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The effect of antibiotics on cytokine production by mononuclear cells and the cross-talk with colon cancer cells

[Efecto de antibióticos sobre la producción de citocinas por células mononucleares y la interacción con células de cáncer de colon]

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Abstract

Context: Antibiotics belong to the powerful weapons applied against microbial infections. It is notable that in addition to their antimicrobial effect they express immunomodulatory and anti-cancer activities.

Aims: To explore the effect of four antibiotics on the immune cross-talk between peripheral blood mononuclear cells (PBMC) and colon carcinoma cells from two human lines.

Methods: Cefotaxime, meropenem, ampicillin and vancomycin were separately added to PBMC co-incubated with cells from two human colon carcinoma cell lines, i.e. HT-29 and RKO. After 24 hours, the level of the following cytokines produced by PBMC was evaluated: IL-6, IL-1ra, IL-1β, TNFα, IFNγ and IL-10.

Results: All four antibiotics did not affect the generation of IL-6 and IL-1ra in both co-cultures. On the other hand, all of them restrained the production of IL-1β by PBMC incubated with HT-29 cells. In the same incubation mixture cefotaxime, vancomycin and meropenem decreased IFNγ and IL-10 production, while ampicillin and vancomycin inhibited TNFα. As for PBMC incubated with RKO carcinoma cells, cefotaxime inhibited the production of IL-1β, IFNγ and mildly of IL-10, whereas vancomycin repressed that of IL-1β, TNFα and IFNγ. Notably, vancomycin increased the production of IL-1β and decreased that of TNFα and IFNγ. The results indicate that the four antibiotics examined exert a modulatory effect on the immune cross-talk between PBMC and human colon cancer cells from two lines expressed by a different impact on pro-and anti-inflammatory cytokines generation.

Conclusions: These findings support the conception that antibiotics may express not only an anti-microbial effect, but also possess an anti-cancer activity that may be considered for integration to the therapeutic arsenal against cancer.

Keywords: Ampicillin; cefotaxime; colon carcinoma cells; cytokines; meropenem; mononuclears; vancomycin.

Resumen

Contexto: Los antibióticos son armas poderosas aplicadas contra las infecciones microbianas. Además de su efecto antimicrobiano expresan actividades inmunomoduladoras y contra el cáncer.

Objetivos: Explorar el efecto de cuatro antibióticos sobre la interacción inmune entre las células mononucleares de sangre periférica (PBMC) y células de dos líneas humanas de carcinoma de colon.

Métodos: Cefotaxima, meropenem, ampicilina y vancomicina se añadieron por separado a PBMC co-incubadas con células de dos líneas de carcinoma de colon humano (HT-29 y RKO). Después de 24 horas, se evaluaron las concentraciones de las siguientes citocinas producidas por PBMC: IL-6, IL-1ra, IL-1β, TNFα, IFNγ e IL-10.

Resultados: Los cuatro antibióticos no afectaron la generación de IL-6 e IL-1ra en ambos co-cultivos. Por otra parte, todos contuvieron la producción de IL-1β por PBMC incubadas con células HT-29. En la misma mezcla de incubación cefotaxima, vancomicina y meropenem disminuyeron la producción de IFNγ e IL-10, mientras que la ampicilina y vancomicina inhibieron TNFα. Cuando PBMC se incubaron con células RKO, cefotaxima inhibió la producción de IL-1β, IFNγ y ligeramente de IL-10, mientras que la vancomicina reprimió las de IL-1β, TNFα e IFNγ. En particular, la vancomicina aumentó la producción de IL-1β y disminuyó las de TNFα e IFNγ. Los resultados indican que los cuatro antibióticos examinados ejercieron un efecto modulador sobre la interacción inmune entre PBMC y las células de cáncer de colon humano a partir de dos líneas, expresadas por un impacto diferente sobre la generación de citocinas pro- y anti-inflamatorias.

Conclusiones: Estos hallazgos apoyan la idea de que los antibióticos pueden presentar no sólo un efecto anti-microbiano, también poseen una actividad anti-cancerígena que pudiera ser considerada para la integración de éstos al arsenal terapéutico contra el cáncer.

