ANTIGENOTOXIC EFFECT OF EPIGALLOCATECHIN-3-GALLATE (EGCG) ON BLEOMYCIN *IN VITRO* INDUCED DNA DAMAGE IN HUMAN LYMPHOCYTES

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Background

Epigallocatechin-3-gallate (CAS 989-51-5) is the main polyphenol present in green tea (Camellia sinensis) which accounts for about 60-70% of the total catechins. The major green tea polyphenols are: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-gallate (ECG) and epicatechin (EC). EGCG is a strong polyphenol catechin antioxidant found in green tea, reported to have broad efficacy against many conditions generated from oxidative damage. Extensive oxidation of low density lipoproteins (LDLs) is correlated to cardiovascular diseases and EGCG is reported to strongly inhibit Cu(2+)-mediated oxidative modification of LDLs. The antiapoptotic proteins Bcl-2 and Bcl-x(L) are observed to suppress apoptosis and (EGCG), which conveys survival to heavily damaged and mutated cells *in vitro*. EGCG (along with the other catechins) is shown to directly inhibit these proteins, reestablishing the normal apoptotic pathway in the cell.

Most experimental studies demonstrating antimutagenic or anticarcinogenic effects have been conducted with water extract of green tea or polyphenolic fraction isolated from green tea. It has been reported that tea extracts have antibacterial and anti-inflammatory (Toda et al., 1989, 1991; El-Mowafy et al., 2010; Abboud et al., 2008), antiviral (Nakayama et al., 1990, Green, 1949), antioxidative (Matsuzaki and Hara 1985), antitumor (Katiyar et al., 1992, 1996, 1993) antimutagenic (Kuroda, 1996, Weisburger et al., 1996; Yen and Chen, 1996; Constable et al., 1996; Kennedy et al., 1998) and anticarcinogenic effects (Lin and Liang, 2000; Chung 1999; Bertolini et al., 2000; Qiao et al., 2011; Stearns et al., 2010; Liang et al., 2010; Farabegoli et al., 2010; Rady et al., 2018). The anticarcinogenic property has been highly attributed to the polyphenolic compounds in the tea. Additionally it was well establish its radioprotective effects (Mun et al., 2018).

Objectives

Considering the reports on the anticarcinogenic effects of green tea polyphenols we recognized the needs to extend the study of the comparative antigenotoxic effects as measured by cytokinesis-block micronucleus assays in human lymphocytes against known classical mutagens and genotoxicants. The aim of this study is to evaluate the *in vitro* protective effects of EGCG in the presence of DNA damage agent bleomycin.

Material and Methods

The cytokinesis-block micronucleus cytome assay was used as an endpoint. This assay is one of the most commonly used tests for measuring DNA damage (Bonassi et al., 2007; El-Zein et al., 2008; ICH 2016; OECD #487, 2016). Biomarkers evaluated include: binucleated cells with micronuclei (BNMN) and micronuclei per cell (MN). Peripheral human lymphocytes were treated with different concentrations of bleomycin as follows: 2, 4, and 8 µg/mL. A comparison of biomarkers has been done with bleomycin plus 1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, as well as 40 µg/mL of EGCG (see Table 1). Briefly, the lymphocyte cultures from three donors were set up in 4.5 mL of RPMI 1640 supplemented with 15% of fetal calf serum, phytohemagglutinin (PHA) and 0.5 mL whole blood from a three healthy donors was used. Cytochalasin B (6µg/ml) was added according to Fenech (2007).The identification of BNMN was according to the criteria described by Fenech (2007). For this assay 1000 binucleated (BN) cells with well-preserved cytoplasm were examined on coded slides. The investigation was approved from the IRB of the University of Findlay.

