Archives of the
International Society of Antioxidants in Nutrition and Health (ISANH)

Vol. 5, Issue 2, 2017
DOI 10.18143/AISANH_v5i2_4
Extended abstract of Vienna Polyphenols 2017

Green Tea Polyphenols Affect Invasiveness of Human Gastric MKN-28 Cells by Inhibition of LPS or TNF-alpha Induced Matrix Metalloproteinases-9/2

ARCONE Rosaria [(1)], PAGLIARA Valentina [(1)], D'ANGELO Stefania [(1)], NASSO Rosarita [(1)], DI DONATO Paola [(2)], MASULLO Mariorosario [(1)]

[1] *Dipartimento di Scienze Motorie e del Benessere, Università di Napoli "Parthenope", Napoli, Italy.*
[2] *Dipartimento di Scienze e Tecnologie, Università di Napoli "Parthenope", Napoli, Italy.*

Corresponding author:

Rosaria Arcone
Dipartimento di Scienze Motorie e del Benessere,
Università di Napoli "Parthenope"
Via Medina 40, 80133 Naples, Italy
rosaria.arcone@uniparthenope.it

Abstract

Green tea polyphenols have been identified as molecules responsible for the beneficial effects showed by the green tea against oxidative stress and cancer risk. We investigated the effects of green tea polyphenol extracts (GTPs) on oxidative stress and cell invasiveness in human gastric MKN-28 cancer cells. The pre-treatment with 10^{-4} M catechin equivalents of GTPs exerts a protective effect on xanthine-xanthine oxidase induced cell cytotoxicity, thus confirming the anti-oxidant properties of GTPs. The effect of GTPs was also extended on the cell migration and invasive ability induced by TNF-alpha or LPS. Results demonstrated that GTPs exposure (10^{-6} M) prevents the increase in cell invasiveness induced by the inflammatory agents. In addition, the treatment with GTPs prevented the TNF-alpha or LPS induced Matrix Metalloproteinases (MMP)-9/2 up-regulation. Our results demonstrated that GTPs reduced the oxidative stress and the invasive potential of gastric MKN-28 cancer cells thus confirming the protective role of these polyphenols against the metastatic process in gastric cancer.

Introduction

Several studies demonstrated a correlation between green tea consumption and a reduced cancer risk (Fang *et al.*, 2015). Green tea polyphenols (GTPs) have been identified as molecules responsible for the beneficial effects showed by the green tea against oxidative stress and cell invasiveness (Maeda-Yamamoto et al. 1999). In the metastatic process, cancer cells invade the surrounding microenvironment through the secretion of various enzymes, and among them, matrix metalloproteinases (MMPs) play a crucial role for their ability to degrade extracellular matrix components. Furthermore, in the early stage of the metastatic process a key step is played by MMP-9/2 through their

Received: July 27, 2017; Accepted: December 7, 2017 -

up-regulation by inflammatory factors, mainly TNF-α and/or LPS (Arcone et al. 2016). As inflammation is closely linked to tumor progression, polyphenols containing dietary substances, exert chemopreventive effects on carcinogenesis through inhibition of tumor cell invasiveness. In this work, we have determined the effect of GTPs on: *i*) oxidative induced cell injury; *ii*) cell migration and invasiveness in absence or presence of pro-inflammatory factors (TNF-α or LPS); *iii*) MMP-9/2 expression levels in human gastric MKN-28 cancer cells.

To examine whether GTPs could affect TNF-α or LPS induced MMP-9/2 secretion, Western blotting analysis was performed on cell conditioned media from untreated or GTPs pre-treated MKN-28 cells.

Materials and Methods

Chemicals and reagents

All chemical reagents were of analytical grade (Carlo Erba, Milan, Italy). Cell culture reagents were from Lonza (Basel, Switzerland). Matrigel and cell culture inserts were purchased from BD Biosciences (Bedford, MA). Tumor necrosis factor-α (TNF-α) and Lipopolysaccharide (LPS) were obtained from Sigma-Aldrich (Milan, Italy). MMP-9 and MMP-2 rabbit polyclonal antibodies were purchased from Epitomics (Burlingame, U.S.A). Other antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Western Chemiluminescent HRP substrate kit and Amicon Ultra 10K filtration units were from Millipore (Burlington, MA).

Preparation of green tea polyphenols extract

Green tea extract was prepared starting from two grams of commercially available green tea as previously reported (Arcone et al. 2016). The concentration of green tea polyphenols (GTPs) was reported as catechin (CAT) equivalents.

Cell culture treatments

The effect of GTPs on cell invasiveness was evaluated in human MKN-28 gastric adenocarcinoma cells (ATCC, CSC-C9494L) treated with 10 ng/ml TNF-α or LPS, as reported (Arcone et al. 2016). In particular, cell migration activity was examined by the three-dimensional Boyden chamber assay, and cell invasion assay was performed using Matrigel Invasion Boyden Chambers (Pagliara et al. 2014).

Gelatin zymography

Gelatin zymography was carried out as described previously (Pagliara et al. 2014). In brief, concentrated cell conditioned media, were analyzed under non-reducing condition onto 9% polyacrylamide gel co-polymerized with 1 mg/ml gelatin (Sigma-Aldrich). After staining, gelatinolytic activity was revealed as clear bands against blue background.

Results and Discussion

Previous studies have reported that 10^{-6} M GTPs exerted a protective effect against oxidative stress induced cell cytotoxicity (Arcone et al. 2016).

