The Effect of Digoxin on the Recovery Rate of the Heart after Exercise in Mice.

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ABSTRACT
The effect of Digoxin on the recovery rate of the heart after exercise was studied in twelve mice. Digoxin is one of the cardiac glycosides, a closely related group of drugs that have in common specific effects on the myocardium. The beneficial effects of Digoxin result from a direct action on cardiac muscle, as well as indirect action on the cardiovascular system mediated by the effects on the autonomic nervous system. Of these Digoxin has two main effects with two different mechanisms of action. The first is the cholinergic effect (vagomimetic action) which slows the heart rate. This is responsible for the effects of Digoxin on the Sinoatrial and Atrioventricular nodes. This is all accomplished through a central mechanism that increases the cholinergic stimulation of the heart. This causes a decrease in conduction through the SA node. The second is the positive inotropic effect. This is a competition between potassium and digoxin. These compete for binding sites on an enzyme, referred to as potassium-ATPase. By blocking potassium from binding to the enzyme, Digoxin causes the heart muscle to be exposed to calcium for a longer period of time resulting in the heart contracting more forcefully. This in turn makes the heart a more efficient pump. In this experiment there were two groups a control and experimental. A carefully calibrated dose of a placebo or Digoxin was administered to the control and experimental groups respectively. The mice then had their resting heart rate measured. They were then exercised and their recovery rate of the heart back to its resting heart rate after exercise was measured. The effects of Digoxin was used to show that there was a significant difference, a decrease in time, in the rate of recovery of the heart after exercise in the Placebo vs. the Experimental groups in two separate trials. This was concluded with 99.74% confidence.

Keywords: Digoxin, Heart Rate, Exercise

INTRODUCTION
The heart is a valved muscular pump that propels blood around the body. This muscle is the key to the function of all of the major systems of the body. The autorhythmic cells of the heart, especially the sinoatrial node and the atrialventricular node, regulate the heartbeat. The heart rate is the measure of these beats in a one minute time period. Heart rate is easy to measure and the main determinant of cardiac output during exercise (Horstmann and Konn 1993-1994). A three-phase relationship has been demonstrated between increasing heart rate and cardiac output at rest. Phase I with cardiac output increasing with increasing heart rate, Phase II a plateau, and Phase III decreasing cardiac output without any further increase in the heart rate (Payne et al. 1997). Then this source continues to state that the, “optimal rate”, can be defined as the rate at the onset of Phase II.

This three-phase relationship applies to Mus domesticus CD 1 strain, the mouse. In a study (P:\S\L Consulting Group Inc. 1997), Dr. Jeffery Leiden, cardiology section chief at the University of Chicago, and his team of genetic researchers recently identified several genes that control mouse heart development and function. By manipulating these gene sequences, researchers at the university have successfully produced mouse models of human heart disease, such as congestive heart failure—a debilitating disease that affects an estimated two million Americans annually. Many ask, “Why Mice?” and this is because the genes that control the mouse heart development and function are similar to those that control the human heart. A mouse with congestive heart failure will display virtually the same physical symptoms as humans (P:\S\L Consulting Group Inc. 1997).

Digoxin is one of the cardiac glycosides, a closely related group of drugs that have in common specific effects on the myocardium (Glaxo Wellcome 1995). These drugs are found in a number of plants. Digoxin is extracted from the leaves of Digitalis lanata (Glaxo Wellcome 1995). It has the molecular formula C_{41}H_{64}O_{14}, a molecular weight of 780.95, and a melting and decomposition points above 235 °C (PDR 1995). This heart drug by Glaxo Wellcome has been around for many years. It was just recently, October 23 1997, that the Food and Drug Administration approved Digoxin 60 years after it was introduced to the U.S. market (Nando 1997). This was due to the fact that the drug was launched in the U.S. market in 1934, four years prior to the passage of the Food Drug and Cosmetic Act of 1938. General uses for Digoxin include the following; prevention of Atrial Fibrillation, and Paroxysmal Atrial Tachycardia. The most common side effects related to the use of Digoxin are toxicity and heart rhythm disturbances (InformationNetwork Inc. 1995-1997).
A few examples would include Arrhythmias, nausea/vomiting/anorexia, vision disturbance, and Central Nervous System effects.

