Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Effects of Minimum and Maximum Doses of Furosemide on Fractional Shortening Parameter in Echocardiography of the New Zealand White Rabbit

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ARTICLE HISTORY

Received: April 22, 2012
Revised: June 23, 2012
Accepted: August 04, 2012

Key words:
Echocardiography
Fractional shortening
Rabbit

ABSTRACT

There is no data on the effect of maximum and minimum doses of furosemide on heart's work performance and amount of fractional shortening (FS) in echocardiography of rabbit. This study was designed to validate probability of the mentionable effect. Twenty-four healthy female New Zealand white rabbits were divided into four equal groups. Maximum and minimum doses of furosemide were used for the first and second groups and the injection solution for the third and fourth groups was sodium chloride 0.9% which had the same calculated volumes of furosemide for the first two groups, respectively. The left ventricle FS in statutory times (0, 2, 5, 15, 30 minutes) was determined by echocardiography. Measurements of Mean±SD, maximum and minimum amounts for FS values in all groups before injection and in statutory times were calculated. Statistical analysis revealed non-significant correlation between the means of FS. The results of this study showed that furosemide can be used as a diuretic agent for preparing a window approach in abdominal ultrasonography examination with no harmful effect on cardiac function.

To Cite This Article: Vali R, MN Masouleh and SM Rafie, 2013. Effects of minimum and maximum doses of furosemide on fractional shortening parameter in echocardiography of the New Zealand white rabbit. Pak Vet J, 33(2): 218-220.

INTRODUCTION

Furosemide is one of the most commonly used drugs in clinical medicine (Suzuki *et al*., 2011). It is the most potent and most common diuretic used in heart failure (Pichette and Du-Souich, 1996; Peddle *et al*., 2012). Congestive heart failure has been reported in pet rabbits (Lord *et al*., 2011). Furosemide a loop diuretic, acts on the loop of henle of the nephron by reversibly inhibiting the sodium/potassium/ chloride co-transfer. This action increases the amount of water in the tubule and subsequently increasing the urinary volume and decreasing the blood volume (Alván *et al*., 1990). It is used for the treatment of canine pulmonary edema secondary to left heart failure (Peddle *et al*., 2012). Increase of the urinary volume and distention of urinary bladder can be useful in diagnosis of this organ's diseases and finding an appropriate window in abdominal ultrasonography. The dose of furosemide in rabbits is 5-10mg/kg body weight, and the routes of administration are subcutaneously (SC), intramuscularly (IM) and intravenously IV (Carpenter *et al*., 2004).

Echocardiography is a non-invasive technique used for the evaluation of cardiac structure and function such as ventricles (Fontes-Sousa *et al*., 2009). Fractional shortening (end-diastolic diameter minus end-systolic diameter divided by end-diastolic diameter) is a measureable parameter that can determine ventricular function (Fontes-Sousa *et al*., 2006).

In routine ultrasonography exams, the sonologist can use the furosemide as a diuretic agent to finding a good window for abdominal investigations, but in the case of presence of cardiac disease and probability of the furosemide influence on cardiac function, what can the clinician do? As the amount of potassium reduction in therapeutic doses of furosemide is unclear, this study was designed to measure the amount of potassium reduction and interaction effects on cardiac function. In addition the probability of difference on the effects of the maximum and minimum doses of furosemide on cardiac function was determined.

MATERIALS AND METHODS

Twenty-four healthy female New Zealand white rabbits weighing (1.51±0.21kg) in four equal groups were used. Rabbits were healthy and free from any sign of