We have then tested if GTPs could affect cell migration and invasiveness in MKN-28 cells, treated with LPS or TNF-α as pro-inflammatory agents.

As reported in *Table 1*, the exposure to TNF-α or LPS slightly affect MKN-28 cell migration. The GTPs pre-treatment (10^{-6} M) did not significantly modify this behavior.

Treatments	***Migration** (%)*	***Invasion** (%)*
Vehicle	100.0 ± 5.3	100.0 ± 2.3
GTPs	77.2 ± 1.7	109.2 ± 1.7
TNF-α	103.7 ± 0.5	198.1 ± 0.7
GTPs + TNF-α	84.5 ± 2.3	124.0 ± 1.0
LPS	102.6 ± 2.0	176.9 ± 1.8
GTPs + LPS	85.4 ± 1.2	124.2 ± 0.8

Table 1. Pretreatment with GTPs prevents the increase of cell invasiveness induced by TNF-α or LPS in MKN-28 cells. *Cells were pre-treated with 10^{-6} M GTPs or vehicle alone for 6 hrs and then incubated for additional 18 hrs with the specific pro-inflammatory agent. The percentage was referred to that of the vehicle alone.*

On the contrary, in MKN-28 cells exposed to TNF-α or LPS, a 2-fold increase in cell invasiveness was observed. In this case, the pre-treatment with GTPs (10^{-6} M) prevents the increase of cell invasiveness induced by both inflammatory stimulus.

The treatment with TNF-α was able to induce an increase in MMP-9/2 expression *(Table 2).* We found that the pre-treatment with GTPs was able to reduce

Received: July 27, 2017; Accepted: December 7, 2017 -

the amount of both MMP-9 and MMP-2 in the culture medium.

Treatments	*Protein fold increase*	
	MMP-9	*MMP-2*
Vehicle	1.0 ± 0.1	1.0 ± 0.7
TNF-α	6.5 ± 0.9	7.2 ± 0.3
GTPs (10^{-8} M) +TNF-α	4.6 ± 0.1	5.9 ± 0.5
GTPs (10^{-6} M) +TNF-α	4.1 ± 0.2	4.6 ± 0.2
GTPs (10^{-4} M) +TNF-α	3.3 ± 0.2	4.2 ± 0.4
GTPs (10^{-4} M)	0.5 ± 0.1	1.8 ± 0.01

Table 2. Pretreatment with GTPs reduces the up-regulation of MMP-2/9 protein levels induced by TNF-α in MKN-28 cells. *The data were reported as MMP-9/2 fold change vs control cells cultured in absence of GTPs and TNF-α (set as 1).*

This effect was concentration dependent and the highest concentration of GTPs (10^{-4} M) induced about one half reduction in MMP-9/2. A similar behaviour was observed when the effect on the GTPs pre-treatment was evaluated on LPS induced MMP-9/2 protein expression level *(Table 3).*

Treatments	*Protein fold increase*	
	MMP-9	*MMP-2*
Vehicle	1.0 ± 0.7	1.0 ± 0.1
LPS	7.8 ± 0.2	7.2 ± 0.4
GTPs (10^{-8} M) + LPS	7.0 ± 0.1	7.2 ± 0.4
GTPs (10^{-6} M) + LPS	5.3 ± 0.2	6.6 ± 0.3
GTPs (10^{-4} M) + LPS	4.9 ± 0.1	3.7 ± 0.3
GTPs (10^{-4} M)	2.0 ± 0.2	2.2 ± 0.2

Table 3. Pretreatment with GTPs reduces the up-regulation of MMP-2/9 protein levels induced by LPS in MKN-28 cells. *The data were reported as MMP-9/2 fold change vs control cells cultured in absence of GTPs and TNF-α (set as 1).*

We also evaluated whether GTPs affect gelatinolytic activity performing gelatin zymography on conditioned media from TNF-α or LPS treated MKN-28 cells. As showed in *Table 4*, TNF-α treatment induced an increase in MMP-9 and MMP-2 gelatinolytic activity. The pretreatment with 10^{-4} M GTPs caused a reduction of both MMP-9/2 gelatinolytic activity. A similar effect was observed in cells treated with LPS.

Treatments	*Gelatinolytic activity*	
	MMP-9	*MMP-2*
Vehicle	1.0 ± 0.8	1.0 ± 0.3
GTPs (10^{-4} M)	1.0 ± 0.4	1.2 ± 0.5
TNF-α	9.8 ± 0.1	3.7 ± 0.1
GTPs (10^{-4} M) + TNF-α	5.0 ± 0.7	2.3 ± 0.2
LPS	9.8 ± 0.2	6.1 ± 0.1
GTPs (10^{-4} M) + LPS	7.3 ± 0.1	3.0 ± 0.1

Table 4. Pretreatment with GTPs reduces the up-regulation of gelatinolytic activity levels induced by TNF-α or LPS in MKN-28 cells. *The data were reported as MMP-9/2 fold change vs control cells cultured in absence of GTPs, TNF-α and LPS (set as 1).*

The pre-treatment with GTPs alone and in the absence of LPS or TNF-α, did not interfere with MMP-9/2 gelatinolytic activity, as compared to control cells.

All these results demonstrated that green tea polyphenol extracts might prevents the increase in cell invasiveness and metalloproteinases activity during an inflammatory process in gastric cancer cells.

Acknowledgements

This research was supported in part by the University of Naples "Parthenope", "Bando per la ricerca individuale, annualità 201 to MM, AR and DS.

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Received: July 27, 2017; Accepted: December 7, 2017 -