

Development of Hypertensive Animal Model Using Ovariectomised Rat Fed with Short-Term 2% Cholesterol Diet

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ABSTRAK

Menopaus merupakan pemberhentian pendarahan haid, ia secara langsung berkaitan dengan penyakit kardiovaskular (CVD) seperti tekanan darah tinggi dan strok. Pengurangan estrogen dan pengambilan diet berkolesterol tinggi mempunyai kesan yang menjejaskan fungsi kardiovaskular dan metabolisma pada wanita. Kajian ini bertujuan untuk menghasilkan model tikus hipertensi dalam tikus *Sprague-Dawley* terovariektomi yang diberi makan dengan 2% diet kolesterol dalam tempoh jangka pendek. Lapan belas tikus *Sprague-Dawley* dibahagikan secara rawak kepada tiga kumpulan iaitu kumpulan kawalan Sham (sham), kumpulan kawalan terovariektomi diberi diet normal (Ovx + N), tikus terovariektomi diberi diet kolesterol 2% (Ovx + Cho) selama empat minggu. Pada akhir kajian, pengambilan makanan dan berat badan diukur. Tekanan darah sistolik, diastolik dan tekanan min darah ditentukan. OVX + Cho menunjukkan peningkatan yang ketara dalam perubahan fisiologi seperti berat badan dan pengambilan makanan berbanding dengan kumpulan Ovx + N. Peningkatan yang ketara dalam tekanan darah sistolik, diastolik dan tekanan min darah diperhatikan dalam kumpulan Ovx + Cho. Berdasarkan hasil kajian di atas, dipercayai bahawa makanan berkolesterol selama empat minggu (jangka pendek) menyebabkan peningkatan ketara dalam tekanan darah, pengambilan makanan dan berat badan dalam tikus terovariektomi.

Kata kunci: tekanan darah, berat badan, kolesterol, diet, terovariektomi, tikus

ABSTRACT

Menopause, a cessation of menstrual bleeding, is directly related to serious illness confined to cardiovascular disease (CVD) such as hypertension and

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stroke. The oestrogen withdrawal and consumption of high cholesterol diet has a detrimental effect on cardiovascular function and metabolism in menopausal women. The present study was aimed to develop the hypertensive rat model in the ovariectomised Sprague Dawley rats fed with 2% cholesterol diet following a short term period. Eighteen Sprague-Dawley rats were randomly divided into three groups; Sham control (sham), ovariectomized control fed with normal diet (Ovx +N), ovariectomized rats fed with 2% cholesterol diet (Ovx + Cho) for four weeks. At the end of the study, the food intake and body weight were measured. The systolic, diastolic and mean blood pressure was determined. OVX+Cho showed significant increase in physiological changes such as body weight and food intake compared to OvX+N group. The significant increase in systolic, diastolic and mean blood pressure was observed in OvX+Cho group. Based on the above findings, it is believed that feeding cholesterol diet for four weeks (short term) results in significant increase in the blood pressure, food intake and body weight in the ovariectomised rats.

Keywords: blood pressure, body weight, cholesterol, diet, ovariectomised, rats

INTRODUCTION

Menopause is the permanent cessation of menstruation resulting from the loss of ovarian follicular activity which occurs after 12 consecutive months of amenorrhoea without any pathological or physiological cause (WHO, 1994). The timing of natural menopause is variable, however, it can occur in the women with age range from 45-55 years. Menopause has detrimental effects on cardiovascular function and metabolism in women (Leong et al. 2008). Menopause is associated with cardiovascular disease (CVD) including hypertension, obesity, stroke, myocardial infarction and renal failure (Bray 2003; Leong et al. 2015). Among all the complications related to the menopause, obesity is an important one to be highlighted. Postmenopausal obesity results in increased risk of many diseases, such as

diabetes, hypertension, heart diseases, osteoporosis and breast cancer (Bray 2003). Several experimental studies used ovariectomised rats as a menopausal model for the tentative researches (Leong et al. 2015).