cardiovascular or respiratory tract diseases on the basis of the physical examination which included careful thoracic auscultation. The animals were housed individually in cages in a controlled environment, at temperature of 20-25°C, with 12 hours of light and 12 hours of dark cycle, and were fed a standard pellet diet and adequate water. Blood samples were obtained from saphenous vein, twenty-four hours before injection of furosemide and echocardiography examination and at Minute 60 after injection. These samples were collected for determining plasma potassium concentration and differences before and after the injection. The normal range of potassium is 3.7-6.8 meq/l (Carpenter *et al*., 2004). The weight of each rabbit was recorded prior to injection of furosemide. The rabbits of the first and second group received maximum (10mg/kg) and minimum (5mg/kg) dosage of furosemide respectively (SC injection). The rabbits of third and fourth group received SC injections of sodium chloride 0.9% as specified for the first and second group. Feeding was withheld a few hours before echocardiography examination to reduce abdominal distension from intestinal fill, which could mechanically compress the diaphragm and lungs, particularly when the abdomen is compressed during the segment of echocardiography examination in which the images were obtained via the sub-costal approach.

Imaging technique: Rabbits were placed in right lateral recumbency to obtain right parasternal view, over a gap in the tabletop through which the ultrasound probe was brought from below and placed on a shaved area on the anterior aspect of the lower portion of the right thoracic wall. Echocardiography measurements were obtained from standard views (Boon, 2011). Transthoracic 2-dimensional and M-mode echocardiography were performed by using an ultrasonography system (Acuson, Cyprus) equipped with a 3-7 MHz (7v3c) neonate transducer. From the right parasternal short-axis view, 2-dimensional guided m-mode tracing was made just below the mitral valve at the level of the papillary muscles for measurements of the left ventricular internal diameter (LVID), in diastole and systole (Dimitrov *et al*., 2011). Fractional shortening was calculated from measurements for the LVID in systole and diastole by use of the following formula:

FS (%) = [(LVIDd – LVIDs)/LVIDd]□100 (Fontes-Sousa *et al*., 2006; 2009). In our study calculations of the FS were easily obtained in all of the animals, before injection (min0) and at 2, 5, 15 and 30 minutes after injection by use of ultrasound system software. All data about each rabbit was recorded. Measurements of mean, standard deviation of (FS) before injection and in statutory times were calculated for all rabbits. Statistical procedures for comparison between mean of FS (min 0) and mean at 2, 5, 15 and 30 minutes after injection were done by using independent-sample t-test. Statistical differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

In the present study, none of the animals died during or after the study. Values for FS before injection (min 0) and after injection (2, 5, 15 and 30 minutes) in 24 New Zealand white rabbits are summarized in Table 1. Minimum, maximum, mean±SD before injection in 24 female New Zealand white rabbits was 32, 53 and 40.8±6.1. Potassium concentrations before injection and after injection (min 60) are summarized in Table 2.

Table 1: Values (mean±SD) fractional shortening in all groups before (min 0) and after injection of drug at various intervals

Time	Furosemide (groups)		Sodium chloride (groups)	
(min)	1	2	3	4
0	40.5±5.1	38.6±6.2	42.2±6.7	41.6±7.2
2	36.3±4.7	44.5±10.5	42±6.5	37.3±5.3
5	39.5±12	47.5±11.3	38.5±4.4	38.2±7.2
15	39.3±7.6	39.5±10.1	43.6±6.5	42.5±8.4
30	39.4±8.8	35.6±6.1	40.6±6.5	43.6±7.7
0/2	0.1	0.3	0.9	0.1
0/5	0.8	0.1	0.3	0. 5
0/15	0.6	0.9	0.7	0.8
0/30	0.9	0. 6	0.7	0.7

1=Group1 received maximum dose of furosemide; 2=Group 2 received minimum dose of furosemide; 3=Group 3 received maximum dose of sodium chloride; 4= Group 4 received minimum dose of sodium chloride.

Table 2: Potassium concentration (Means±SD) before and after drug injection

drug	Dose	Drug Injection		P value
		Before	After	
Furosemide	Max	5.1±0.8	4.5±0.6	0.7
	Min	5.2±0.6	4.5±0.2	
Sodium chloride	Max	5.3±0.4	4.5±0.4	0.9
	Min	5.5±0.6	4.4±0.3	

There are several reasons that the New Zealand white rabbits are a good model for cardiovascular research, such as; the ability to perform repeated measurements in the same animal, low cost to procure and maintain, easy approach to arterial and venous system, relatively large heart size in relation to thoracic volume, The possibility of using standard recording equipment and easy management of the rabbits, as a result, these rabbits are commonly used in cardiac research (Salemi *et al*., 2005; Fontes-Sousa *et al*., 2006). In addition their similarity to humans with regard to cardiac anatomy, physiology and age related changes, are other reasons for using rabbits in these kinds of research (Fontes-Sousa *et al*., 2009).