Palabras Clave: Ampicilina; cefotaxima; células de carcinoma de colon; citocinas; meropenem; mononucleares; vancomicina.

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INTRODUCTION

Since centuries the way to prevent and cure infections has been one of the major health tasks holding human mind attention. The main concern of the health providers, from ancient healers till nowadays modern medicine, has been the way to overcome inflammation and the subsequent damage caused by invisible invaders. The antibacterial arsenal underwent a number of evolutionary stages starting with natural products up to the revolutionary introduction of antibiotics, all of them with the same goal – to reinforce the intricate self-defense of the organism. The saving of a great number of human lives achieved by sulfonamides launched as antimicrobial agents cannot be forgotten. Unfortunately, due to bacterial capacity to evade the action of the antimicrobial agents by developing antibiotic-resistant species, the "battle" between intruders and continuous modification of antibiotics' structure and pharmacodynamics continues till present. As a result of this incessant competition the number of antibiotics with broad spectrum activity adept to divert or abort bacterial damage is on a permanent rise. It is of interest that antibiotics, in addition to their antimicrobial effect may promote cellular host defense by stimulating the immune system and particularly cytokine production. Lahat et al. (2007) have found that administration of ciprofloxacin, a second generation fluoriquinolone, to rats with colitis induced by trinitrobenzene-sulfonic acid (TNBS) caused a decrease of IL1-β, IL-8 and TNFα levels in colon homogenates. Using a similar animal model Garrido-Mesa et al. (2015) have shown that doxycycline exerted a decreased production of IL-8 by intestinal epithelial cells. Bode et al. (2015) have reported that incubation of human monocytes with linezolid, vancomycin and daptomycin under sepsis-like conditions caused a different regulation of sepsis-associated cytokine expression. Linezolid increased the generation of the pro-inflammatory cytokines TNFα, IL-1β and IL-6, while the mRNA level of the anti-inflammatory cytokine IL-10 was decreased. Vancomycin stimulated mRNA expression of TNFα and IL-6 and daptomycin induced upregulation of IL-6 and downregulation of IL-10 mRNA level. Immunomodulatory properties have been observed with other antibiotics such as tobramycin, ceftriaxone and levofloxacin (Calbo et al., 2008; Guchhait et al., 2015).

In addition, the capacity of antibiotics to interact with cancer cells has drawn attention. Ren et al. (2014) have shown that rapamycin attenuated cell proliferation, cytokine production, as well as additional functions of head and neck squamous carcinoma cells stimulated by lipopolysaccharide (LPS). According to the authors this effect was achieved through inhibition of the oncogenic Toll-like receptor 4 (TLR4). It has been reported that antiproliferative activity and apoptosis of MCF-7 cells might be significantly increased by formamidinodoxorubicins, a group of doxorubicin derivatives (Marczak et al., 2015).

A thorough review of the fluoroquinolones as potential anticancer agents has been reported by Sharma et al. (2013).

The way antibiotics may modulate the immune reactions and modify carcinogenesis is intriguing. However, a certain limitation for investigation of this phenomenon *in vivo* is linked to a relatively large number of various constituents associated with both inflammatory responses and cancer development. It was therefore the aim of the present work to examine the effect of four broad spectrum antibiotics currently used in everyday practice on cytokine production by human peripheral blood mononuclear cells (PBMC) and their capacity to modulate the cross-talk between immune and cancer cells. We have chosen three antibiotics of the β-lactam sub-group i.e. cefotaxime, as a member of the third generation of cephalosporins, meropenem from sub-group of carbapenems and ampicillin from the aminopenicillin group. Vancomycin was chosen as a representative of the glycopeptides' antibiotics being active mostly against Gram-positive bacteria.

MATERIAL AND METHODS

Cell preparation

Peripheral blood mononuclear cells (PBMC) were obtained from venous blood withdrawn from adult blood bank donors after signing an informed consent. The cells were separated by Lymphoprep-1077 (Axis-Shield PoC AS, Oslo, Norway) gradient

centrifugation. The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin, 10% heat inactivated fetal calf serum (FCS), and was designated as complete medium (CM). All culture conditions were endotoxin free.

