(–)-Epicatechin attenuates high glucose-induced inflammation by epigenetic modulation in human monocytes.

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Abstract

Purpose: Diabetes is a pro-inflammatory state associated with an increased monocyte activity. NF- κ B is the master switch of inflammation and is activated during diabetes. (-)-Epicatechin (EC), the main cocoa flavanol, displays anti-inflammatory and anti-diabetic effects under high glucose conditions. Recently, it has been suggested that dietary polyphenols might modulate chromatin remodelling by epigenetic changes and regulate monocyte NF- κ B activation and cytokine expression under diabetic conditions. The aim of the study was to test the potential anti-inflammatory role of EC via inducing post-translational histone changes in the presence of a high glucose (HG) concentration.

Methods: Human monocytes (THP-1 cells) were pre-treated with EC (5 μ M) and 4 h later exposed to 25 mM glucose (HG) for a total of 24 h. Control cells were grown under normoglycemic conditions (NG, 5.5 mM glucose). Acetyl CBP/p300, HDAC4, total histone 3 (HH3), H3K9ac, H3K4me2 and H3K9me2, and phosphorylated and total levels of p65-NF κ B were analysed by Western blot. Histone acetyltransferase (HAT) activity was measured in nuclear lysates and TNF- α release was evaluated in culture media.

Results: EC incubation restored to control levels (NG) the changes induced by HG in p-p65/p65-NF- κ B ratio, acetyl CBP/p300 values and HAT activity. Moreover, EC pre-treatment counteracted the increased acetylation of H3K9 and H3K4 dimethylations and attenuated the diminished H3K9 dimethylation triggered by HG. EC also significantly decreased HG-enhanced HDAC4 levels and TNF- α release, respectively.

Conclusions: EC induces epigenetic changes and decreased NF- κ B and TNF- α levels in human monocytes cultured in HG conditions.

Keywords: Diabetes, epicatechin, epigenetics, inflammation, human monocytes THP-1 cells, post-translational histone modification.

Introduction

Diabetes is characterized by a sustained hyperglycaemia that stimulates the inflammatory response [1]. High glucose leads to the activation of the transcriptional factor nuclear factor- κ B (NF- κ B) and activates the expression of inflammatory cytokines, including tumour necrosis factor (TNF)- α and interleukin (IL)-6, in THP-1 cells and monocytes from diabetic individuals [1, 2]. p65 protein is the key transcriptionally active component of NF- κ B, and requires CBP/p300 for its transactivation to modulate gene expression [1].

Epigenetic histone modifications are considered major underlying mechanisms for sustained pro-inflammatory phenotype of diabetic cells [3, 4]. Histone acetylation stimulated by histone acetyltransferases (HATs) increases accessibility to transcription factors to induce gene transcription. On the other hand, histone deacetylation by histone deacetylases (HDACs) promotes DNA winding and therefore gene repression [1, 3]. Histone methylation has variable effects on transcription regulation; thus, Histone H3 lysine 4 dimethylation (H3K4me2) has been associated with gene activation, whereas Histone H3 lysine 9 dimethylation (H3K9me2) seems to be related to gene repression [3, 5]. In concert, hyperglycaemia enhances monocyte activation by increasing H3K9 acetylation (H3K9ac) and H3K4me2, and decreasing H3K9me2, allowing the binding of NF- κ B to inflammatory genes [1, 2, 5].

(-)-Epicatechin (EC) is one of the most abundant flavonoids in human diet, being present in high concentrations in grapes, cocoa, tea, and many other fruits and vegetables [6]. EC exerts anti-diabetic, anti-oxidant and anti-inflammatory effects [6]. Consequently, EC decreased p65-NF- κ B induction evoked by lipopolysaccharide stimulation in monocytes [6]. However, the precise mechanism for the anti-inflammatory effects of EC is not fully understood.

In view of all these evidences it could be hypothesised that EC could exert an anti-inflammatory effect in diabetes through epigenetic changes. Thus, the protective effect of EC on inflammatory markers, namely NF- κ B and TNF- α , acetylation-close-related events and post-translational modifications of key histone and in high-glucose challenged human monocytes were studied.