Fractional shortening parameter has been determined for the mouse, Syrian hamster, rabbit, guinea pig and Chinchilla (Table 3). In the present study, the mean of FS value in all groups before injection is about (40.8±6.1). This value compared with the results of other studies on rabbits has shown an increase (table3). This difference could be due to the use of anesthetic drugs, since research has shown that anesthetic drugs may reduce fractional shortening (Yang *et al*., 1999; Baumgartner *et al*., 2010; Pelosi *et al*., 2011). No significant correlation was found between means of FS values before injection and at 2,5,15 and 30 minutes after injection by using independent-sample t-test, and the p-level for statistical significance was set at 0.05 (Table 1).

Normal serum potassium levels are between 3.7 and 6.8 mEq/L (Carpenter *et al*., 2004; Bielecka-Dabrowa *et al*., 2012). In our study Means of serum potassium concentration before injection and after injection (min 60) in all groups was compared and no significant correlation was found, (Table 2). Although potassium concentration reduced after injection, drug type and dose has no

Table3: Values for Fractional shortening in rodents as reported by various workers

Animal	Age (weeks)	BW (kg)	Anesthetic type	FS%	Reference
Mouse	9-10	0.02-0.26	Isoflurane	35-38	Yang *et al.* (1999)
Hamster	10	0.07-0.13	Penthobarbital	44.7±6	Salemi *et al.* (2005)
Rabbit	16-18	2.2-3.2	Ketamine/Medetomidine	30.1±3	Fontes-Sousa *et al.* (2006)
Rabbit	16-20	2.3±0.4	Ketamine/Midazolam	36±4.3	Fontes-Sousa *et al.* (2009)
Rabbit	14-16	1.5±0.3	Atropine	37.3±3.3	Nasrollahzadeh-Masouleh *et al.* (2010)
Guinea pig	-	0.5-0.7	Xylazine/Ketamine	35.6±2.6	Cetin *et al.* (2005)
Chinchilla	Adult	-	Isoflurane	40-64	Linde *et al.* (2004)

significant effect on serum potassium before and after injection. This reduction could be due to furosemide administration, because diuretics like furosemide are known to inhibit sodium and chloride co-transport from the luminal side of thick ascending limb of the loop of Henle. The increased electrolyte excretion will then increase tubular flow and urinary volume. The effect on potassium is considered to be transient and mainly secondary to increase tubular flow (Alván *et al*., 1990). Drugs such as furosemide may precipitate hypokalemia by accelerating renal loss (Bielecka-Dabrowa *et al*., 2012).

There are three types of hypokalemia. In mild hypokalemia potassium concentration is 3-3.5mEq/L and most often asymptomatic. In moderate hypokalemia potassium concentration is 2.5-3.5mEq/L and non specific symptoms like weakness, malaise and constipation appear as well. Potassium concentration in severe hypokalemia is below 2.5 mEq/L and cardiac arrhythmias increase (Rastegar and Soleimani, 2001). Changes in the potassium concentration in this study are in normal range of serum potassium concentration (3.7 to 6.8 mEq/L) and it has no effect on cardiac function.

Conclusion: In conclusion, the results of this study indicate that a single dose of furesemide administration in therapeutic doses (minimum or maximum) has no significant effect on reducing the potassium concentration and changes in the Fractional shortening parameter. The results of this study conclude that furosemide can be used for preparing a window approach in abdominal ultrasonography with no harmful effect on mechanical cardiac performance and only prolonged furosemide administration, may induce reduction of serum potassium and cause severe hypokalemia.

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