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(RESEARCH ARTICLE)

Assessment of steviol on pro-inflammatory cytokines release in human CD14+ cells

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Abstract

Steviol is a major metabolite of stevioside which is a natural noncaloric sweetener. The aim of this present study was to investigate an inhibitory activity of steviol on pro-inflammatory cytokines release from lipopolysaccharide (LPS)-stimulated human CD14+ cells. CD14+ cells were incubated with different concentrations of steviol in the absence or presence of LPS. TNF-α, IL-1β and IL-6 level were determined by using human enzyme-linked immunosorbent assay (ELISA) kits. Steviol at the concentration of 1-100 μM significantly inhibit (P<0.05) the release of TNF-α (966.0±66.0, 906.3±36.7 and 659.3+52.3 vs 1190.3+75.5 pg/ml) and IL-1β (2834.0+67.2, 2440.0+70.5 and 2181.3+143.3 vs 3226.7 +106.6 pg/ml) in a dose dependent manner when compared to LPS-treated CD14+ cells. While the inhibition of IL-6 (P<0.05) was found at 10 μM and 100 μM (2171.7+44.3 and 1902.3+58.0 vs 2580.3+105.2 pg/ml). In conclusion, steviol, a major metabolite of stevioside, possess an anti-inflammatory activity in human CD14+ cell.

Keywords: Steviol; TNF-α; IL-1β; IL-6; CD14+ cell

1. Introduction

Steviol (SVO) is an aglycone of stevioside which is a natural non-caloric sweetener isolated from *Stevia rebaudiana* Bertoni. Stevia leaves and its extracts, including stevioside, have traditionally been used as a sweetener to sweeten a variety of foods especially for those who suffering from obesity and diabetes mellitus [1]. Stevioside is degraded into steviol by the bacterial flora of the cecum [2]. Steviol, a major metabolite of stevioside, has a molecular weight of 318.44 Da with the chemical formula of $C_{20}H_{30}O_3$. It is therefore the colonic cell line (Caco-2) was shown to absorb steviol but not stevioside [3].

Inflammatory process is triggered mainly by immune cells and it is involved with cytokines release [4]. CD14+ monocytes are immature phagocytic cells circulating in the blood that phagocytize and degrade microbes. Bacterial lipopolysaccharide (LPS) was shown to stimulate the immune cell to release pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6[5]. The inhibition of pro-inflammatory cytokines can be used as a tool to determine anti-inflammatory activity of the natural products. Therefore, the aim of this study was to examine the effects of steviol, a major metabolite of stevioside, on anti-inflammatory activity using an *in vitro* model LPS-stimulated CD14+ cells.

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2. Material and methods

2.1. Preparation steviol

Crude stevioside was supplied by Thai Pharmacognosy Research Laboratory, Chaing Mai, Thailand. Steviol (approx. 90% purity) was obtained by oxidation of stevioside as described by Ogawa et al. (1980) [6]. Purity of steviol were determined by using High-performance liquid chromatography conducted with a Waters model 510 liquid chromatograph (Waters, Millipore Corp., Milford, MA).

2.2. Isolation of peripheral blood mononuclear cells (PBMCs) and $CD14^+$ cells preparation

A 5 ml of blood was kindly donated from healthy volunteers. Peripheral blood mononuclear cells (PBMC) were separated from blood samples according to the method described by Boyum (1968) [7]. $CD14^+$ cells were removed from PBMC by using Dynabeads (M-450 $CD14^+$; Dynal Inc., Oslo, Norway) according to the manufacturer's instruction. The Dynabeads-bound cells ($CD14^+$ cells) were washed three times with phosphate-buffered saline (PBS). $CD14^+$ cells were adjust to 1 x 10^6 cells/ml and grown in RPMI 1640 medium containing 25 mM HEPES, 2 mM L-glutamine (Sigma, St. Louis, MO, USA), 10% heat inactivated fetal calf serum (Gibco BRL, USA), penicillin (100 U/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. Cell viability assay

