

POTENCY OF CV12 ACUPUNCTURE AS OBESITY PREVENTION THROUGH MODULATION OF α AND β ESTROGEN RECEPTORS EXPRESSION ON THE ADIPOSE TISSUE OF OVARIECTOMIZED MICE

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui potensi CV 12 akupunktur sebagai pencegahan kenaikan berat badan di ovariektomized mice (OVX). Perempuan tikus galur BALB/c, 28 tikus, 3-4 minggu, 25-35 gram dibagi menjadi empat kelompok; yaitu K1 (n = 8, palsu dan injeksi vehiculum), K2 (n = 8, OVX dan injeksi vehiculum), K3 (n = 7, OVX dan injeksi estrogen intraperitoneal 10 mg/kg/hari) dan K4 (n = 6, OVX dan CV12 akupunktur dengan frekuensi 2 Hz di 10 mA terus menerus lonjakan gelombang saat ini). Injeksi dan akupunktur diberikan setiap hari selama empat minggu. perubahan berat badan di K1 (0,75 ± 2,8) gram, K2 (2,71 ± 2,4) gram, K3 (0,71 ± 0,95) g dan K4 (0,93 ± 2,40) gram dengan perbedaan yang signifikan dalam K2 dan K4 (p = 0,011). Ekspresi α reseptor di K1 (12,25 ± 2,96), K2 (5,43 ± 2,64), K3 (18,86 ± 1,57), K4 (17 ± 2,00) dengan perbedaan yang signifikan dengan K2 K1, K3 dan K4 dengan p < 0,05. Ekspresi β reseptor di K1 (11,38 ± 1,92), K2 (4 ± 1,41), K3 (16,86 ± 1,86), K4 (14,17 ± 5,24) dengan signifikan berbeda ekspresi α reseptor di massa lemak visceral pada kelompok K2 ke K1, K3 dan K4 dengan p < 0,05. CV 12 akupunktur increases ekspresi α dan β reseptor estrogen di jaringan adiposa viseral tikus OVX ekuivalen dan mampu mencegah kenaikan berat badan karena penarikan estrogen. Hal ini berpotensi dapat digunakan sebagai salah satu pencegahan manajemen obesitas menopause. (FMI 2017;53:24-28)

Kata kunci: tikus, ovariektomi, akupunktur CV 12, ER α , ER β

ABSTRACT

This study aims to determine the potency of CV 12 acupuncture as prevention of weight gain in ovariectomized mice (OVX). Female mice strain BALB/c, 28 mice, 3-4 weeks, 25-35 grams were divided into four groups; namely K1 (n = 8, sham and vehiculum injection), K2 (n = 8, OVX and vehiculum injection), K3 (n = 7, OVX and injection of estrogen intraperitoneal 10 mg/kg/day) and K4 (n = 6, OVX and CV12 acupuncture with a frequency of 2 Hz in 10 mA of continuous spike current wave). Injeksi dan akupunktur given every day for four weeks. Weight change in K1 (0.75 ± 2.8) grams, K2 (2.71 ± 2.4) grams, K3 (0.71 ± 0.95) g and K4 (0.93 ± 2.40) gram with a significant difference in the K2 and K4 (p = 0.011). α receptor expression in K1 (12.25 ± 2.96), K2 (5.43 ± 2.64), K3 (18.86 ± 1.57), K4 (17 ± 2.00) with a significant difference to the K2 K1, K3 and K4 with p < 0.05. β receptor expression in K1 (11.38 ± 1.92), K2 (4 ± 1.41), K3 (16.86 ± 1.86), K4 (14.17 ± 5.24) with significantly different α receptor expression in visceral fat mass on the group K2 to K1, K3 and K4 with p < 0.05. CV 12 acupuncture increases the expression of α and β estrogen receptor in visceral adipose tissue of OVX mice equivalently and able to prevent the increase of weight due to the estrogen withdrawal. This point could potentially be used as one of the management of obesity prevention in menopause. (FMI 2017;53:24-28)

Keywords: mice, ovariectomy, acupuncture CV 12, ER α , ER β

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INTRODUCTION

Obesity is an epidemic disease that has struck the world. Based on data of WHO, one billion people worldwide were overweight, and at least 300 million are clinically obese diagnosed (Mori et al., 2008). The populations that experiencing the rapid increment of obesity prevalence is the post-menopausal women (Allende-Vigo, 2008; Geer and Shen, 2009). Obesity in women is expected because of the withdrawal of estrogen levels in the blood.

