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Effect of (-)-epigallocatechin-3-gallate on lipogenesis in 3T3-L1 Cell

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Abstract

We studied the effect of epigallocatechin-3-gallate(EGCG) on the adipose conversion of 3T3-L1 cells by insulin. In the 10 days of culture with insulin, the fat cells exhibited the increased and larger intracytoplasmic lipid droplets. In contrast, the levels of triglyceride(TG), a marker of adipose conversion, were decreased. However, the levels of glucose were decreased in the adipose conversion. In addition, levels of cholesterol were decreased in the differentiated 3T3-L1 cells.

Key words : EGCG, Lipogenesis, 3T3-L1, Triglyceride

Introduction

Tea is one of the most popular beverage in the world because of its attractive flavor, aroma, and taste. Over the 300 different kinds of tea are now available, but there are only 3 general forms of tea, that is, the unfermented green tea, the partially fermented paochong tea or oolong tea and the fermented black tea. Green tea is manufactured by steaming or drying fresh tea leaves to prevent oxidation of the green tea polyphenols[1].

Green tea is a popular beverage world-wide. The epicatechin derivatives, which are commonly called "polyphenols", are the active ingredients in green tea and possess antioxidant[2]. Many biological functions of polyphenols within tea have been studied[3] including anti-oxidative activity[4,5], anti-inflammation[6], antimutagenic[7], and anti-carcinogenic effects[8], lowering of plasma cholesterol and triglyceride levels, and reduction of blood pressure and platelet aggregation[9] in several system.

This experiment was carried out to investigate the role of epigallocatechin-3-gallate(EGCG) on lipolysis of well-differentiated 3T3-L1 cells. The results in present study suggest that EGCG inhibited lipogenesis.

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Materials and Methods

Chemicals

Epigallocatechin-gallate(EGCG) was provided by Sigma(USA). (-)-Epigallocatechin gallate was dissolved in 200 ㎕ ethanol. The dissolved EGCG was then diluted in 2,100 ㎕ of double distilled Water (DDW) after vortexing. The reagent was stored under -20℃ after filtration by 0.2 ㎛ syringe filter. Final volume was 2,300 ㎕(10 mM).

Dulbecco's Modified Eagle's Medium (DMEM), bovine insulin, dexamethasone, 3-isobutyl-1-methyl-xanthine(IBMX), penicillin-streptomycin solution and 2.5% trypsin-EDTA were purchased from Sigma(USA). Fetal bovine serum(FBS) was purchased from Biowhittaker(Cambrex. USA).

The stock solution of dexamethasone was at the concentration of 1 mM solution in ethanol. IBMX was prepared to 100 mM in DMSO. They were stored at -20℃. Insulin stock solution was stored at the concentration of 10 ㎎/㎖ in DDW. The reagent was stored under -20℃ after filtration by 0.2 ㎛ syringe filter. The medium to initiate differentiation of 3T3-L1 cells consists of Dexamethasone 0.5 ㎖ and IBMX 2.5㎖ in DMEM(500 ㎖) containing 10% FBS.

Cell Culture

Cells were grown in DMEM containing 10% FBS and 1% penicillin-streptomycin at 37℃ in a humidified CO_2(5%) incubator. At confluence(day 0), the medium was changed to DMEM-dexamethasone-IBMX containing 10% FBS to initiate differentiation of 3T3-L1 cells(D1 treatment). Differentiation was induced with 10 ㎍/㎖ inulin(D2 treatment). The medium containing 10㎍/㎖ insulin was changed for the differentiation of 3T3-L1 cells at 2 days interval. EGCG was present at the confluence day of the culture.

Cells were harvested at D1 treatment, D2 treatment(first day), D2 treatment(3rd days), 5th days, 7th days and 9th days, respectively.

Assays

Total cholesterol, glucose, and triglyceride from sonicated cell extracts were measured by spectrophotometry(Bio-tek instruments, USA). The protein content of the homogenate was measured by using a GeneQuant Calculator(Amersham Bio, USA). Bovine serum albumin was used as a standard.