To detect viability of cells, the method of Mosmann (1968) [8] using the MTT (3-(4, 5-dimethylthaizol-2-yl)-2, 5-diphenyltetrazolium bromide) colorimetric assay was performed. In brief, $CD14^+$ cells (1 x 10^6 cells/ml) were incubated with varying concentrations of steviol in the absence or presence of LPS (1 µg/ml)(Sigma). Formazan dye was dissolved with dimethylsulfoxide (DMSO) and the absorbance of each well was measured at 540 nm in an automatic microplate reader (Wallac Victor 1420, Perkins Elmer).

2.4. Pro-inflammatory cytokines determination

$CD14^+$ cells (1 x 10^6 cells/ml) were seeded into a 96-well plate and incubated with different concentrations of steviol in the absence or presence of LPS (1 µg/ml) for 24 h in a humidified atmosphere of 5% CO_2 at 37 ºC. Supernatant fluids were collected and stored at -80º C until pro-inflammatory cytokine was assayed by using a commercial human TNF-α, IL-1β and IL-6 enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA).

2.5. Statistical analysis

Data from five individual experiments were analyzed and presented as mean $\pm$ S.E.M. Statistical significance was determined by using one-way ANOVA and student Newman-Keuls, with a value of $p<0.05$ as being statistically significant.

3. Results and discussion

3.1. Effect of steviol on cell viability

To confirm non-toxic concentration of steviol in the determination of pro-inflammatory cytokines release, cytotoxic effects of steviol on $CD14^+$ cells was determined by using MTT assay. This method has been widely used to monitor cell viability. The metabolic activity is measured in populations of cells by incubating the cells with a tetrazolium salt (MTT) that is cleaved into a colored formazan product by cellular metabolic activity [9]. Steviol, in doses ranging from 0.1-100 µM had no cytotoxic effect in $CD14^+$ cells. Cell viability was about 100% (Figure 1). Previous study in four intestinal cell lines (T84, HT29, Caco-2 and IEC-18 cells) investigated the effects of steviol on cell viability and reported that steviol at concentration of 0.1-100 µM had no cytotoxic effect in T84, HT29, Caco-2 and IEC-18 cells. Moreover, steviol at higher concentrations, 0.2 mM and 0.8 mM, decreased cell viability, cell viabilities were 80-90% and 7-34% respectively [10].

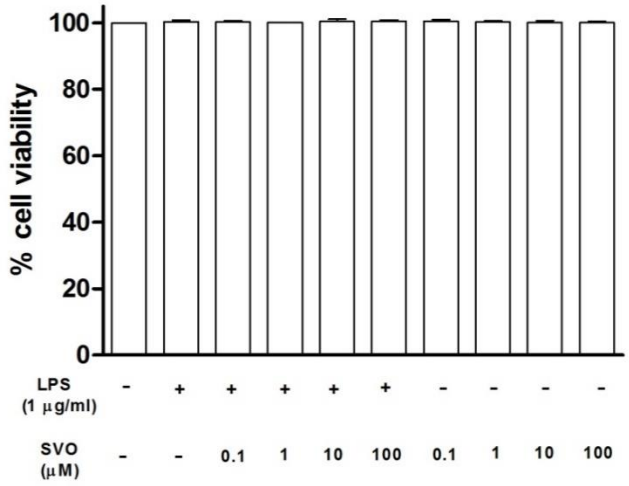

Figure 1 Cytotoxic effect of steviol (SVO) on CD14⁺ cells

CD14⁺ cells were cultured with the indicated concentrations of steviol (SVO) alone or in combination with LPS (1 μg/ml). Data are expressed as the mean ± SEM of five independent experiments.

