EPIGALLOCATECHIN GALLATE OF GREEN TEA INHIBITS PROLIFERATION, DIFFERENTIATION AND TNF-α IN THE PRIMARY HUMAN VISCERAL PREADIPOCYTES CULTURE

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ABSTRACT

Objectives: Green tea EGCG (epigallocatechin gallate) have shown to cause loss of body fat and also inhibit the growth of cancer cell. Therefore the aim of this study is to determine whether green tea EGCG can directly inhibit proliferation, differentiation and TNF-α (tumor necrosis factor-α) of the human visceral preadipocyte culture.

Methods: Primary human visceral preadipocytes culture were treated with EGCG in several doses 0 µM (control group), 10 µM, 50 µM, 100 µM and 200 µM for 24 hours respectively. The human visceral tissues specimens were derived from the Surgery dept. of Dr. Syaiful Anwar General Hospital in Malang. Cellular account of the adipogenesis processes (proliferation, differentiated and undifferentiated cells) was determined using microscope after hematoxyline staining. TNF-α concentration derived from primary human preadipocytes culture also observed using ELISA Kit Assay design. Results: It was found that there were a dose dependent respond to the cellular account for proliferation in EGCG control group (78.8 ± 5.88%), EGCG 10 µM (66.32 ± 4.63%), EGCG 50 µM (48.72 ± 3.84%), EGCG 100 µM (28.2±4.63%) and EGCG 200 µM (62.68±3.67%). There were also significant decrease of the percentages in the differentiated cells in EGCG control group (66.43± 3.62%), EGCG 10µM (67.56±3.61%), EGCG 50 µM (62.75±2.42%), EGCG 100 µM (21.52±3.44%), EGCG 200 µM (28.04±1.24%). There were not any changes in the percentages of undifferentiation cell in the groups of EGCG 10 µM and 50 µM, however there was significant (p<0.001) increase in ECGC 100µM (78.47±3.44%) and ECGC 200µM (71.95±1.24%) compared to EGCG control group (33.56±3.62%). The concentration of TNF-α also decrease in the dose dependent of EGCG. It was showed that the ECGC dose 100 µM yielded maximum effect in the inhibition of the adipogenesis process. Conclusions: Green tea EGCG inhibits adipogenesis processes in the level of proliferation, differentiation cells and TNF-α, in dose dependent manner.

Key words: green tea epigallocatechin gallate (EGCG), human preadipocytes, proliferation, differentiation, TNF-α

INTRODUCTION

The prevalence of overweight and obesity has reached epidemic proportion in the recent years (Deitel 2003). For example in the US about two-thirds adult are currently overweight or obese. Whilst in Asia such as: South Korean adult the overweight is 20.5% and obesity is 1.5%. In Indonesia the national survey indicates about 8.1% adult men are overweight, about 13.5% adult women are overweight, and about

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6.5% adult women are obese. Evidence indicates that overweight and obesity are strongly associated with major health risks such as cardiovascular diseases, diabetes and cancer. These obesity-related adverse health consequences have lead to an increased number of preventable deaths (Fontaine et al., 2003). Therefore the prevention and treatment of obesity are critical to curtail the rising incidence of morbidity and mortality.

The cause of obesity is usually attributed to a combination of genetic and environmental factors, and the disorder itself involves specific biological factors that regulate energy homeostasis. Recent studies indicate that adipose tissue plays important role in energy homeostasis, and its function is not limited to a site of energy storage and mobilization, but it is extended as an active endocrine organ (Ahima & Filer, 2000). Adipose tissue secretes peptides, such as leptin and tumor necrosis factor-α (TNFα), that are involved in food intake and energy expenditure. Adipose mass, at any given time, reflects average adipocyte size and total adipocyte number. It was once thought that adipocyte acquisition was permanent and that weight gain/loss caused only variation in cell size (Ailhaud, 1992). However, it is now believed that adipose tissue undergoes dynamic-remodelling throughout adulthood.