High Blood Pressure is defined as systolic blood pressure (SBP) greater than 140 mmHg and/or diastolic blood pressure (DBP) greater than 90 mmHg (Bray 2003). Increase in the blood pressure is the most common symptom in the CVD and is a main source of morbidity and mortality worldwide. Approximately, 90-95% cases of hypertension are idiopathic. It is a complex, multifactorial disorder, including genetic and lifestyle modifications (Leong et al. 2015). Based on the epidemiological surveys, several factors are linked with an increase in the blood pressure and these include age, sex, race, socioeconomic status, emotional disturbances, nutrition,

increase in the body weight, alcohol consumption, physical activity and exposure to various environmental agents. In Malaysia, data from the National Health and Morbidity Survey reported that 37% of the population developed hypertension (Institute of Public Health 2008).

The occurrence of increase in blood pressure and CVD are less in women compared to men of similar age and this is due to the sufficient oestrogen level present in the reproductive age of the women (Zago et al. 2004). However, the oestrogen levels gradually diminish at the age of menopause and predispose to several diseases including obesity and hypertension. Oestrogen has been proven to exhibit cardioprotective effects in pre-menopausal women. A number of studies reported that the incidence of hypertension and menopausal are highly correlated (Zago et al. 2004; Deroo & Korach 2006; Adam et al. 2009). Few studies presumed that the consumption of improper diet (high sodium diets, high cholesterol diet) has a high risk of developing elevated blood pressure in the postmenopausal state.

There are in vivo and in vitro studies which were conducted to observe the effect of cholesterol rich diet on major blood vessels (Jaarin et al. 2015; Nurul-Iman et al. 2013; Leong et al. 2015). Significant changes were observed in the pathophysiology of the aorta following the cholesterol rich diet in ovariectomised rats. It is noteworthy that the commercial cholesterol rich diet is cost expensive. Prolonged consumption of cholesterol diet

(more than 24 weeks) led to develop hypertension in the ovariectomised rats (Jaarin et al. 2015; Leong et al. 2015). It was important to observe the effect of short term consumption of cholesterol diet on hypertension which may result in low consuming of cholesterol diet. The effect of 2% cholesterol diet on the blood pressure was also to be investigated. Hence, the present study was aimed to develop hypertensive ovariectomised rat model following the consumption of 2% cholesterol in the normal diet in a short term period.

MATERIALS AND METHODS

ANIMALS AND DIETS

Eighteen female Sprague-Dawley rats with weighing 250 ± 50 g were obtained from Animal unit, Universiti Kebangsaan Malaysia. The rats were kept in stainless steel cage at temperature 20–22°C and 12:12 h light cycle). The rats were acclimatized for 7-days and were given free access to food and water. Prior ethical approval was obtained. Two percent cholesterol diet was purchased from Next Gene Scientific Sdn Bhd, Singapore.

STUDY DESIGN

The rats were randomly divided into three groups of six animals (n=6) each; sham control (Sham), ovariectomised control fed with standard rat chow (Ovx+N) and ovariectomised control fed with 2% cholesterol (Ovx+Cho). Following the ovariectomy, the treatment was continued for 4 weeks.

PROCEDURE FOR OVARIECTOMY

All the animals except Sham group were ovariectomised following intraperitoneal injection of ketamine hydrochloride and xylazine (50 and 10 mg/kg body weight, respectively). Bilateral ovariectomies were performed from a dorsal approach. Following 2 weeks of ovariectomy, Sham and Ovx+N groups received normal diet. Normal diet with additional 2% cholesterol was given to Ovx+ Cho (Adam et al. 2009).

MEASUREMENT OF FOOD INTAKE AND BODY WEIGHT

Food intake and body weight were measured at weekly intervals for four weeks in all the groups. Measurement of food intake was calculated by subtracting the total amount provided to the remaining amounts in the cage (Abdulla et al. 2011). Body weight was measured by using the electronic weighing scale. The increase in the body weight was calculated by deducting the final weight from the initial weight.

BLOOD PRESSURE MEASUREMENT

Prior to measure the blood pressure, the rats were made unconscious by inhalation of diethyl ether. Blood pressure of the unconscious rats were measured at the baseline and at the end of the experiment. It was measured by using the non-invasive tail-cuff method with sphygmomanometer technique using CODA system (a computerized monitor system) (Aida Azlina et al. 2009). Minimum of three measurements were taken

consecutively and the average was then determined as a final measurement for SBP, DBP and mean blood pressure (Aida Azlina et al. 2009).

STATISTICAL ANALYSIS

All data analyses were conducted using SPSS version 22. The normality of the data was determined by Kolmogorov–Smirnov test. The food intake and body weight changes among groups were compared using two-way ANOVA with Tukey post-hoc test. Statistical significance was defined as $P < 0.001$. Data are expressed as means (\pm SEM).