Materials and methods

Materials and chemicals

(-)-EC (>95% of purity) and D-glucose were purchased from Sigma (St Louis, MO, USA). Anti-TATA was obtained from Abcam (Cambridge, MA, USA). All other antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). HAT activity colorimetric assay kit and milliplex MAP Kit were obtained from Biovision (Mountain View, CA, USA). NE-PER nuclear and cytoplasmic extraction reagents and BCA kit were purchased from Pierce (Rockford, IL, USA). Histone purification kit was obtained from Active Motif (Carlsbad, CA, USA).

Cell culture and treatment

Human THP-1 cells were grown in RPMI medium supplemented with 10% foetal bovine serum and 1% antibiotics. Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂. Cells were maintained under normoglycemic conditions (NG, 5.5 mM glucose) or pre-incubated with 5 µM EC and 4 h later, exposed to 25 mM glucose (high glucose, HG) for a total of 24 h, and then harvested. Mannitol was used as hyperosmolar control.

TNF- α release measurement

TNF- α levels were measured with a Milliplex MAP Assay kit according to the manufacturer's instructions. Values were calculated based on a standard curve constructed for the assay [1].

Preparation of nuclear lysates

Nuclear lysates were prepared using a commercial kit. Lysates were collected, assayed for protein concentration by BCA protein assay kit according to the manufacturer's specifications, aliquoted and stored at -80° C [1].

Measurement of HAT activity

Nuclear lysate protein (50 µg) was taken for determination of HATs activity according to the manufacturer's instructions. Absorbance was measured at 440 nm [1].

Histone purification

Histone purification was performed with a commercial kit following the manufacturer's instructions. The pellet was air-dried, histones resuspended in sterile water and stored at -80°C.

Western blot analysis

Equal amounts of nuclear lysates or purified histones were separated by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membranes and probed with the corresponding primary

antibody followed by incubation with peroxide-conjugated anti-rabbit or anti-mouse immunoglobulin. Blots were developed with the ECL system (GE Healthcare). Normalization of Western blot was ensured by TATA binding protein and bands were quantified using a scanner and accompanying software.

Statistics

Prior to statistical analysis, data were tested for homogeneity of variances by the test of Levene; for multiple comparisons, one-way ANOVA was followed by the Bonferroni test when variances were homogeneous or by the Tamhane test when variances were not homogeneous. $P < 0.05$ was considered significant. A SPSS version 22.0 program has been used. Data were obtained by performing 3 independent experiments in duplicate.

Results

HG significantly enhanced p-p65-NF- κ B levels in nuclear lysates and this increase was abrogated by pre-treatment with EC (Fig. 1A). There was no alteration in total p65-NF- κ B values resulting in significant attenuation of p-p65/p65 ratio with EC treatment (Fig 1B). In agreement, HG incubation increased TNF- α release in THP-1 cells, whereas EC pre-treatment partly prevented the enhancement in the cytokine secretion (Fig. 1C). These results suggested that EC alleviates the inflammatory response induced by HG in monocytes.

Fig. 2A and 2B show that HG incubation increased acetyl CBP/p300 levels, but this effect was totally repressed by EC pre-incubation. Additionally, EC pre-treatment prevented the enhanced HDAC4 levels evoked by HG (Fig. 2C). HDAC1, HDAC2 and HDAC3 levels were not modified by any treatment (data not shown). Moreover, HAT activity was increased in HG-challenged cells, but EC pre-treatment restored HAT activity to control levels (Fig. 2D).

The analysis of the post-transcriptional histone modifications showed that HG treatment enhanced H3K9ac and H3K4me2 levels and decreased H3K9me2 levels, which could suggest a plausible explanation for stimulation of TNF- α gene expression (Fig. 3). However, EC pre-treatment prevented all these alterations, and restored all values to control levels, indicating a protective effect against the epigenetic changes induced by HG.

Discussion

Chronic inflammation is implicated in the majority of diabetic vascular complications [4]. Several studies indicate that high glucose-mediated activation of NF- κ B and cytokine expression in monocytes is related to epigenetic changes [1, 2, 4]. In this regard, lifestyle and diet can affect epigenetic alterations and might exert protective effects against the maintenance of the metabolic memory [7]. Here we show that EC alleviates HG-induced inflammatory response by preventing the increase of p-p65-NF- κ B levels and TNF- α release in THP-1 cells. In concert, other authors have reported that EC avoided p65-NF- κ B activation in human monocytes under pro-inflammatory conditions [6]. Moreover, EC pre-treatment prevented HG-induced H3K9ac and H3K4me2 increases, and H3K9me2 decrease in THP-1 cells. These features could be related to a beneficial effect evoked by EC against HG-induced inflammation (enhanced NF- κ B and TNF- α levels). In agreement, other polyphenols such as curcumin, epigallocatechin-3-gallate and resveratrol exerted anti-inflammatory effects under HG conditions, and these outcomes were related to chromatin remodelling, histone acetylation inhibition and modulation of histone methylation in monocytes and hepatic cells [1, 7, 8]. Additionally, cocoa, which is a rich source of EC, decreased global DNA methylation of peripheral leukocytes in humans with cardiovascular risk factors [9].