3.2. Effect of steviol on TNF-α, IL-1β and IL-6 release

It is known that pathogenic bacteria can activate monocytes or macrophages directly, initiating a cytokine cascade in the inflammatory process and the immunological response [11]. Stimulated monocytes release a broad spectrum cytokines. TNF-α, IL-1β and IL-6 are biologically active peptides produced by monocytes [12]. Thus, the interference in the production of TNF-α, IL-1β and IL-6 can be employed as criteria to evaluate anti-inflammatory effects. As shown in figure 2, level of TNF-α demonstrated a drastically increased when CD14⁺ cells were cultured with LPS (1190.3 ± 75.5 pg/ml). In the absence of LPS, steviol itself (0.1-100 μM) has no effect on TNF-α release. Interestingly, when CD14⁺ cells were cultured with steviol at concentration of 1-100 μM in the presence of LPS, the level of TNF-α was significantly decreased (P<0.05) in a dose dependent manner when compared to LPS-treated CD14⁺ cells (966.0 ± 66.0, 906.3 ± 36.7 and 659.3 ± 52.3 vs. 1190.3 ± 75.5 pg/ml).

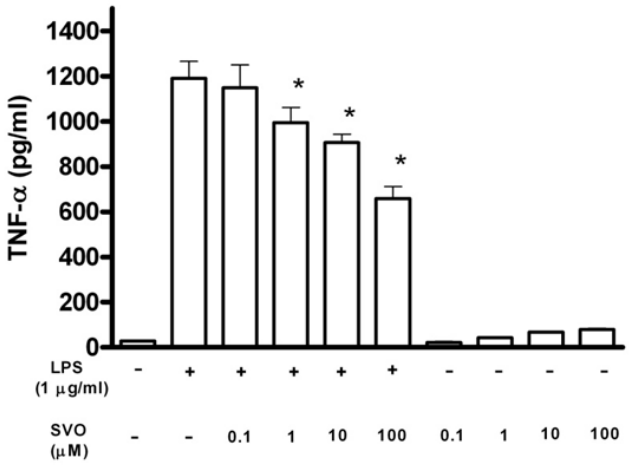

Figure 2 Effects of steviol on the production of TNF-α

CD14⁺ cells were cultured with the indicated concentrations of steviol (SVO) alone or in combination with LPS (1 μg/ml). Data are expressed as the mean ± SEM of five independent experiments. (*) Statistically significant difference in cytokine release (p<0.05), as compare with LPS-treated group.

Steviol at concentration of 0.1-100 μM has no effect on IL-1β release (Figure 3). LPS stimulated CD14⁺ cells resulted in an increase of IL-1β production (3226.7 ± 106.6 pg/ml). Similarly to TNF-, when CD14⁺ cells were cultured with steviol (1-100 μM) in the presence of LPS results demonstrated that IL-1β significantly decreased (P<0.05) when compared

to LPS-treated CD14+ cells (2834.0 ± 67.2, 2440.0 ± 70.5 and 2181.3 ± 143.3 pg/ml vs. 3226.7 ± 106.6 pg/ml) (Figure 3).

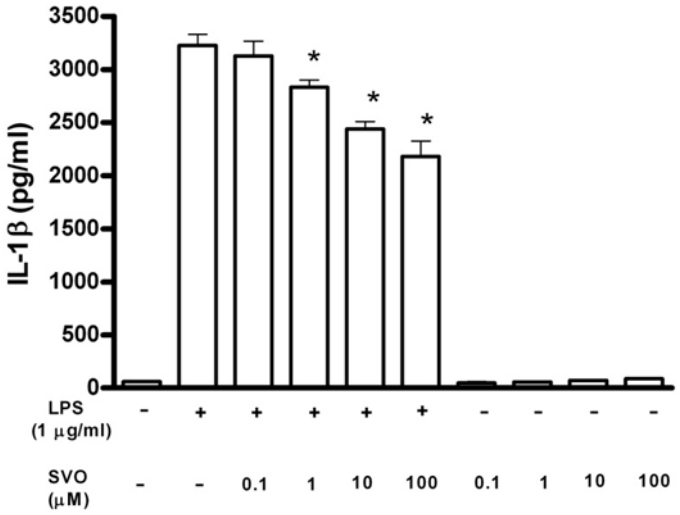

Figure 3 Effects of steviol on the production of IL-1β

CD14+ cells were cultured with the indicated concentrations of steviol (SVO) alone or in combination with LPS (1 μg/ml). Data are expressed as the mean ± SEM of five independent experiments. (*) Statistically significant difference in cytokine release ($p<0.05$), as compare with LPS-treated group.