RESULTS

FOOD INTAKE AND BODY WEIGHT

In the present study, the changes in the food intake and body weight of the animals following 4 weeks of consumption of 2% cholesterol diet was determined (Table 1). The food intake between Ovx+Cho and Ovx+N; Ovx+N and Sham groups were compared. It was shown that Ovx+Cho group (food intake 252.7 ± 9.17 g; body weight 278.43 ± 13.9 g) had a significant increase in food intake and body weight at the end of 4th week of the study compared to Ovx+N group (172.9 ± 9.67 g; 247.43 ± 15.23 g), respectively) ($p < 0.001$). Although, there was a significant increase in food intake in Ovx+N group (172.9 ± 9.67 g) compared to sham group (152.9 ± 6.83 g), the non-significant difference in body weights was found between Ovx+N (247.43 ± 15.23 g) and Sham group (240.43 ± 12.23 g) ($p > 0.001$). Less

Table 1: Food intake and body weight gain in rats fed with respective normal and 2% cholesterol diet

Groups	Food intake (g/weeks)					Body Weight (g)				
	0	1	2	3	4	0	1	2	3	4
Sham	142.5	147.6	150.2	153.4	152.9	200.43	210.17	217.36	230.32	240.43
	± 5.61	± 5.38	± 5.41	± 6.98	± 6.83	± 14.13	± 15.22	± 13.95	± 14.78	± 12.23
Ovx+ N	140.7	145.6	154.2	160.4	172.9	203.43	214.17	220.36	234.32	247.43
	± 9.67	± 7.35	± 8.34	± 8.98	± 9.67 ^{bc}	± 12.16	± 17.22	± 18.97	± 14.56	± 15.23 ^a
Ovx + Cho	144.7	156.6	169.5	205.3	252.7	208.43	218.23	235.22	256.17	278.43
	± 8.13	± 4.67	± 7.37	± 5.67	± 9.17 ^b	± 13.90	± 11.65	± 15.30	± 17.5	± 13.9 ^b

Each value is expressed as the mean \pm SEM (n=6). ^a p>0.05: Sham Vs Ovx+N; ^b p<0.05: Ovx+Cho Vs Ovx+N, ^c p<0.05: Sham Vs Ovx+N.

significant findings of food intake and body weights were observed between the groups at the different weeks of the study (week 0,1,2 and 3).

SYSTOLIC BLOOD PRESSURE MEASUREMENT

The weekly systolic blood pressure was measured in normal and cholesterol fed groups with normal diet fed ovariectomised rats. The results were compared between the groups at the end of the study (i.e at 4th week of experimental period) (Figure 1). It was observed that Ovx+Cho group showed a significant increase in systolic blood pressure (128.2 ± 9.8 mmHg) compared to Ovx+N group (115.1 ± 8.7 mmHg) ($p < 0.001$). No significant increase in systolic blood pressure was observed in the Ovx+N group compared to Sham group (112.2 ± 8.3 mmHg) ($p > 0.001$). There was no significant difference noted in the systolic blood pressure of Ovx+ Cho and Ovx+N groups; Ovx+N and sham groups at different week interval (week 0,1,2 and 3).

DIASTOLIC BLOOD PRESSURE MEASUREMENT

The weekly diastolic blood pressure was measured in normal and cholesterol diet fed ovariectomised rats and the results were compared between the groups at the end of the study (Figure 2). It was observed that the Ovx+Cho group showed a significant increase in diastolic blood pressure (95.2 ± 9.8 mmHg) compared to Ovx+N group (76.4 ± 9.2 mmHg) ($p < 0.001$). No significant increase in diastolic blood pressure was observed in the Ovx+N group compared to Sham group (77.5 ± 9.7 mmHg) ($p > 0.001$). The non-significant differences in diastolic blood pressure were observed at different week interval between the groups.