HATs and HDACs are involved in chromatin remodelling and play an important role regulating the pro-inflammatory response [1]. NF- κ B coactivator, CBP/p300, possesses intrinsic HAT activity leading to histone acetylation and facilitating pro-inflammatory gene expression [1]. Thus, H3K9 deacetylation at chromatin contributes to NF- κ B signalling termination [10]. Here it is shown that EC pre-treatment restored CBP/p300 levels and HAT activity to control values, which could contribute to decrease p-p65 levels and TNF- α release. Similarly, curcumin was shown to prevent HAT (p300) activation in THP-1 cells under HG challenge as prevented p300 recruitment in TNF- α and IL-6 promoters [1]. Therefore, it could be inferred that the chromatin remodelling induced by EC prevented p65-NF- κ B binding to TNF- α promoter and expression during HG, but further studies are needed.

HDACs play very distinct functions [11]. Upregulated HDAC4 has been associated with inflammatory and podocyte loss in response to common detrimental factors in diabetes [11]. Although further studies are needed, we show that HDAC4 inhibition induced by EC pre-treatment could be related with a protective effect in HG-challenged THP-1 cells.

It could be highlighted that EC dose employed in this work is realistic as in human serum 6 μ M EC has been detected after 26 g of cocoa intake [12] and this concentration is within the range recommended for *in vitro* studies [13].

In summary, EC induced epigenetic changes on post-translational histone modifications, modulated HAT (p300) and HDAC4 levels as well as decreased NF- κ B expression levels and TNF- α release in THP-1 cells under HG conditions. Altogether, these data indicate that EC may be helpful in reducing vascular inflammation and diabetic complications. Future studies will involve a complete characterization of epigenetic changes that EC causes *in vivo* in diabetics.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Figure legends

Figure 1. EC prevents p-p65-NF- κ B increase and alleviates TNF- α release induced by high-glucose in THP1 cells. THP-1 cells were cultured in presence of NG (5.5 mM) or HG (25mM) conditions in absence or presence of EC (5 μ M) for 24 h. A) Bands of representative experiments. B) Percentage data of p-p65/p65-NF- κ B ratio relative to controls (NG). C) TNF- α was measured in the media with a Milliplex MAP Assay kit. Values are expressed as a percentage relative to the control condition (NG) and are means $\pm$ SD, n=5-6. ^{a,b,c} Means with unlike letters were significantly different ($P<0.05$).

Figure 2. EC inhibits high-glucose induced upregulation of Acetyl CBP/p300 and HDAC4 levels and HAT activity in THP-1 cells. THP-1 cells were cultured in presence of NG (5.5 mM) or HG (25 mM) conditions in absence or presence of EC (5 μ M) for 24 h. A) Bands of representative experiments. Densitometric quantification of B) acetyl CBP/p300 ratio and C) HDAC4. Values are expressed as a percentage relative to control condition (NG) and are means $\pm$ SD, n=5-6. Equal loading of Western blots was ensured by TATA protein. D) HAT activity measured in nuclear lysate (means n=5-6 $\pm$ SD) and expressed as O.D. measured at 440 nm. ^{a,b} Means with unlike letters were significantly different ($P<0.05$).

Figure 3. EC restores histone acetylation and methylation to control values under HG challenge. THP-1 cells were cultured in presence of NG (5.5 mM) or HG (25 mM) conditions in absence or presence of EC (5 μ M) for 24 h. H3K9ac, H3K4me2 and H3K9me2 were analysed after histone purification A) Bands of representative experiments. Percentage data of B) H3K9ac/H3, C) H3K4me2/H3 and D) H3K9me2/H3 ratios relative to controls (NG). Values are means $\pm$ SD, n=5-6. ^{a,b} Means with unlike letters were significantly different ($P<0.05$).

Figure 1

Figure 2

Figure 3