Adipogenesis occurs when extra fat mass is needed for significant caloric gain or for cellular homeostasis (Rosen 2002). In obesity or overweight people, the increased of fat mass can be resulted of either increase size cell (hypertrophy) or increase fat cell number (hyperplasia) or both. Adipose tissue hyperplasia is a result of an increase in adipogenesis, which includes preadipocyte proliferation and adipocyte differentiation (Lin, et al. 2005). Therefore potential therapeutic substances, especially from low-toxicity natural products, that have the ability to reduce or inhibit adipogenesis or increase cell death by apoptosis could have an advantage impact as a strategy for treating or preventing obesity and related metabolic disorder (Wolfram, et al. 2006), such as the potential of green tea. In vitro studies some researchers have shown that the most active substances derived from green tea called epigallocatechin-3-gallate (EGCG) inhibit adipogenesis, induce apoptosis, inhibit mRNA resistin gen, inhibit proliferation and differentiation in 3T3-L1 adipocyte (Liu et al. 2006; Hung et al., 2005; Diepvens et al. 2007). The effect of EGCG in apoptosis has approved in cell cancer (Hsu et al., 2005; Landis-Piwowar et al, 2007). Tumour necrosis factor α (TNF-α) as one of signaling molecule for apoptosis (Aggarwal 2000) mediated apoptosis in brown adipocytes (Nissoll et al., 2000). However TNF-α expression was elevated in experimental obesity models (Hofmann et al 1994; Porras et al. 1997) and obese human
Our previous studies have shown that crude extract of green tea (Camellia sinensis) decreased interleukin-1 in Rattus norvegicus, decreased interleukin-6 in primary culture of HUVEC (human umbilical vein endothelial cell). However it has not known yet whether EGCG could inhibit adipogenesis in human visceral preadipocyte. Therefore, the objective of this study was to investigate whether EGCG inhibit adipogenesis and TNF-α in the primary culture of the human visceral preadipocytes.

METHOD

The reason we used primary culture of the human visceral fat was to have a model that as close as in human. The specimen of the human visceral preadipocyte tissues was obtained from Surgery Department of the Syaiful Anwar General Hospital and Mutiara Bunda Hospital in Malang, after met the Ethical Clearance. Briefly, after the adipose tissue cleaned from surrounding tissues, then adipose tissue was washed and moulded. In order to get the preadipocyte isolation, suspended tissue was incubated with Collagenase type-1 0.2%mg/ml (Sigma) for 45 minutes, 37°C with shaking. Incubation was stopped using culture medium DMEM/F12 (1:1) that added by 15 mmol/l HEPES, 14 mmol/l NaHCO3, 33 µmol/l biotin, 17 µmol/l D-pantothenate and 10% FBS. After it was filtered using nylon mesh (250 µm), cell suspended was centrifuged in 1500 rpm for 7 minutes. After two days, human preadipocyte culture was growth in adipogenic medium (DMEM/F12 added by 100 U/ml Penicillin, 100 U/ml Streptomycin, 66 mM Insulin, 100 nM dexamethasone, 0.5 mM IBMX and 10 µg/ml transferin) to have cell differentiation. Cell suspended was growth in culture plate incubated in 37°C, 5% CO2 for 24 hours. Cells was washed every 3 days. (Samad et al. 2004, Indra 2006)

EGCG (Sigma) was incubated in the preadipocytes culture after reaching monolayer states for 24 hours. There were several doses of EGCG i.e. 0 µM, 50 µM, 100 µM and 200 µM. Then cell were cropped in the following day (day two) (Lin et.al., 2005). In order to count the percentages of the cellular proliferation and differentiation, the cells were counted every day after the treatment, in 25 different fields (Hang et.al., 2000). TNF-α derived from primary preadipocyte human visceral media was measured by Human TNF-α ELISA kit (Bioassay). Then all of the results were analyzed statistically using Anova in computerized SPSS version 13 program.
RESULTS

In order to measure the proliferation of adipocyte based on the total amount of the differentiated and undifferentiated cells. Preadipocyte differentiated cells were having fibroblast-like adipocyte, then became a mature adipocyte cell was having polygonal form. Unlike the cellular differentiation, the undifferentiated cell can be detected by no changing in its morphology. (Figure 1)

There were a significant ($p=0.000$) decrease of the cellular account for proliferation in EGCG control group (78.8 ± 5.88%), EGCG 10 µM (66.32 ± 4.63%), EGCG 50 µM (48.72± 3.84%), EGCG 100 µM (28.2±4.63%) and EGCG 200 µM (62.68±3.67%). There were also significant decrease of the percentages in the differentiated cells in EGCG control group (66.43± 3.62%), EGCG 10µM (67.56±3.61%), EGCG 50 µM (62.75±2.42%), EGCG 100 µM (21.52±3.44%), EGCG 200 µM (28.04±1.24%). However, there were not any changes in the percentages of undifferentiation cell in the groups of EGCG 10 µM and 50 µM, but there was significant ($p<0.001$) increase in ECGC 100µM (78.47±3.44%) and ECGC 200µM (71.95±1.24%) compared to EGCG control group (33.56±3.62%) (Figure 2). It was showed that the ECGC dose 100 µM was yielded maximum effect in the inhibition of the adipogenesis process