The beneficial effects of Digoxin result from a direct action on cardiac muscle, as well as indirect action on the cardiovascular system mediated by the effects on the autonomic nervous system. Of these Digoxin has two effects with two different mechanisms of action. The first is the cholinergic effect or vagomimetic action, which slows the heart rate, this is responsible for the effects of Digoxin on the Sinoatrial and Atrioventricular nodes. This is all accomplished through a central mechanism that increases cholinergic stimulation to the heart. This causes a decrease in conduction through the SA node. Since the nerve which provides the heart with cholinergic activity is the Vagal nerve, this is often referred to as an increase in Vagal tone. The next is the positive inotropic effect. This effect results from competition between potassium and digoxin. These compete for binding sites on an enzyme, referred to as potassium-ATPase. So, digoxin is a potassium blocker or antagonist. By blocking potassium from binding to the enzyme, Digoxin causes the heart muscle to be exposed to calcium for a longer period of time resulting in the heart contracting more forcefully (Marshall Univ. School of Medicine 1995-1997). There is an increase in the force and velocity of myocardial systolic contraction (positive inotropiacl action) (PDR 1995). Gastrointestinal absorption of Digoxin is a passive process.

Absorption from an elixir formulation has been demonstrated to be 70 to 85 percent compared to an identical intravenous dose (PDR 1995). The distribution of Digoxin is as follows: following administration, a 6-8 hour distribution phase is observed. This is followed by a much more gradual serum concentration decline, which is dependent on digoxin elimination from the body (Glaxo Wellcome 1995). The approximate time to onset of effect and to peak effect for Digoxin is 0.5-2.0 hours and 2-6 hours respectively. Elimination of Digoxin follows first-order kinetics, that is the quantity of digoxin eliminated at any time is proportional to the total body content (PDR 1995).

There have been many studies involved with this drug. They mainly focus on the uses to prevent different heart disorders, such as congestive heart failure, atrial fibrillation, atrial flutter, and paroxysmal atrial tachycardia. I would like to focus on the use of this drug and exercise.

The overall purpose would be for people who have had heart attacks to be able to function better in everyday life without as much stress put on the heart. Also, that they can exercise and strengthen the heart without the high impact of stress associated with this exercise.

Objectives:

1. The initial goal of this study is to take the mice and exercise them until a steady average of their rate of recovery of the heart back to its resting heart rate is determined.
2. The next goal of this study is to evaluate the effects that Digoxin has on the recovery rate of the heart after exercise to its resting heart rate.

Hypothesis:

Digoxin with its cholinergic and positive inotropic effect will cause a decrease in the amount of time in the rate of recovery of the heart back to its resting heart rate after exercise in the treated mice.

MATERIALS AND METHODS

In this study 12 male, 60 day old *Mus domesticus*, (mice), CD 1 strain from Charles River Laboratories were used. The drug used in this study was Digoxin, a cardiac glycoside, that was obtained from Sigma (100 mg). Upon arrival the mice were immediately placed in the Animal Facilities of Harnly Hall at McPherson College. Four cages were obtained from the McPherson College Science Department that had been prepped with bedding, food, and water. The mice were divided into two groups. One of the groups was the control that was to have the Placebo administered to them and the other was the experimental group that was to have the calibrated dose of Digoxin administered to them. The cages were labeled 1-4 and three mice were placed in each. The two cages with the control group were labeled, “Placebo,” and the other two cages were labeled with, “Digoxin”, for the experimental group. The mice were acclimatized to their surroundings for 3-5 days. In the acclimatization process the mice were handled regularly to ensure that would not cause an increase in their heart rate later on in the study.

Preparation:

Digitalization may be accomplished by either of two general approaches that vary in dosage and frequency of administration, but reach the same endpoint in terms of total amount of digoxin accumulated in the body. The first approach is rapid digitalization that may be achieved by administering a loading dose based upon projected peak body digoxin then calculating the maintenance dose as a percentage of the loading dose. The other approach is the more gradual digitalization that may be obtained by beginning with an appropriate maintenance dose, thus allowing digoxin body stores to accumulate slowly. The more gradual approach is what was used in this study due to the narrow window of toxicity of this drug (0.8-2.1 ng/ml).

The dose for this experiment was calibrated by using the known blood volume of a mouse at 5ml. Then it was figured that approximately 55% of the blood was blood serum.
The usual amount of digoxin that a 70 kg patient requires to achieve 8-15 ug/kg per peak body stores is 750 to 1250 ug (0.75-1.25 mg). So, having used the bottom end of the peak body stores (0.75 mg), this was divided by 70 to obtain the conversion factor for the mass dosage. Then the conversion factor was taken and multiplied by the average weight of a mouse (30g) to obtain the average dose. Then this average dose was scaled down to a volume that was easily injected via feeding needles (Fisher Science). This was accomplished using a serial dilution. Starting with 5mg of digoxin per ml of sucrose buffer. Then there was a 1:500 dilution to 10ug/ml followed by a 1:5 dilution to the desired 2.0ug/0.150 ml. The sucrose buffer was made by using 2ml of 5X PBS (phosphate buffer saline) adding 2.0 mg sucrose and then diluting it to volume (10.0ml) with water.