Estrogen receptor activation produce a linear result of the obesity prevention and treatment. Estrogen receptor activation increases the expression of uncouple proteins 1 and 2 thus increasing thermogenesis and heat output. Activation of estrogen also decreases the expression of SREBP and lipoprotein lipase which acts on lipogenesis resulting in decreased synthesis of adipose mass (Yepuru et al., 2010). Therapy of estrogen hormone replacement in postmenopausal women known to affect the initiation of cancer in the uterus and should not be

given to women who still have a uterus. It also increases the risk for stroke, blood clots, breast cancer, gallstones, ovarian cancer and dementia. The addition of progestin in hormone replacement therapy slightly reduce the risk but raise the prices. Thus it is necessary to develop the appropriate modalities of therapy to address this.

Acupuncture is one of treatment method which began to be developed throughout the world. In some previous studies, acupuncture proved to modulate leptin in models of polycystic ovary syndrome mice. Acupuncture also play a role of preventing weight gain in mice exposed to a high-fat diet or the ovariectomy mice. This study aims to determine the effects of acupuncture on the expression of estrogen receptors in visceral fat tissue that is still unsolved.

MATERIALS AND METHODS

Twenty eight female mice (BALB/c) aged 3-4 weeks old weighing 25-35 grams were purchased from Pusat Veterinaria Farma Surabaya. Animals were kept at 25-30°C room temperature, with free access to food and water and a 12-hour light/dark cycle. The mice were divided into four groups: sham + vehiculum injection; OVX + vehiculum injection; OVX + intraperitoneal injection of estrogen (10 mg/kg/day); and OVX + CV 12 acupuncture.

All the procedures performed were approved by the Research Ethics Commissions of Faculty of Veterinary Medicine, Universitas Airlangga. Mice were ovariectomized by the dorsal approach under general anesthesia. Once anesthetized, abdominal incision was performed, the fallopian tubes were ligated, the ovaries were removed, and the incision was closed with suture procedure. Sham surgery was performed with the same abdominal surgery without removal of the ovaries. Recovery period for each mouse was seven days.

Estrogen (β -estradiol; Sigma Aldrich) was diluted in dimethyl sulfoxide (DMSO 78; Sigma Aldrich) for intraperitoneal injection (10 mg/kg/day) (Modderet al., 2004). DMSO 78 solution was used for vehiculum injection. Estrogen and vehiculum treatments were administered 0,5 ml every day for one month.

The acupuncture point for AES treatment was CV 12 (中脘 or zhōng wǎn) located in the midline of the body, midway between xiphoid process and umbilicus. Needles with a length of 0.5 inch or 1.25 cm were inserted 2 mm depth at an angle of 85 degrees. Stimulation of 2 Hz, 10 mA continuous spike waves was administered for ten minutes every day for one month without anesthesia. Acupuncture instrument used in this study

was electro stimulator (AES-04) produced by the Faculty of Science and Technology UNAIR. The positive electrode was connected to electro stimulator while negative electrodes was connected on the ground.

All mice were weighed before and after one month treatments. At the end of the experiment, the mice were anesthetized and the abdomen was incised. Omentum fat, reproductive organs and all intraperitoneal organs were removed and the mice were decapitated. The visceral abdominal fat mass was separated, weighed and examined for the expression of estrogen receptors α and β by immunohistochemistry.

Statistical comparisons were performed by randomized-design ANOVA using Statistical Package for the Social Sciences (SPSS) software version 16. The analysis used was descriptive and analytic/inferential analysis. Inferential analysis was used to look at the test of adipose differential for each group, differential expression of α and β estrogen receptor in each group, as well as the correlation between adipose mass with expression of α and β estrogen receptor.

RESULTS

Pre-treatment weight loss can be observed in Table 1. In tests of homogeneity variation of initial body weight group, the value obtained was 0.436 (> 0.05), which means that the data were normally distributed.

Table 1. Initial body weight in all four groups

Group	n	Body Weight (gram)		
		Mean \pm SD	Min	Max
Sham + vehiculum	8	27.00 \pm 5.16	23	39
OVX+vehiculum	7	26.29 \pm 3.15	20	29
OVX + estrogen	7	25.14 \pm 2.67	21	29
OVX + acupuncture	6	28.83 \pm 1.94	26	31

Statistical descriptive of weight after treatment can be seen in Table 2. The results of the post-treatment weight measurements used for test the homogeneity of variances and obtained $p = 0.918$, which means that the data was homogeneous.

Table 2. Weight after treatment in all four groups

Group	n	Weight (gram)		
		Mean \pm SD	Min	Max
Sham+vehiculum	8	26.63 \pm 2.67	24	30
OVX+vehiculum	7	26.29 \pm 3.15	26	32
OVX + estrogen	7	25.14 \pm 2.67	22	31
OVX + acupuncture	6	28.17 \pm 2.14	25	30

Change (delta) of weight is the weight difference of the post-treatment weight reduced by pretreatment weight. Detailed data can be seen in Table 3. The normality test was obtained $p = 0.73$. Thus, it could be concluded that the group distribution was normal and could be used at ANOVA test. The ANOVA result was $p = 0.76$, means there was no different in all four groupgroup.

