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Quantitative combination of natural anti-oxidants prevents metabolic syndrome by reducing oxidative stress

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ABSTRACT

Insulin resistance and abdominal obesity are present in the majority of people with the metabolic syndrome. Antioxidant therapy might be a useful strategy for type 2 diabetes and other insulin-resistant states. The combination of vitamin C (Vc) and vitamin E has synthetic scavenging effect on free radicals and inhibition effect on lipid peroxidation. However, there are few studies about how to define the best combination of more than three anti-oxidants as it is difficult or impossible to test the anti-oxidant effect of the combination of every concentration of each ingredient experimentally. Here we present a math model, which is based on the classical Hill equation to determine the best combination, called Fixed Dose Combination (FDC), of several natural anti-oxidants, including Vc, green tea polyphenols (GTP) and grape seed extract proanthocyanidin (GSEP). Then we investigated the effects of FDC on oxidative stress, blood glucose and serum lipid levels in cultured 3T3-L1 adipocytes, high fat diet (HFD)-fed rats which serve as obesity model, and KK-ay mice as diabetic model. The level of serum malondialdehyde (MDA) in the treated rats was studied and Hematoxylin-Eosin (HE) staining or Oil red slices of liver and adipose tissue in the rats were examined as well. FDC shows excellent antioxidant and anti-glycation activity by attenuating lipid peroxidation. FDC determined in this investigation can become a potential solution to reduce obesity, to improve insulin sensitivity and be beneficial for the treatment of fat and diabetic patients. It is the first time to use the math model to determine the best ratio of three anti-oxidants, which can save much more time and chemical materials than traditional experimental method. This quantitative method represents a potentially new and useful strategy to screen all possible combinations of many natural anti-oxidants, therefore may help develop novel therapeutics with the potential to ameliorate the worldwide metabolic abnormalities.

1. Introduction

Metabolic syndrome is a disorder of energy utilization and storage, and a constellation of cardiovascular risk factors including abdominal obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, dyslipidemia, and low high-density cholesterol (HDL) levels, etc. Insulin resistance and abdominal obesity are present in the majority of people with metabolic syndrome. Obesity is a major risk factor for Type 2 diabetes mellitus (T2DM) [6] which has two most common features: high blood glucose (hyperglycemia) and hyperlipidemia. Hyperglycemia is a result of the body being unable to produce enough insulin, or not being able to use the insulin properly (insulin resistance).

Obesity-related disorders is linked with the change of redox status with increased oxidative stress in adipose tissue. Oxidative stress in adipocytes plays a central role in T2DM and obesity-caused insulin resistance [26]. Reactive oxygen species (ROS), the radical forms of oxygen and the by-products of mitochondrial respiration and enzymatic oxidases, can act as signaling molecules. ROS are also a common feature of insulin resistance [13]. Therefore, antioxidant therapy might be a useful strategy for type 2 diabetes and other insulin-resistant states. Studies showed that many anti-oxidants exhibited protective effect on preadipocytes exposed to oxidative stress [11]. Some anti-oxidants, such as N-acetylcysteine (NAC) and manganese (III) tetrakis (4-benzoic

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acid) porphyrin (MnTBAP), showed dose-dependent suppression of insulin resistance [13], while others, such as green tea catechins (GTCs), GTP, GSEP, etc. which are associated with a lower risk of obesity, can modulate fat metabolism [27], and ameliorate pancreatic beta-cell dysfunction and death in HFD-induced diabetic rats [4]. Some antioxidants, such as Vc supplementation, can ameliorate some aspects of the obesity-diabetes syndrome in fat hyperglycemic mice [1] and may be beneficial in preventing the development of diabetic nephropathy.

It has been known that, the combination of Vc and vitamin E has synthetic scavenging effect on free radicals and inhibition effect on lipid peroxidation. However, there are few studies about how to define the best combination of more than three anti-oxidants because it is difficult to test the anti-oxidant effect of the combination of every concentration of each ingredient experimentally.. The question whether the combined administration of these anti-oxidants appears to be essential to reinforce the anti-oxidative effect of single component keeps open.

To address this question, we at first present a math model which is based on the classical Hill equation to get the best combination, which is called FDC, of several natural anti-oxidants Vc, GSEP and GTPs, after the measurement of scavenging effect of these components with different doses on hydroxyl free radicals.

Complex diseases, such as metabolic syndrome, can be induced by either genetic factors or environmental and lifestyle factors, or a combination of both. We investigated the effects of FDC on oxidative stress, blood glucose and serum lipid levels in cultured 3T3-L1 adipocytes, Diet-Induced Obese rats (DIO rats) and KK-ay mice. DIO rats here serve as an obesity model caused by environmental factors, while KK-ay mice a diabetic model caused by genetic factors. We tested the hypothesis that FDC reduces the fat mass and oxidative stress in 3T3-L1 adipocytes, KK-ay mice and DIO rats. Finally, the level of serum MDA in the treated rats was studied and the HE or Oil red slices of liver and adipose tissue in the rats were examined to study whether FDC can inhibit lipid peroxidation and reduce fat accumulation in different tissue.