Colon cancer cell lines

HT-29 and RKO human colon cancer cell lines were obtained from American Type Cultural Collection, Rockville, MD. The cells were maintained in CM containing Mc-COY'S 5A medium and modified Eagle medium (MEM- Biological Industries Co, Beth-Haemek, Israel) respectively, supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and antibiotics (penicillin, streptomycin and nystatin - Biological Industries Co, Beth-Haemek, Israel). The cells were grown in T-75 culture flasks at 37°C in a humidified atmosphere containing 5% CO2. Cell viability was examined by trypan blue dye exclusion by the use of the XTT Cell Viability Assay Kit Protocol (Thermo Fisher Scientific Inc. Walthman, MA, USA) to quantify cell numbers based on metabolic activity in microplates (Thermo Fisher Scientific, Walthman, Ma, USA) (Bessler et al., 2000).

Preparation of antibiotics

Cefotaxime (cefotaxime sodium, Patheon UK Limited-manufacturer, UK) and ampicillin (ampicillin as sodium salt, Sandos Ltd, Austria) were dissolved in distilled water at 200 mg/mL, aliquoted and stored at -20°C until used. At the day of experiment, frozen antibiotic samples were thawed and further diluted in CM. Meropenem (as trihydrate, AstraZeneca Ltd, UK) was freshly dissolved in distilled water at 50 mg/mL, and further diluted in CM. The final concentrations of the three drugs used were 20, 100 and 500 µg/mL. Vancomycin (as hydrochloride, TEVA, Israel) was dissolved in distilled water at 125 mg/mL aliquoted and stored at -20°C until used. At the day of experiment, a frozen antibiotic sample was thawed and further diluted in CM. The final concentrations of vancomycin were 20, 50 and 125 µg/mL.

Effect of antibiotics on cytokine production

PBMC (0.5 mL of 4 x 10^6 cells/mL of CM) were incubated with an equal volume of CM or one of the colon cancer cell types (4 x 10^5 cells/mL of CM) suspended in appropriate CM. Antibiotics were added at the onset of cultures at concentrations as described. Control cultures contained CM. The cultures were maintained for 24 h at 37°C in a humidified atmosphere containing 5% CO2. At the end of the incubation period the cells were removed by centrifugation at 1500 rpm for 10 min, the supernatants were collected and kept at -70°C until assayed for cytokines content.

Cytokine content in the supernatants

The concentration of the following cytokines: TNFα, IL-1β, IL-6, IFNγ, IL-10, and IL-1ra in the supernatants was tested using ELISA kits specific for these cytokines (Biosource International, Camarillo, CA, USA) as detailed in the guide-line provided by the manufacturer. The detection levels of these kits were: 15 pg/mL for IL-6 and 30 pg/mL for the remaining ones.

Statistical analysis

A linear mixed model with repeated measures and the assumption of compound symmetry (CS) was used to assess the effects of the drug type, drug concentration and cell line on cytokine levels. SAS ver. 9.4 was used for this analysis. Paired t-test was used to compare between the level of cytokine produced with various concentrations of each drug and that found in control cultures (incubated without antibiotics). Probability values of $p<0.05$ were considered as significant. The results are expressed as mean ± SEM.

RESULTS

Effect of antibiotics on colon cancer cell viability

Twenty four hours of incubation of HT-29 colon cancer cells with concentrations of cefotaxime, ampicillin or meronem between 20 and 500 µg/mL had no significant effect on cell viability as tested by XTT assay ($p=0.098$, $p=0.276$, and $p=0.086$, respec-

tively), but was significantly reduced when the cells were incubated with vancomycin at concentrations between 20 and 125 μg/mL (p=0.008). RKO cell viability was not significantly affected by any of the four drugs tested (p>0.05). (Table 1).