Table 3. Change (delta) of initial body weight in all four groups

Group	n	Weight (gram)			One way Anova
		Mean±SD	Min	Max	
Sham+vehiculum	8	0.75 ± 2.82	-4	4	F=2.594
OVX+vehiculum	7	2.71 ± 2.43	0	2	p=0.076
OVX + estrogen	7	0.71 ± 0.95	-1	2	
OVX + acupuncture	6	-0.67 ± 2.07	-3	3	

Weight change is a problem that is often complained by women who reach menopause. Decreased estrogen levels as a result of discontinued producing of estrogen in the blood thought to be one reason for the increase in weight in this phase. Selection of female mice with the age of 3-4 weeks with weight of 25-35 grams was the age range of adult mice that could serve as a model of menopause by removing ovaries (ovariectomy = OVX).

Mice in the K1 group was performed the dorsal abdominal opening and then closed again without took the ovaries. Results that were expected in this group was the image of mice that had normal levels of estrogen due to the intact ovaries. Mice in K2 group got ovariectomy after one-weekperiod of adaptation. A week later then given estrogen injection vehiculum as the treatment with the same total volume injected into the K3. Results that were expected in this group was the image of mice that had underwent menopause without estrogen replacement therapy or other activities that caused boost of estrogen levels in the blood.

In K3 group, mice performed ovariectomy after one-weekperiod of adaptation and then given estrogen injections with predetermined volume which was dose of 10 mg/kg/day for four weeks. Results that were expected in this group was mice underwent menopause but had estrogen hormone replacement therapy. K4 group mice was treated as menopause similar to K2 and K3 groups but this group was expected to provide an overview of menopause mice that received acupuncture therapy.

From the data of weight after treatment and delta of weight above, although there was no significant difference in the group of 1,2, 3 and 4, but it could be seen that mice in the K2 group had the highest weight gain compared to other groups. It indicated that the decrease of estrogen had a big role in the incensement of body weight in ovariectomized mice. Group 1 and 2 had a

weight gain that was lower than K1, because these two groups had estrogen level that was equivalent to condition of reproductive mice, which in the K1 group had intact ovaries; while at K3, estrogens obtained from the external through the injection maintaining internal estrogen levels.

α Estrogen receptor is receptor that is expressed in almost all tissues of the body in both adipose tissue and muscle. This study aims to know α receptor expression in visceral adipose tissue. For the expression of the α receptor (ER α) in each group can be seen in Table 4.

Table 4. Expression of Receptor α in adipose Network

Group	n	Expression			One way Anova
		Mean±SD	Min	Max	
Sham+vehiculum	8	12.25 ± 2.96 ^a	7	14	F=2.179
OVX+vehiculum	7	5.43 ± 2.64	2	9	p=0.031
OVX + estrogen	7	18.86 ± 1.57 ^{ab}	16	21	
OVX + acupuncture	6	17.00 ± 2.00 ^{ab}	14	19	

In Table 5 and Figure 1 we could see there were significant differences in the expression of ER α in K1 compared to K2, K1 was also different than K3 and K4. K2 ER α expression which was a result of mice underwent menopause was different compared to all group. Meanwhile, no significant differences were found in K3 and K4. This indicates that in mice with intact ovaries and mice with injected estrogen the ER α expression was higher than the ovariectomized mice. Increased expression of ER α in K4 had the possibility of immense occurrence due to the same conditions in the K1 and K3, which indicated that CV 12 acupuncture also modulate estrogen in the blood which was proven by its ability to modulate the ER α expression in visceral adipose tissue of mice.

Research by knockout (KO) the isoform of estrogen receptor showed the importance of this isoform in lipid and glucose homeostasis. The ER α of KO mice would show insulin resistance (Foryst et al., 2008). This suggests the importance ER α role in the prognosis of menopausal obesity and further metabolic syndrome.

Table 5. Expression of β receptors in adipose Network

Group	n	Expression			One way Anova
		Mean±SD	Min	Max	
Sham+vehiculum	8	11.38 ± 1.92 ^a	9	15	F=2.179
OVX+vehiculum	7	4.00 ± 1.41 ^b	2	6	p=0.031
OVX + estrogen	7	16.86 ± 1.86 ^c	14	19	
OVX + acupuncture	6	14.17 ± 2.99 ^c	10	19	

β Estrogen receptor (ER β) was expressed on specific areas, even though it also found in adipose tissue, hypothalamus, lung and female reproductive tract. This

study aims to know the ER β expression in visceral adipose tissue. For ER β expression in each group can be seen in Table 5 and Figure 2.