Our quantitative model can be used to determine the best combination of many natural anti-oxidants. It represents a potentially novel and useful strategy to screen all possible combinations of many natural anti-oxidants, therefore may help develop novel therapeutics with the potential to ameliorate the worldwide metabolic abnormalities.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture media and supplements were obtained from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM) and newborn calf serum were purchased from Gibco BRL (Grand Island, NY). 2',7'-dichlorofluorescin diacetate (DCFH-DA) was purchased from Sigma Chemical Company (St. Louis, MO, USA). All anti-oxidants, including Proanthocyanidins (20% glycerin dissolved), Vitamin E powder, Vitamin C, Grape seed extract, Grape skin extract, Oligo Proanthocyanidin (OPC > =85%) and Tea Polyphenol, were purchased from TianJin JianFeng Natural Product R&D Co., Ltd., Tianjin, PR China. The precise composition and purity of these compounds are shown in Table S1 (for OPC), S2 (for Tea Polyphenol) and S3 (for Grape skin extract), respectively. All other chemicals made in China were of analytical grade.

2.2. Assay for the scavenging effect on hydroxyl radical generated from Fenton reaction system

The $HO^{\bullet}$ scavenging activity was assessed according to previous reference with slight modification [16]. $HO^{\bullet}$ was generated by a Fenton-type reaction at room temperature. The reaction mixture (1.0 mL) contained 600 μL of luminol (0.1 mM, diluted in the carbonic acid-buffered saline solution (CBSS, pH 10.2), 100 μL of sample solution (with different concentrations, replaced with CBSS in the control), 200 μL of Fe^{2+}–EDTA (3 mM), and 100 μL of H_2O_2 (1.2 mM). Initiation of the reaction was achieved by adding Fe^{2+}–EDTA and then H_2O_2 into the mixture.

The $HO^{\bullet}$ scavenging abilities of all anti-oxidants mentioned above were assessed by the chemiluminescent method. The chemiluminescence (CL) intensity integral was recorded and the inhibitory rate was obtained according to the formula: inhibitory rate (%)=$[(CL_{control}-CL_{sample})\times 100]/CL_{control}$.

2.3. Math model to determine FDC

The process of Initiation and inhibition of free radical processes can be described by the Hill equation [12].

$$f(x)=\frac{k^n}{k^n+(a-\log_{10}x)^n},\quad 0<x\le 10^a,\quad n>0$$

$$f(x)=1,\quad x>10^a$$

where x is the final concentration of FDC (unit: μg/ml); $f(x)$ is the free radical scavenging percentage when the concentration of FDC is x; a, the log value of the FDC concentration, is the concentration of x when the curve reaches the maximum; n determines the curvature of the curve ($n>0$). y=0.5 when n=0; y can be 0 or 1 when $n=+\infty$; and k determines the IC_{50}, the concentration of x when 50% of free radical were scavenged:

$$f(x_{IC50})=0.5,\quad k=a-\log_{10}x_{IC50}$$

For more details, please check the Supplementary online materials.

2.4. Cell culture and treatment

3T3-L1 fibroblastic cells (preadipocytes) were cultured and maintained in culture medium (DMEM supplemented with 100 μg/ml streptomycin, 75 μg/ml penicillin, and 10% newborn calf serum) at 37 °C in humidified 5% CO_2/95% air atmosphere. 3T3-L1 preadipocytes were induced and differentiated into adipocytes as described before with slight modification [26].

Completely confluent plates were incubated in DMEM containing 10% FBS with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 10 μM dexamethasone and 0.5 μg/ml insulin. Two days after incubation, the medium was replaced with DMEM containing 5 μg/ml insulin. Fresh culture medium was added after 2 days and then every other day till the cells attained adipocyte morphology identified by Oil Red O stai4ning.

2.5. Measurement of intracellular ROS induced by DEX or TNF-α in adipocytes

Fully differentiated adipocytes were incubated with DEX or TNF-α to induce intracellular ultra ROS generation as a model in high level oxidative stress state [27]. 3T3-L1 cells treated with DEX or TNF-α and FDC were used to measure the effect of FDC, while cells without any treatment as control. Fully differentiated adipocytes were incubated with cell-permeable, oxidation-sensitive and non-fluorescent reagent DCFH-DA (final concentration 10 μmol/L for 30 min at 37 °C). Oxidation of DCFH-DA by peroxides yielded fluorescent derivative dichlorofluorescein (DCF), which acts as a control for changes in uptake, ester cleavage, and efflux, to detect intracellular ROS content change.