Effect of antibiotics on IL-6 and IL-1ra secretion

The production of IL-6 or IL-1ra by non-stimulated PBMC, or by cells stimulated by either HT-29 or RKO colon cancer cell was not affected significantly by 24 h incubation with any of the four antibiotics tested at concentrations as indicated (data not shown).

Table 1. Effect of antibiotics on IL-1β secretion.

IL-1β (ng/mL)				
Spontaneous				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 8)**	**Meropenem (n = 8)**	**Vancomycin (n = 8)**
0	0.88 ± 0.17	0.67 ± 0.22	0.32 ± 0.09	0.42 ± 0.12
20	0.65 ± 0.16***	0.87 ± 0.30	0.30 ± 0.09	0.33 ± 0.12
100/[a]50	0.56 ± 0.14***	0.82 ± 0.29	0.29 ± 0.10	0.29 ± 0.10**
500/[a]125	0.40 ± 0.12***	0.73 ± 0.24	0.27 ± 0.10	0.29 ± 0.09**
HT-29-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	5.07 ± 0.56	4.65 ± 0.57	4.77 ± 0.72	5.64 ± 0.68
20	4.22 ± 0.58***	3.98 ± 0.55***	3.53 ± 0.52***	4.44 ± 0.70***
100/[a]50	4.01 ± 0.57***	3.93 ± 0.56***	3.68 ± 0.63***	4.65 ± 0.75***
500/[a]125	2.88 ± 0.47***	3.62 ± 0.57***	3.72 ± 0.63***	4.71 ± 0.63***
RKO-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	3.62 ± 0.47	3.46 ± 0.44	2.90 ± 0.51	3.54 ± 0.56
20	3.78 ± 0.45	3.59 ± 0.41	3.21 ± 0.40	4.39 ± 0.70***
100/[a]50	3.36 ± 0.45	3.34 ± 0.40	3.09 ± 0.50	4.20 ± 0.75**
500/[a]125	1.88 ± 0.28***	3.10 ± 0.43**	2.51 ± 0.44*	3.75 ± 0.63

PBMC were incubated for 24 h without (spontaneous) or with one of human colon cancer cell lines HT-29 or RKO as described in Materials and Methods in the absence or presence of cefotaxime, ampicillin and meropenem at concentrations of 20 -500 μg/mL or vancomycin at 20-125 μg/mL. [a]Represents vancomycin concentration added. IL-1β was tested in the supernatants. Asterisks represent comparison between cells incubated without or with antibiotics;*p<0.05; **p<0.01; ***p <0.001.

Effect of antibiotics on pro-inflammatory cytokines production

IL-1β (Table 1)

Incubation of non-stimulated cells with elevated doses of cefotaxime or vancomycin caused a dose dependent inhibition of IL-1β secretion by PBMC (p<0.001, and p=0.03, respectively), whereas ampicillin, or meropenem had no significant effect (p=0.133, and p=0.254, respectively). A dose dependent inhibition of HT-29 induced IL-1β secretion by PBMC was found when cells were incubated with increased doses of cefotaxime (p<0.001), ampicillin (p<0.001), meropenem (p<0.001) or vancomycin (p=0.001). The secretion of IL-β induced by RKO cells was reduced significantly when PBMC were incubated with higher doses of cefotaxime (p<0.001), ampicillin (p=0.011), or meropenem (p=0.005), but was slightly stimulated following incubation with vancomycin (p=0.0012).

TNFα (Table 2)

The production of TNFα by non-stimulated PBMC was not affected significantly either by incubation with cefotaxime (p=0.095), ampicillin (p=0.554), or vancomycin (p=0.126), but it was significantly affected by meropenem (p=0.011). HT-29 and RKO-induced TNFα secretion by PBMC was not affected significantly by cefotaxime (p=0.158, and p=0.58, respectively), meropenem (p=0.249, and p=0.772, respectively) or vancomycin (p=0.259, and p=0.667, respectively). However, ampicillin caused a dose dependent inhibition of both HT-29 or RKO induced TNFα production (p<0.0001, and p=0.0025, respectively.