As shown in figure 4, in the absence of LPS, steviol (0.1-100 μM) has no effect on IL-6 release whereas the level of IL-6 was drastically increased in LPS stimulated CD14+ cells (2580.3 ± 105.2 pg/ml). However, when CD14+ cells were cultured with steviol at concentration of 10 μM and 100 μM in the presence of LPS, IL-6 significantly decreased ($P<0.05$) in a dose dependent manner when compared to LPS-treated CD14+ cells (2171.7 ± 44.3 and 1902.3 ± 58.0 pg/ml vs. 2580.3 ± 105.2 pg/ml).

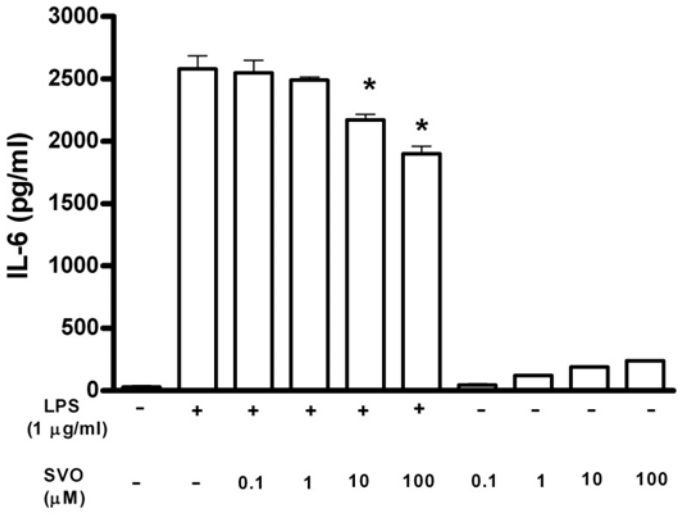

Figure 4 Effects of steviol on the production of IL-6

CD14+ cells were cultured with the indicated concentrations of steviol (SVO) alone or in combination with LPS (1 μg/ml). Data are expressed as the mean ± SEM of five independent experiments. (*) Statistically significant difference in cytokine release ($p<0.05$), as compare with LPS-treated group.

Inflammatory cytokines are important for host defense mechanism from infection [13]. In this study, steviol alone (0.1-100 μM) had no effect on TNF-α IL-1β and IL-6 release (Figure 2-4). In addition, our results also demonstrated that steviol (1-100 μM) significantly decreased the production of TNF-α, IL-1β and IL-6 (Figure 2-4) in LPS-stimulated $CD14^+$ cells cells. These activities were not attributed to cell cytotoxicity. These results are consistent with earlier observation of oral ingestion of stevioside that has an inhibitory effect on the release of TNF-α from LPS-stimulated PBMCs isolated from treated rats [14]. Since stevioside was degraded by normal flora in intestine to steviol [2]. Therefore, it is possible that the inhibitory effect on inflammatory cytokines release could be from the activity of this major metabolite, steviol.

4. Conclusion

The present study demonstrated that steviol at the concentration of 0.1-100 μM had no cytotoxic effect on $CD14^+$ cells. In addition, steviol possess an inhibitory activity on pro-inflammatory cytokines release in $CD14^+$ cells. Therefore, we can concluded that steviol has an anti-inflammatory activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Rattanasrisomporn Jatuporn, Boonkaewwan Waraporn, Kayan Autchara and Boonkaewwan Chaiwat declare that they have no conflict of interest.

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