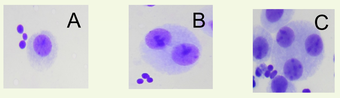
Results

Our bleomycin dose-effect investigation revealed log dependency for BNMN and MN yields (Fig. 1-A and 1-B). We tested EGCG ability to reduce bleomycin's initial genotoxic effect within the same dose range (Table 1). Our data clearly suggests that, the EGCG presented in *in vitro* culture decreased the micronuclei and BNMN cell yields, showing a clear protective effect from bleomycin-induced DNA damage. The scale of in vitro protective effect of EGCG in µg/mL measured by binucleated cells with micronuclei is as follow: 5 > 10 > 20 ≥ 40. The lowest dose of 1 µg/mL was not protective. Additionally our results demonstrated that the higher EGCG dose (40 µg/mL) combined with bleomycin induced lower than expected levels of MN and BNMN cells and this is perhaps a consequence of an overt toxic effect of both drugs on lymphocytes (Fig. 1 A, B). The toxic effect is also seen from the MN and BNMN lymphocyte cell yields resulted after EGCG treatment alone.

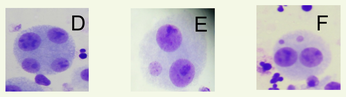
Table 1. *In vitro* protective effect of EGCG on human lymphocytes exposed to bleomycin

DRUG µg / ml	NDI Mean ± SD	BNMN Mean ± SD	MN Mean ± SD
Control	**2.1 ± 0.06**	**5.3 ± 0.5**	**6.7 ± 1.2**
EGCG			
1.0 EGCG	2.1 ± 0.08	5.1 ± 0.3	5.4 ± 0.2
5.0 EGCG	2.3 ± 0.17	4.3 ± 1.2	5.0 ± 0.8
10.0 EGCG	2.2 ± 0.12	6.0 ± 0.8	6.0 ± 0.8
20.0 EGCG	1.96 ± 0.05	7.8 ± 3.3	8.3 ± 3.7
40.0 EGCG	1.9 ± 0.08	8.3 ± 1.2*	9.0 ± 2.2*
2.0 Bleo	**1.76 ± 0.05**	**26.7± 2.5**	**29.3 ± 3.4**
2.0 Bleo + 1.0 EGCG	1.83 ± 0.05	30.0 ± 0.21	34.8 ± 5.3
2.0 Bleo + 5.0 EGCG	2.0 ± 0.08	8.0 ± 0.8***	9.7 ± 1.2***
2.0 Bleo + 10 EGCG	1.93 ± 0.05	11.0 ± 0.82***	12.0 ± 0.81***
2.0 Bleo + 20 EGCG	1.86 ± 0.04	12.3± 2.0***	13.7 ± 1.7***
2.0 Bleo + 40.0 EGCG	1.8 ± 0.06	17.0 ± 2.9**	19.7 ± 5.4**
4.0 Bleo	**1.6 ± 0.08**	**37.7± 3.3**	**43.0 ± 2.2**
4.0 Bleo + 1.0 EGCG	1.63 ± 0.12	38.6 ± 3.8	43.6 ± 3.1
4.0 Bleo + 5.0 EGCG	1.93 ± 0.05	13.0 ± 1.4***	13.7 ± 1.7***
4.0 Bleo + 10.0 EGCG	1.86 ± 0.05	15.66 ± 1.2***	19.67 ± 1.31***
4.0 Bleo + 20.0 EGCG	1.73 ± 0.03	22.3 ± 2.0***	25.7 ± 2.5***
4.0 Bleo + 40.0 EGCG	1.66 ± 0.04	26.3 ± 2.6***	32.7 ± 3.3***
8.0 Bleo	**1.36 ± 0.09**	**52.7± 1.7**	**74.7± 4.2**
8.0 Bleo + 1.0 EGCG	1.4 ± 0.08	51.3 ± 1.9	68.3 ± 3.9
8.0 Bleo + 5.0 EGCG	1.83 ± 0.05	13.0 ± 1.4***	15.7 ± 1.9***
8.0 Bleo + 10.0 EGCG	1.8 ± 0.06	18.7 ± 1.7***	23.3 ± 2.3***
8.0 Bleo + 20.0 EGCG	1.5 ± 0.08	30.3 ± 2.0***	40.0 ± 4.9***
8.0 Bleo + 40,0 EGCG	1.43 ± 0.09	23.7 ± 2.6***	28.7 ± 1.24***