IFNγ (Table 3)

Cefotaxime or ampicillin added to non-stimulated PBMC at concentrations as indicated had no significant effect on IFNγ production (p=0.985, and p=0.89, respectively), whereas meropenem and vancomycin reduced significantly the secretion of IFNγ by non-stimulated PBMC (p<0.0001, and p=0.001, respectively). Cefotaxime and meropenem caused a concentration dependent inhibition of IFNγ secretion by PBMC induced by HT-29 cells (p=0.0032, and p<0.001, respectively) or by RKO cells (p<0.001, and p=0.01, respectively). The secretion of IFNγ generated by HT-29 was not affected significantly by ampicillin (p=0.13), whereas RKO-induced IFNγ synthesis was concentration dependently inhibited (p<0.001). Vancomycin significantly reduced secretion of IFNγ prompted by HT-29 cells (p<0.001). Vancomycin had no significant effect on RKO-induced IFNγ production (p=0.22).

Effect of antibiotics on anti-inflammatory cytokine production

IL-10 (Table 4)

The production of IL-10 by non-stimulated PBMC was reduced following incubation with elevated concentrations of cefotaxime (p=0.0003) and meropenem (p=0.0004) but was not affected by addition of ampicillin (p=0.26) or vancomycin (p=0.061). The secretion of IL-10 by PBMC prompted by HT-29 cells was concentration dependent inhibited when cultures were incubated with increased concentrations of cefotaxime (p<0.0001), meropenem (p<0.023) or vancomycin (p<0.033), whereas that induced by RKO cells was only borderline inhibited by cefotaxime (p=0.049) and was not affected by meropenem (p=0.07) or vancomycin (p=0.13). Ampicillin had no significant effect on either HT-29 or RKO-induced IL-10 production (p=0.082, or p=0.38, respectively).

Table 2. Effect of antibiotics on TNFα secretion.

TNFα (pg/mL)				
Spontaneous				
Drug, μg/mL	**Cefotaxime (n = 8)**	**Ampicillin (n = 8)**	**Meropenem (n = 8)**	**Vancomycin (n = 8)**
0	261 ± 39	253 ± 43	267 ± 51	358 ± 77
20	216 ± 49	220 ± 50	303 ± 43	285 ± 85
100/[a]50	213 ± 53	234 ± 56	230 ± 56	251 ± 54
500/[a]125	218 ± 54	208 ± 45	208 ± 51	241 ± 53
HT-29-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	804 ± 119	690 ± 98	965 ± 194	1309 ± 241
20	721 ± 133	575 ± 112**	878 ± 207	1216 ± 279
100/[a]50	819 ± 154	543 ± 96**	896 ± 215	1093 ± 226
500/[a]125	934 ± 203	499 ± 83**	902 ± 209	1109 ± 216
RKO-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	821 ± 174	854 ± 150	953 ± 188	1320 ± 249
20	876 ± 163	857 ± 123	937 ± 172	1326 ± 242
100/[a]50	834 ± 184	604 ± 124**	894 ± 186	1428 ± 279
500/[a]125	901 ± 210	577 ± 93**	1014 ± 214	1435 ± 236

PBMC were incubated for 24 h without (spontaneous) or with one of human colon cancer cell lines HT-29 or RKO as described in Materials and Methods in the absence or presence of cefotaxime, ampicillin and meropenem at concentrations of 20 -500 μg/mL or vancomycin at 20-125 μg/mL. [a]Represents vancomycin concentration added. TNFα was tested in the supernatants. Asterisks represent comparison between cells incubated without or with antibiotics; *p<0.05; **p<0.01; **p <0.001.

Table 3. Effect of antibiotics on IFNγ secretion.