Results

In 2 days after culture with dexamethasone, IBMX, and insulin, a few of intracytoplasmic lipid droplet in 3T3-L1 was observed at control and EGCG treatment(Fig 1, b, and c). In the 10 days of culture with insulin, the fat cells exhibit larger intracytoplasmic lipid droplets(Fig 1. d-k). When 20 ㎛M of EGCG was added, lipogenesis due to insulin was inhibited(Fig 1. e, g, i and k), compared with normal condition(Fig 1. d, f, h and j). During this period, triglyceride (TG) level, which was used as a marker of differentiation, and a significant decrease in TG level was observed(Fig 2). Glucose levels were significantly decreased on the adipose conversion(Fig 3). The levels of total cholesterol(TG) were decreased in differentiated 3T3-L1 cells(Fig 4).

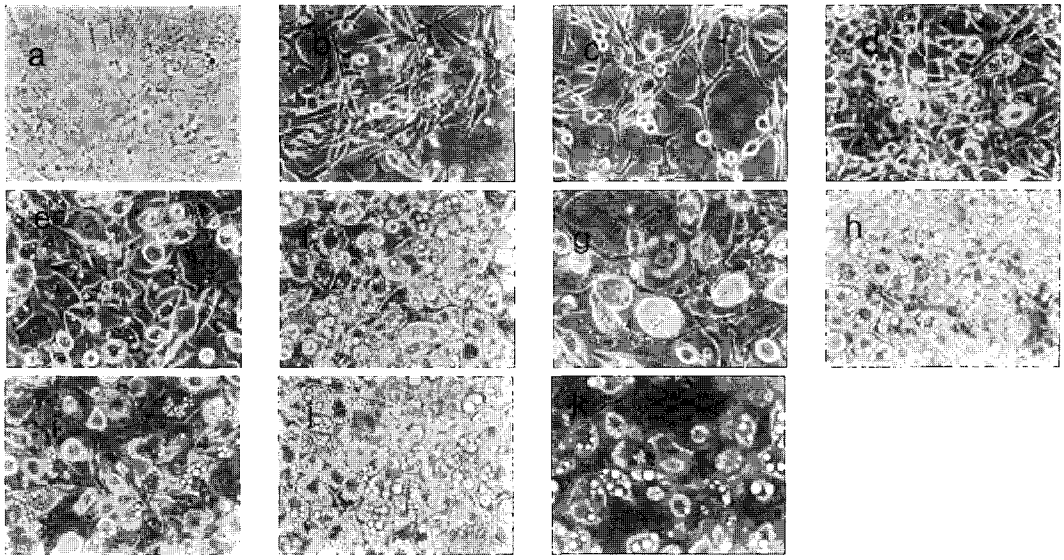

Fig 1. Effect of EGCG on insulin-induced adipose conversion of 3T3-L1 preadipocytes.
a : Not differentiated, b : DMEM + Dexamethasone(1 μM) + IBMX (0.5 mM) + Insulin(10 ㎍/㎖),
c : DMEM+Dexamethason(1μM)+IBMX (0.5 mM) + Insulin(10 ㎍/㎖) + EGCG(20 μM),
d : DMEM + Insulin(10 ㎍/㎖) for 2 days, e : DMEM + Insulin(10 ㎍/㎖)+ EGCG(20 μM) for 2 days,
f : DMEM + Insulin(10 ㎍/㎖) for 4 days, g : DMEM + Insulin(10 ㎍/㎖) + EGCG(20 μM) for 4 days,
h : DMEM + Insulin(10 ㎍/㎖) for 6 days, I : DMEM + Insulin(10 ㎍/㎖) + EGCG(20 μM) for 6 days,
j : DMEM + Insulin(10 ㎍/㎖) for 8 days, k : DMEM + Insulin(10 ㎍/㎖) for 8 days.