The concentration of TNF-α also decrease in the dose dependent of EGCG (Figure 3). TNF-α decreased in the preadipocyte cell exposed to 200 µM EGCG. The concentration of TNF-α in control group was significant different (394.85 ± 146.93 pg/ml) compared to the others. Even the group of EGCG 200 µM was decrease (16.99 ± 11.73 pg/ml) significantly ($P< 0.01$) compared to the control group and EGCG 10 µM (142.47 ± 23.09 pg/ml), but there was no significant different when compared to that in EGCG 50 µM (83.09 ± 25.07 pg/ml) and EGCG 100 µM (43.79 ± 11.23 pg/ml)
Figure 1. Morphology aspect of the proliferation and differentiation of the human primary culture of visceral preadipocyte induced by differentiation factors and EGCG 50 µM, 100 µM, 200 µM for 24 hours (Photomicroscope Olympus CKX31, 400X)

Figure 2. The percentages of total cell proliferation, differentiation, and undifferentiation in the primary culture of the human visceral preadipocyte induced by differentiation factors and EGCG in several doses (10 µM, 50 µM, 100 µM, 200 µM) for 24 hours.
DISCUSSION

Our study have approved that green tea EGCG inhibited adipogenesis in primary human visceral adipocyte culture. These results are similar to that of Liu et.al. (2006), Lin et.al (2005) and Hung et.al. (2005), even they have used 3T3-L1 adipocyte culture instead. In this study has shown that EGCG 100 µM is the most effective dose to inhibit the proliferation and the differentiation of the primary preadipocyte culture. However in the study by Lin et.al. (2005) have shown that EGCG 200 µM the most effective to decrease the lipid droplet size, the percentage of total lipid droplet number and total cell counts of 3T3-L1 adipocyte culture. Using Laser Scanning Cytometry, Lin et al (2005) also showed that 24 hour EGCG treatment caused significant increase of adipocyte apoptotic especially at the EGCG 50 µM and EGCG 200 µM concentration, although the increase with EGCG 100 µM was not significant. In addition to the inhibitory effect on differentiation, apoptosis was also increased by incubation with EGCG during differentiation, suggesting that EGCG can reduce adipose tissue mass both by inhibiting maturation and by increasing cell death (Lin et.al. 2005). However there was no significant apoptosis after 24 hours EGCG
incubation in 3T3-L1 preadipocyte culture (Lin et al., 2005), this is not the same as our results. This discrepancy could not be explained yet this time, except that we have used a primary human visceral preadipocyte culture. It is a deemed to elaborate and design more details study.

Possible mechanism of action for EGCG-induced inhibition of adipogenesis could involve mitogen activated protein kinase (MAPK), especially the extracellular signal-regulated kinases (ERKs) which are stimulated by growth-related signals (Lin et al., 2005). EGCG has showed the most effective in reducing the amounts of phospho-ERK1/2 protein (Hung et al., 2005; Liu et al., 2006). It has shown that EGCG did not alter Cdk2 protein expression at 24 hours of incubation in preadipocyte culture 3T3-L1, but it was found to dose (20-100 µM) dependently decrease the activity of Cdk2, which is well known as a downstream protein regulating cell mitogenesis (Hung et al., 2005).

TNF-α expression was elevated in experimental obesity models (Hofmann et al 1994; Porras et al. 1997) and obese human (Hube et al., 1999). Hofmann et al (1994) reported that there was an overexpression of TNF-α and its receptors in animal models for obesity linked to insulin resistance. This indicated that TNF-α may be overproduced in adipose tissue, where it has direct autocrine or paracrine effects via local receptors. Hube et al. (1999) have showed that the mean mRNA levels of both TNF receptors (p60 TNF and p80 TNF) two-three fold higher in adipose tissue samples from obese than non-obese women. Thus it is suggested that mRNA TNF-α expression to be up-regulated in adipose tissue. Therefore when the number of the adipose cells decrease, TNF-α also decrease. These results are similar to our study, especially when the preadipocyte culture have exposed to the ECGC 50 µM, 100 µM and 200 µM.

Our study indicated that there is an inhibitory effect of EGCG on differentiation, proliferation and TNF-α in the primary culture of the human visceral preadipocyte in vitro. These results support the basic theory that EGCG may prove to be a valuable natural product in the prevention and or treatment obesity. However more constructive in vitro and in vivo studies are still needed.

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