Once the dilution was made the mice in each cage were all weighed individually and then the average weight from each cage was calculated. This in turn was divided by the weight of the average mouse (30g) and this gave a conversion factor for the actual dosage. This conversion factor was then multiplied by the 0.150ml to find the final volume of the dose for the mice. In this study two separate trials were performed. In trial 1 the dosages were 0.21ml for both the digoxin and the placebo due to almost identical weights of the mice. For trial 2 the dosages were 0.190 and 0.168 ml for the digoxin and placebo respectively.

Next, after prepping the digoxin, the Heart Rate Sensor and the computer must be calibrated. The PASCO CI0-6543B Heart Rate Sensor works with a PASCO Science Workshop computer interface to monitor a heart rate. This particular heart rate sensor monitors the flow of blood through a part of the body, the tail in this study, by shining an infrared light through the tail and monitoring the change in light intensity. The light source is a small infrared light-emitting diode. The sensor consists of a Heart Rate Sensor amplifier box, a cable with DIN connectors for connecting to a PASCO computer interface, and a sensor clip. Additional equipment was a computer (PC or Macintosh), Science Workshop software version 2.2 or higher.

Once the Heart Rate Sensor, computer, and Digoxin were prepped, the mice were prepared. Each mouse had rubber tubing superglued to its tail. This was for immobilization of the mouse once it was placed on the data collecting table. This table consisted of a 4X6 inch piece of wood, brass hooks, cardboard, and 20 gauge galvanized wire. The cardboard was used to make a square cage with a hole in the backside. The hole was to slip the mouse’s tail out of so it could be wired down. On the backside of the wood the hooks were screwed in and the wire was wrapped firmly around the hooks. When the tubing was glued to their tails it contained a half inch space one inch from the rear of the mouse where the tail was to be exposed so the Heart Rate Sensor could be clipped.

Each mouse was given a calibrated dose of either the Digoxin of the Placebo, 2 hours prior to its testing. Two hours was selected due to the fact that the peak time to onset for Digoxin is two hours. After two hours the mouse was immobilized in the data collecting table. The Heart Rate Sensor clip was clipped onto the mouse’s tail in the exposed space between the rubber tubing. The mouse’s resting heart rate was established by monitoring its heart rate on the computer at three and six minutes. Once its resting heart rate was established it was covered in shampoo and placed in a three foot tall 10 gallon tube filled half full with 32 °C water. Baby shampoo was used because if the mouse was placed into the water its fur would trap air and it would float. With the shampoo the mouse is guaranteed to exercise. The mouse swam for 8 to 10 minutes in order to increase its heart rate. After the exercise the mouse was immediately removed, dried off, and quickly replaced onto the data collecting table. Then its heart rate was monitored by the computer until its heart rate after exercise returned back to approximately 14% of its resting heart rate. This was then repeated for each mouse.

RESULTS

In this experiment two completely separate trials were performed. First the mice were separated into two groups one was the placebo and the other was the experimental. Then each was given a carefully calibrated dose of a placebo or digoxin 2 hours prior to them being exercised. Two hours because this was the peak time to onset for digoxin. The mice had their resting heart rate measured and then were exercised. After exercise the recovery rate of the heart back to its resting heart rate was measured.

The time it took the mice with the Digoxin to recover to the original resting heart rate vs. the Placebo was the variable that was recorded in this study. In Figure 1 A and B it is shown that the recovery rate of the heart after the exercise was significantly reduced in the experimental group due to the administration of the digoxin. It is also easily discernable from Figure 2 that the Experimental group (Digoxin administered) recovered significantly quicker than the Control group (Placebo administered).
As seen in Figure 3 there is a comparison of the mean times of the recovery rate of the heart after exercise. Also there is no overlap in the standard error bars showing that the recovery rates are significantly different.

