study that there is a difference between the effects of calcium and dopamine as cardioprotectants. Administration of calcium has provided 100% cardioprotective effect while the use of Dopamine has resulted to only 80% survival rate. This study also showed that small animals like mice could be used as suitable animal model. This can be easily prepared and set up. As shown in this study, acute propranolol toxicity resulting in heart failure and death without intravenous administration of propranolol can be introduced though intraperioneal route. This methodology also does not require anesthesia. The more difficult set up of using bigger animal like rats and rabbits and intravenous administration of propranolol and test

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compounds requires anesthesia and sophisticated hemodynamic measurement and wash-out period for each experiment. The methodology used in this study was easy to perform and may be used for exploratory study for multiple drug discovery effort using numerous natural products**.**

5. Conclusion and Recommendations

This study showed that calcium gluconate and dopamine have significant cardioprotective effects against acute propranolol toxicity. The administration of calcium gluconate and dopamine significanty reduces the mortality rate of mice given intraperitoneally. Moreover, the efficiency of Calcium gluconate in counteracting the toxicity of propranolol is far more pronounced than dopamine. Calcium gluconate and dopamine administered intraperitoneally may serve as cardioprotective agents in acute toxicity studies utilizing mice model. Moreover, this study demonstrated the use of mice model and intraperitoneal route of drug administration in screening for cardioprotective properties of various pharmaceutical and natural products.

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