IFNγ (ng/mL)				
Spontaneous				
Drug (μg/mL)	**Cefotaxime (n = 4)**	**Ampicillin (n = 4)**	**Meropenem (n = 8)**	**Vancomycin (n = 8)**
0	0.39 ± 0.04	0.42 ± 0.05	0.38 ± 0.02	0.41 ± 0.03
20	-	-	0.28 ± 0.01**	0.35 ± 0.03*
100/[a]50	-	-	0.27 ± 0.01**	0.31 ± 0.01**
500/[a]125	0.36 ± 0.02	0.51 ± 0.17	0.28 ± 0.01**	0.29 ± 0.01**
HT-29-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	1.37 ± 0.36	1.09 ± 0.33	1.02 ± 0.16	1.12 ± 0.19
20	1.03 ± 0.33*	0.95 ± 0.37	0.73 ± 0.10**	0.88 ± 0.15**
100/[a]50	0.99 ± 0.36*	1.00 ± 0.42	0.73 ± 0.10**	0.84 ± 0.15**
500/[a]125	0.78 ± 0.13**	0.92 ± 0.31	0.72 ± 0.09**	0.80 ± 0.13**
RKP-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	2.36 ± 0.44	2.15 ± 0.39	2.1 ± 0.39	2.38 ± 0.44
20	1.96 ± 0.39	1.72 ± 0.24*	2.3 ± 0.39	2.64 ± 0.45
100/[a]50	1.91 ± 0.36*	1.63 ± 0.29**	2.24 ± 0.42	2.55 ± 0.47
500/[a]125	1.41 ± 0.30**	1.22 ± 0.22**	1.65 ± 0.28*	2.43 ± 0.43

PBMC were incubated for 24 h without (spontaneous) or with one of human colon cancer cell lines HT-29 or RKO as described in Materials and Methods in the absence or presence of cefotaxime, ampicillin and meropenem at concentrations of 20 -500 μg/mL or vancomycin at 20-125 μg/mL. [a]Represents vancomycin concentration added. IFNγ was tested in the supernatants. Asterisks represent comparison between cells incubated without or with antibiotics; *p<0.05; **p<0.01; **p <0.001.

Table 4. Effect of antibiotics on IL-10 secretion.

IL-10 (ng/mL)				
Spontaneous				
Drug (μg/mL)	**Cefotaxime (n = 8)**	**Ampicillin (n = 8)**	**Meropenem (n = 8)**	**Vancomycin (n = 8)**
0	0.43 ± 0.08	0.36 ± 0.05	0.33 ± 0.09	0.37 ± 0.11
20	0.30 ± 0.07**	0.38 ± 0.10	0.34 ± 0.12	0.30 ± 0.11
100/[a]50	0.27 ± 0.06**	0.31 ± 0.07	0.25 ± 0.08*	0.30 ± 0.11
500/[a]125	0.24 ± 0.04***	0.31 ± 0.07	0.21 ± 0.06***	0.24 ± 0.06
HT-29-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	1.94 ± 0.19	1.81 ± 0.17	1.18 ± 0.26	1.52 ± 0.36
20	1.74 ± 0.19*	1.66 ± 0.14	1.06 ± 0.24	1.17 ± 0.29*
100/[a]50	1.57 ± 0.17***	1.64 ± 0.19	0.98 ± 0.26*	1.14 ± 0.23*
500/[a]125	1.13 ± 0.12***	1.81 ± 0.19	0.87 ± 0.21**	1.18 ± 0.22*
RKO-induced				
Drug (μg/mL)	**Cefotaxime (n = 12)**	**Ampicillin (n = 12)**	**Meropenem (n = 7)**	**Vancomycin (n = 7)**
0	1.37 ± 0.18	1.33 ± 0.11	0.84 ± 0.18	0.88 ± 0.17
20	1.42 ± 0.17	1.28 ± 0.13	0.93 ± 0.23	1.00 ± 0.21
100/[a]50	1.27 ± 0.17	1.42 ± 0.15	1.04 ± 0.23	0.88 ± 0.18
500/[a]125	1.16 ± 0.16*	1.38 ± 0.16	0.73 ± 0.20	0.87 ± 0.17

PBMC were incubated for 24 h without (spontaneous) or with one of human colon cancer cell lines HT-29 or RKO as described in Materials and Methods in the absence or presence of cefotaxime, ampicillin and meropenem at concentrations of 20 -500 μg/mL or vancomycin at 20-125 μg/mL. [a]Represents vancomycin concentration added. IL-10 was tested in the supernatants. Asterisks represent comparison between cells incubated without or with antibiotics; *p<0.05; **p<0.01; ***p <0.001.