MEAN BLOOD PRESSURE MEASUREMENT

The weekly mean blood pressure was measured in normal and cholesterol diet fed ovariectomised rats and the results were compared between the groups at the end of the study (Figure

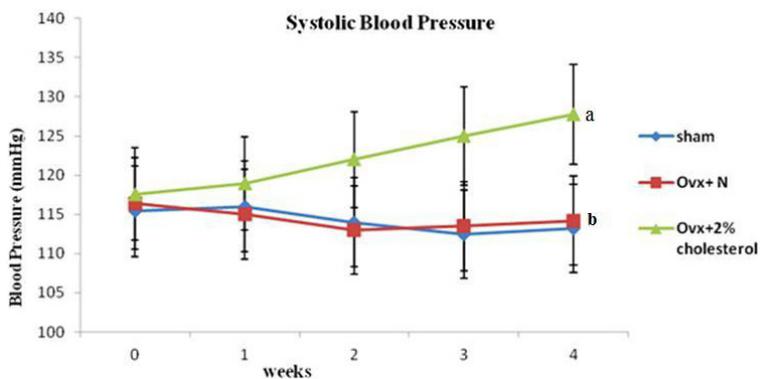


Figure 1: Measurement of systolic blood pressure in normal and cholesterol diet fed ovariectomised rats

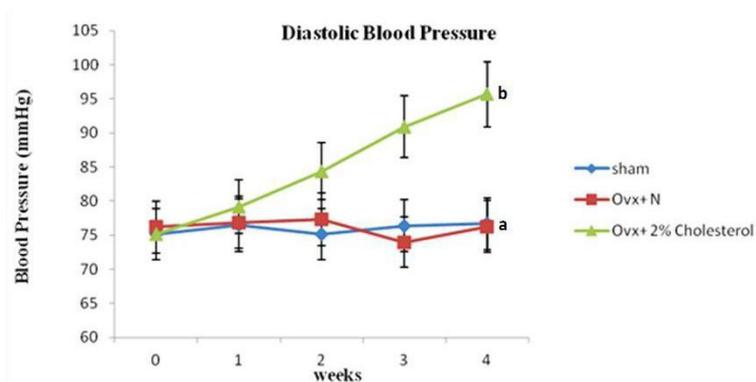


Figure 2: Measurement of diastolic blood pressure in normal and cholesterol diet fed ovariectomised rats

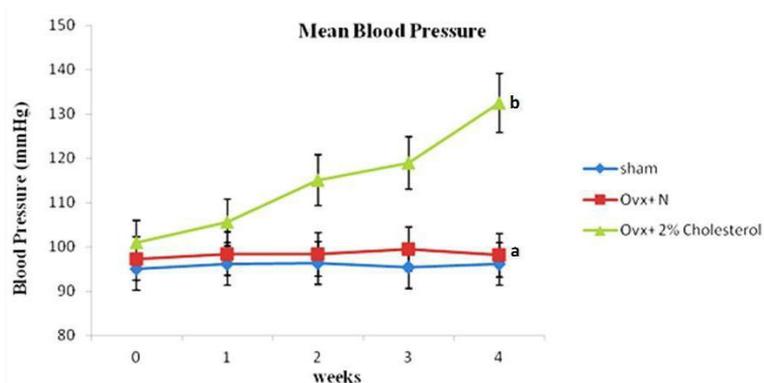


Figure 3: Measurement of mean blood pressure in normal and cholesterol diet fed ovariectomised rats

3). It was observed that the Ovx+Cho group had a significant increase in the mean blood pressure (135.2 ± 7.3 mmHg) compared to the Ovx+N

group (96.7 ± 4.9 mmHg) ($p < 0.001$). No significant increase in mean blood pressure was observed in the Ov_x+N group compared to Sham group (95.5 ± 5.3 mmHg) ($p > 0.001$).

DISCUSSION

Several clinical and experimental studies were carried out to investigate the complications that occurred in the postmenopausal state. Ovariectomised Sprague Dawley rats are used as the animals resemble the postmenopausal state of the human (Latour et al. 2001). With regard to the duration of the study, it is worth to know that there are several studies of cholesterol diet on short and long term period. According to the literature, short term period is presumed from 7 days to 4 weeks/less than 5 weeks and long term period is between 5-24 weeks of dietary cholesterol consumption. These durations are widely used in the atherosclerosis related cholesterol diet researches (Lichtenstein et al. 1994; Brown et al. 1991).