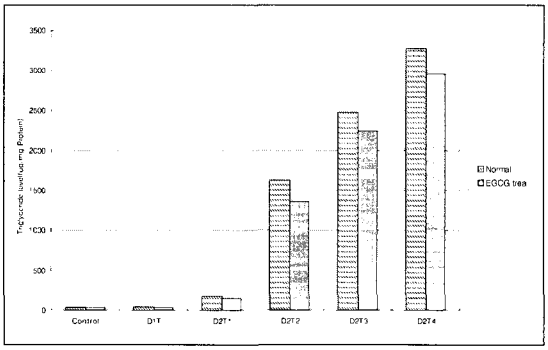

Fig 2. Effect of EGCG on Isulin-induced Triglyceride level in 3T3-L1 cells
D1T : DMEM + Dexamethason (1μM) +IBMX (0.5 mM) + Insulin (10 ㎍/㎖).
D2T1 : DMEM + Insulin (10 ㎍/㎖), 1st time.
D2T2 : DMEM + Insulin (10 ㎍/㎖), 2nd time.
D2T3 : DMEM + Insulin (10 ㎍/㎖), 3rd time.
D2T4 : DMEM + Insulin (10 ㎍/㎖), 4th time.
Normal : Not treatment EGCG in DMEM.
EGCG : Treatment 20 μM EGCG in DMEM.

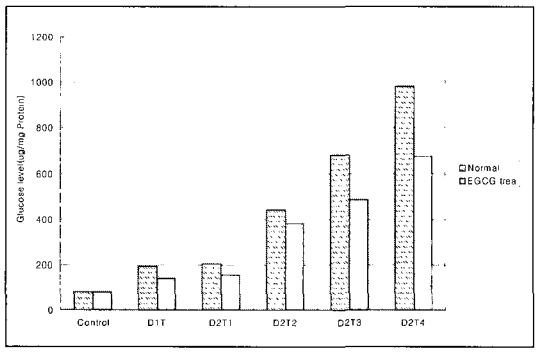

Fig 3. Effect of EGCG on Insulin-induced Glucose level in 3T3-L1 cells
D1T : DMEM + Dexamethason (1μM) +IBMX (0.5 mM)+ Insulin (10 ㎍/㎖).
D2T1 : DMEM + Insulin (10 ㎍/㎖), 1st time.
D2T2 : DMEM+Insulin (10 ㎍/㎖), 2nd time.
D2T3 : DMEM + Insulin (10 ㎍/㎖), 3rd time.
D2T4 : DMEM+Insulin (10 ㎍/㎖), 4th time.
Normal : Not treatment EGCG in DMEM.
EGCG : Treatment 20 μM EGCG in DMEM.

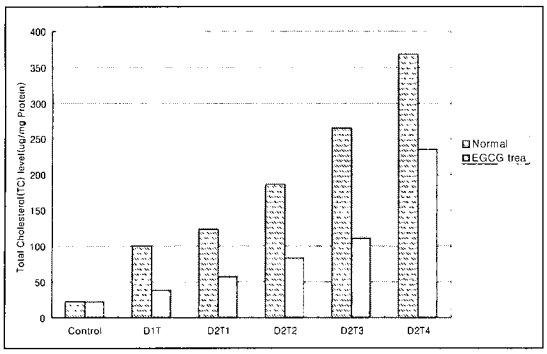

Fig 4. Effect of EGCG on Insulin-induced Total Cholesterol(TC) level in 3T3-L1 cells
D1T : DMEM + Dexamethason (1 μM) +IBMX (0.5 mM) + Insulin (10 μg/mℓ).
D2T1 : DMEM + Insulin (10 μg/mℓ), 1st time.
D2T2 : DMEM + Insulin (10 μg/mℓ), 2nd time.
D2T3 : DMEM + Insulin (10 μg/mℓ), 3rd time.
D2T4 : DMEM + Insulin (10 μg/mℓ), 4th time.
Normal : Not treatment EGCG in DMEM.
EGCG : Treatment 20 μM EGCG in DMEM.

Discussion

Polyphenols are plentiful in all kind of the beverage. Polyphenol is divided into catechin, epicatechin, epigallocatechin, and epigallocatechin gallate. In adipocytes, cAMP exerts a key role in differentiation process[10]. Together with the above findings, EGCG was suggested to inhibit insulin-induced lipogenesis through the prevention of the cAMP-dependent biological process. When mature adipocytes were exposed to EGCG, smaller intracytoplasmic lipid droplets were observed. TG was decreased after exposure to EGCG, suggesting lipolytic substance as shown previously[11]. Glucose and TC were also decreased in the differentiated 3T3-L1 cells. Present study shows that the antiadipogenic effect of EGCG is mediated via the decreased level of cAMP. In addition to other beneficial effects, EGCG can also be useful in preventing obesity for maintaining optimal health or the control of diabetes.

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