DISCUSSION

The present results indicate that the production IL1-β, TNFα, IFNγ and IL-10 was reduced in a different way when non-stimulated PBMC were incubated with the antibiotics used in the study. However, while cefotaxime affected IL1-β and IL-10, meropenem inhibited TNFα, IFNγ and IL-10. The capacity of antibiotics to influence the immune system has been well documented. It has been reported that β-lactams may affect immunity by inhibition of IFN-γ generation, although a certain diversity in their activity has been observed - the clavulanic acid, cefoxitin and cefaloridine being the most potent, whereas meropenem and aztreonam were the least effective (Brooks et al., 2005). Zwolinska-Wccislo et al. (2014) have described an inhibitory effect of ampicillin on mRNA expression for IL-1β and TNF-β in the colonic mucosa of rats with experimental colitis. Considering the pivotal role of TNFα as an activator of cytokine cascade in cases of sepsis, antibiotics capable to attenuate its production may express greater antimicrobial activity. Siedlar et al. (1997) have found that vancomycin, a glycopeptid antibiotic, was capable to down-regulate the TNFα production by LPS stimulated monocytes. On the other hand, it has been reported

that vancomycin increased pro-inflammatory responses by inducing IL-1β release from LPS stimulated macrophages (Ha et al., 2015), similar to the effect observed in the current study in RKO-stimulated PBMC.

The third generation of cephalosporins has been recognized as potential immunomodulators. It has been shown that incubation of adult human PBMC with cefotaxime enhanced the production of TNFα and IL-2 but attenuated IL1-β generation (Besssler et al., 2000). As for the activity of antibiotics on pro-inflammatory cytokine production, Choi et al. (2003) have observed attenuated TNFα and IL-6 generation by stimulated human PBMC incubated with moxifloxacin, a fourth generation fluoroquinolone. It has been suggested that some antibiotics, such as vancomycin and daptomycin, affect cytokine production by modulation of Toll-like receptors (Bode et al., 2015). In a thorough review regarding the mechanism by which macrolides affect inflammatory response, Kanok and Rubin (2010) conclude that their effect is directed on inhibition of pro-inflammatory cytokines secretion, mainly IL-8.

The aptitude of antibiotics to affect carcinogenesis is enthralling. Apparently, there are several mechanisms by which antibiotics may limit cancer development and spreading. A series of antibiotics have been found to possess the capacity to target either malignant cell mitochondria (Lamb et al., 2015) or ribosomal protein synthesis with a consequent restrain of cancer cells owning stem cell-like properties (Cuyàs et al., 2015). Rapamycin, which is an antifungal agent, has been shown to attenuate the development of head and neck squamous cell carcinoma by inhibition of Toll-like receptor 4, which is markedly represented in these cells (Ren et al., 2014). Formamidinodoxorubicin derivates of doxorubicin have been found to decrease significantly the proliferative activity of human breast cancer cells by promoting their apoptosis (Marczak et al., 2015).

The question if antibiotics may affect the way by which macrophages and cancer cells maintain an immune dialog is of interest. Schmall et al. (2015) have reported that co-culturing of macrophages with mouse Lewis lung carcinoma cells and with human lung cancer cells from different lines resulted in an up-regulation of chemokine production in both macrophages and cancer cells. In a previous work from our laboratory we have shown the existence of an immune dialog between human PBMC and cells from two human colon carcinoma lines (HT29 and RKO). This dialog was expressed by a modified cytokine production by the immune cells proceeding through a close contact between mononuclear and tumor cells (Bessler and Djaldetti, 2010). Beury et al. (2014) supported the existence of a cross-talk between macrophages and tumor cells expressed by an increased IL-6 production by macrophages. It is of interest that this equilibrium could be affected by a number of factors reviewed by Djaldetti and Bessler (2014).