**Trial 1:**
The mean \( \bar{X} \) was measured for both the Digoxin and Placebo groups and were 5 minutes 52 seconds (331 sec) and 7 minutes 44 seconds (446 sec) respectively. This was followed by the standard deviation (\( \sigma \)) that was calculated to be 29 seconds for the mice treated with the digoxin and 55 seconds for the mice treated with the Placebo. After these initial statistical tests were assessed the standard error (\( S.E. \)) and two confidence intervals were administered to the collected data. The standard error was 12 seconds and 22 seconds for the Digoxin and the Placebo respectively. Then the two confidence intervals calculated were:

1. These are the 95.44% confidence interval for the time rate of return of the heart rate back to the resting heart rate after exercise.

\[
0 \pm 2(S.E.)
\]

- **Digoxin:** 355 and 307 seconds
- **Placebo:** 490 and 402 seconds

2. These are the 99.74% confidence intervals for the time rate of return of the heart rate back to the resting heart rate after exercise.

\[
0 \pm 3(S.E.)
\]

- **Digoxin:** 367 and 295 seconds
- **Placebo:** 512 and 380 seconds
The Empirical Rule was used to show that the data was normal. In the second trial the sampling options were made more accurate by lowering the Hertz on the computer program.**

** Trial 2:**

The mean \( \bar{x} \) was calculated for the Digoxin and the Placebo and are as follows respectively: 108.75 and 140.75. This was followed by the standard deviation for both groups. Digoxin was 1.7 sec and the Placebo was 13.5 sec. Using the standard deviation the standard error was calculated to be 0.85 sec and 6.75 sec for the Digoxin and the Placebo respectively.

Now using the standard error and the fact that the data was normal due to the Empirical Rule two confidence intervals were calculated.

1. The first was for 95.44% confidence:
   \[ \bar{x} \pm 2(\sigma/\sqrt{n}) \]
   
   Digoxin: 110 and 107 seconds
   Placebo: 154 and 127 seconds

2. The second was for 99.74% confidence:
   \[ \bar{x} \pm 3(\sigma/\sqrt{n}) \]
   
   Digoxin: 111 and 106 seconds
   Placebo: 161 and 121 seconds

**DISCUSSION**

In this study, the rate of recovery of the heart after exercise back to its resting heart rate was affected by digoxin. It was shown by the collected data in trial 1 that with a 99.74% confidence that the rate of recovery was reduced by 85 seconds in the test, digoxin administered, group (Fig. 3). Also in trial 2 the heart rate was shown to be reduced by 32 seconds with the same amount of confidence (Fig. 3). This was caused by the cholinergic effect (vagomimetic action) that digoxin, a cardiac glycoside, produces. This effect slows the heart rate due to the effects of digoxin on the SA and AV nodes. This was all ultimately accomplished through a central mechanism that increases cholinergic stimulation to the heart. Since the nerve that provides the heart with cholinergic activity is the Vagal nerve, this is often referred to as an increase in Vagal tone. The next was the positive inotropic effect. This effect is a competition between potassium and digoxin. These compete for binding sites on an enzyme, referred to as potassium ATPase. So, digoxin is a potassium blocker or antagonist. By blocking the potassium from binding to the enzyme, digoxin causes the myocardium to be exposed to calcium for a longer period of time resulting in the heart contracting more forcefully. The cholinergic effect is primarily responsible for the fast recovery after exercise with some indirect effects by the positive inotropic effect. The cholinergic effect is negated by exercise at first due to the body's response to the exercise. This is controlled by the Medulla that stimulates the SA node due to a rise in the CO\(_2\) levels produced by the exercise. Once these are under control the cholinergic effect kicks in and causes the vagal nerve to increase production of acetylcholine which effects the SA node by slowing down its conductivity. This causes the heart to reduce its heart rate much faster. Also the positive inotropic effect causes the heart to be a more efficient pump and this may allow the heart to not beat as fast during the exercise depending on if maximum volume is reached in the heart.

Even though this study was conducted on mice it may have implications in the future in humans. Mice are good human models as stated earlier by the research of Dr. Jeffery Leiden and his team of genetic researchers at the University of Chicago especially for a study involving the response of the heart to some variable or drug, like in this study, because they are so closely related. This drug may allow people who have had heart attacks and or other heart disorders to function better and do more without as much stress being placed on the heart. The overall purpose would then be for people who have had heart attacks and can't do many thing due to their limited heart strength and people with heart disorders to be able to not only exercise, but strengthen the heart without the high impact of stress associated with this exercise.

**ACKNOWLEDGEMENTS**

Special Thanks to the following:
Dr. Andy Bobb
Dr. Jonathan Frye
Dr. Kent Noffsinger

**LITERATURE CITED**

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