In Table 5 and Figure 2 we could see that the K2 group was different from the three other groups. K1 was different compared to K3 and K4, while no significant difference was found in K3 and K4. This indicates that in the acupuncture group experienced the conditions that similar to K3 resulting in increased expression of ER β .

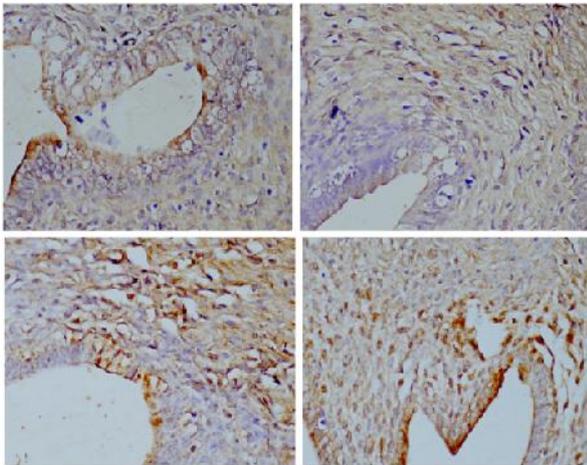


Fig. 1. Expression of α receptors in adipose tissue

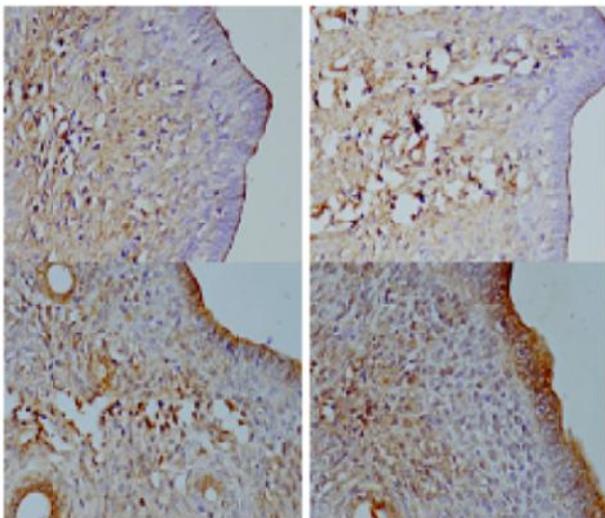


Fig. 2. The expression of β receptor in adipose tissue

To see how big the adipose mass correlated to the expression of both types of receptors, the test was followed by correlation test. The result can be seen in Table 7

Table 6. Correlation of lipid mass and receptor expressions

Group	Res α expression	Res β expression
Sham + vehiculum	0.128	0.152
OVX + vehiculum	0.302	-0.215
OVX + estrogen	0.366	0.743*
OVX + acupuncture	0.387	0.852*

From the table above, it showed that OVX group that were injected with estrogen and got CV12 acupuncture had significant correlation, with a value of R = 0.743 and R = 0.852. Previous research conducted by knockout (KO) the isoform of estrogen receptor showed the importance of this isoform in lipid and glucose homeostasis. In the ER α KO mice would show insulin resistance while ER β KO mice and given a high-fat diet showed the higher obesity possibility (Foryst et al., 2008).

Increased incidence of obesity after menopause, visceral obesity on andropause and in sex differences indicate the importance of hormonal metabolic diseases in regulating body weight (Allende-Vigo, 2008; Geer and Shen, 2009). Estrogen played an important role in increasing the use of energy, a decrease in adipogenesis and regulation of body weight (Ariazi and Jordan, 2006). The binding of estrogen to its receptor would decrease the reactive oxygen species (ROS) in the body that acted as an antioxidant, as it was known that ROS was one pathway in the pathophysiology of obesity.

Activation of estrogen also increased the IKB α protein expression which would inhibit NFKB incensement. Decreased levels of NFKB will reduce inflammation. In addition, decreased levels of NFKB activation will also reduce PPARG and C/EBPs thus there will be a decrease in adipogenesis. Decrease NFKB also reduce levels of TNF α and interleukin 6 that had impact on the production of cytokines by inflammatory cells (Miller et al., 2011).

In the prevention and treatment of obesity, targeting the ER β is more profitable than ER α . This is because the stimulation ER α will increase the risk of uterine cell growth and increase the probability of occurrence of uterine cancer. In the study conducted by Yepuru et al. (2010), activation of ER β with selective ligand will increase the uncouple protein-1 and uncouple protein-2 by two to three fold. Both of these proteins are known to play a role in enhancing thermogenesis and increase the heat output. In addition, activation of this receptor also decreased the expression of PPARG that play a role in adipogenesis.

CONCLUSION

CV12 Acupuncture prevent weight gain induced by ovariectomy. This was due to the ability of increasing the expression of α and β estrogen receptor significantly.

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