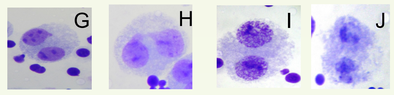
Statistical differences from negative control: Student's t-test *p<0.05; **p<0.01, ***p<0.0001
1.Control and different concentrations of EGCG
2. Bleomycin alone and different combinations of Bleo + EGCG



A. Mononucleated cell;
B. Binucleated cell;
C. Trinucleated



D. Quadrinucleated cell;
E. Binucleated cell with micronuclei;
F. Binucleated cell with two micronuclei



G. Binucleated cell with nuclear bud
H. Binucleated cell with plasmic bridge;
I. Necrotic cell ; J. Apoptotic cell;

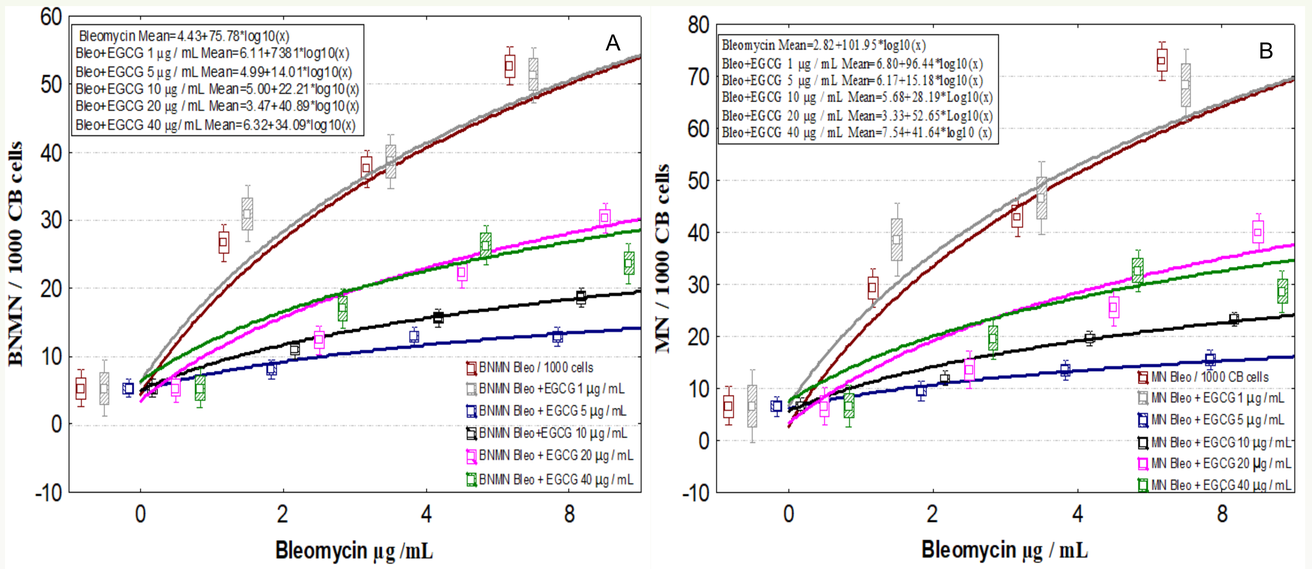


Fig. 1 A-B. *In vitro* protective effect of EGCG on human lymphocytes exposed to bleomycin. A-relationship between combined exposure of bleomycin + EGCG and binucleated cells. B-relationship between combined exposure of bleomycin + EGCG and micronuclei.

Conclusion

1. The log dose-dependency relationship between MN - BNMN cells and bleomycin concentration was established for the whole investigated dose-range.
2. EGCG can reduce bleomycin's *in vitro* genotoxic effect on human lymphocytes. A clearly expressed protective effect of EGCG concentrations were observed after combined treatment of bleomycin + EGCG. The lowest dose of 1 µg/mL EGCG was not protective, the next of 5 µg/mL the most protective.
3. The higher dose of 40 µg/mL EGCG together with bleomycin revealed not only protective but also toxic effect on lymphocytes.

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