The present study used the ovariectomised rats to determine the changes in the blood pressure, body weight and food intake following 4 weeks of consumption of 2% cholesterol diet. The food intake and body weight was compared between Ov_x+ Cho and Ov_x+N groups: Ov_x+N and sham groups. It was found out that there was a significant increase in the body weight and food intake in Ov_x+Cho group compared to Ov_x+N group (Table 1). The underlying mechanism related to the increased food intake in ovariectomised group fed with cholesterol is poorly

understood. Yet, it was stated that cholesterol levels are strongly linked with the dietary intake. According to the literatures, the individual consuming more cholesterol in daily diet inclines to have increase food intake compared to the individuals with normal diet (Kresser 2013). Our findings agreed with the previous study which reported the significant increase in food intake in experimental rats within the first few weeks of ovariectomy (McElroy & Wade 1987). The increase in the body weight following an increase in food intake might be most probably due to the gradual reduction in lean body mass and progressive fat accumulation in different body regions. Progressive fat accumulation induces oxidative stress and was proven to cause obesity, hypertension and cardiovascular diseases (Park et al. 2016). Previous study observed the weight gain in the ovariectomised rats are due to the oestrogen deficiency resulting an increase in the fat accumulation (Latour et al. 2001, Shinoda et al. 2002). However, the findings are not in line with the present results as Ov_x+N group did not show any significance increase in the body weight compared to the sham group. It can be explained that oestrogen has less influence on the body weight in early onset of postmenopausal state. Based on the present study, the increase in body weight mainly reflect the consumption of cholesterol diet (Table 1). It is proved that the effect of oestrogen deficiency worsens with additional cholesterol intake.

Oestrogen deficiency causes a significant increase in the total

cholesterol and triglycerides, which affects lipoprotein metabolism, platelet aggregation and vessel resistance (Gorodeski & Gorodeski 2007; Jenkins et al. 2006). Previous studies observed that the intake of cholesterol rich food develops obesity and several cardiovascular complications in various animal models (Ali et al. 2006; Kennedy et al. 2010). This high cholesterol diet induces damage to the endothelium of large arteries and heart causing hypertension or increase in the blood pressure and subsequently, produce atherosclerosis resulting coronary heart disease (Ryou et al. 2012).

In the present study, Figure 1, 2 and 3 showed an increase in the systolic, diastolic and mean blood pressure of the Ovx+Cho group. It was observed that consumption of additional 2% cholesterol diet for 4 weeks caused significant changes in the blood pressure measurement. The oestrogen deficiency following ovariectomy and increase cholesterol intake are the predisposing factors of the obesity. In the state of obesity, the fat containing adipose tissue elevates circulating blood volume, plasma volume, and cardiac output, which dilates cardiac cavities and enhances in wall tension resulting in systemic and pulmonary hypertension and sudden death (Poirier et al. 2006). This incidence of hypertension frequently occurs in obese women with poor diet control, modern life style, existing family history and menopausal age (American Heart Association 2007). More than 73% of women aged between 65 to 74 years were reported to have high blood pressure (American Heart Association

2008; Yamakawa et al. 1995). Therefore, it is most likely that ovariectomised rats fed with 2% cholesterol diet (Ovx+Cho group) developed hypertension. No significant changes observed in Ovx+N group was most likely due to its early onset of postmenopausal state. Oestrogen deficiency is believed to effect the physiological changes in the prolonged duration. However, lack of oestrogen along with consumption of cholesterol diet may worsen the above findings even in the early onset.

In a routine research on cardiovascular disease, the ovariectomised rats fed with 2% cholesterol were used to develop the atherosclerosis animal model. The experiments related with this type of animal models are needed to be carried out for a prolong period (up to 24 weeks) (Lichtenstein et al. 1994; Brown et al. 1991) in order to achieve the significant outcomes of atherosclerosis or hypertension. Based on the present findings, it is proven that the use of 2% cholesterol diet induced a significant increase in food intake, body weight and blood pressure in ovariectomised rats over 4 weeks (short term period) following ovariectomy. This study showed that consumption of cholesterol diet in early onset of postmenopausal state leads to develop hypertension in the experimental animals. Moreover, this study highlighted the serious adverse effects of cholesterol on postmenopausal women. The development of hypertensive ovariectomised rat model in short term period with the consumption of 2% cholesterol diet is expected to give interesting results.

CONCLUSION

Based on the observations, we concluded that the hypertensive model is established with consumption of 2% cholesterol diet for short term period in ovariectomised rats. The model was easily developed, cost effective and less time-consuming. However, future studies are required to observe the detailed mechanisms related to the cholesterol diet and its potential hypertensive activity.

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