The present work demonstrates that the antibiotics used in the study were able to disturb cytokine generation by affecting the cross-talk between immune and colon cancer cells. Thus, addition of antibiotics to PBMC incubated with HT-29 cells inhibited IL-1β production. A decrease in TNFα secretion was achieved by ampicillin and vancomycin, whereas that of IFNγ and IL-10 was constrained by vancomycin and meropenem. It is notable that the results obtained hereby *in vitro* conditions present a certain limitation of the study since they do not necessarily reflect the *in vivo* situation. Summarizing the findings it appears that although certain antibiotics belong to the same group, as with β-lactams in our case, they may affect differently individual cytokines produced by PBMC. The inhibitory effect of the antibiotics used in the study on pro-inflammatory cytokine production by PBMC co-cultured with malignant cells points out to an additional mechanism by which antibiotics may affect carcinogenesis. Therefore, considering the close relationship between chronic inflammation and cancer development it is conceivable that antibiotics, due to their dual effect as both antimicrobial agents and immunomodulators, could be considered to be included in the inventory of drugs for cancer therapy.

CONCLUSIONS

The results indicate that the four antibiotics used in the study were able to modulate and restrict cytokine production by PBMC co-cultured with colon cancer cells from two human lines, the most affected being IL-1β, TNFα, IFNγ and IL-10. The only exception was enhanced IL-1β production ob-

served following addition of vancomycin to PBMC incubated with RKO cancer cells. Modified immune balance among mononuclear and cancer cells caused by antibiotics suggests the existence of an additional way by which they may affect carcinogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Bessler H, Djaldetti M (2010) Role of the equilibrium between colon cancer and mononuclear cells in cytokine production. Biomed Pharmacother 64: 706–711

Bessler H, Gurary N, Aloni D, Vishne TH, Sirota L (2000) Effect of cefotaxime on cytokine production in newborns and adults in vitro. Biomed Pharmacother 54: 410-414.

Beury DW, Parker KH, Nyandjo M, Sinha P, Carter KA, Ostrand-Rosenberg S (2014) Cross-talk among myeloid-derived suppressor cells, macrophages, and tumor cells impacts the inflammatory milieu of solid tumors. J Leukoc Biol 96: 1109-1118.

Bode C, Muenster S, Diedrich B, Jahnert S, Weisheit C, Steinhagen F, Boehm O, Hoeft A, Meyer R, Baumgarten G (2015) Linezolid, vancomycin and daptomycin modulate cytokine production, Toll-like receptors and phagocytosis in a human *in vitro* model of sepsis. J Antibiot (Tokyo). 68: 485-490.

Brooks B M, Hart C A, Coleman J W (2005) Differential effects of beta-lactams on human IFN-gamma activity. J Antimicrob Chemother 56: 1122-1125.

Calbo E, Alsina M, Rodríguez-Carballeira M, Lite J, Garau J (2008) Systemic expression of cytokine production in patients with severe pneumococcal pneumonia: effects of treatment with a beta-lactam versus a fluoroquinolone. Antimicrob Agents Chemother 52: 2395-2402.

Choi JH, Song MJ, Kim SH, Choi SM, Lee DG, Yoo JH, Shin WS (2003) Effect of moxifloxacin on production of proinflammatory cytokines from human peripheral blood mononuclear cells. Antimicrob Agents Chemother 47: 3704-3707.

Cuyàs E, Martin-Castillo B, Corominas-Faja B, Massaguer A, Bosch-Barrera J, Menendez JA (2015) Anti-protozoal and anti-bacterial Antibiotics that inhibit protein synthesis kill Cancer subtypes enriched for stem cell-like properties. Cell Cycle 14: 3527-3532.

Djaldetti M, Bessler H (2014) Modulators affecting the immune dialogue between human immune and colon cancer cells. World J Gastrointest Oncol 6: 129-138.

Garrido-Mesa J, Algieri F, Rodriguez-Nogales A, Utrilla MP, Rodriguez-Cabezas ME, Zarzuelo A, Ocete MA, Garrido-Mesa N, Galvez J (2015) A new therapeutic association to manage relapsing experimental colitis: Doxycycline plus *Saccharomyces boulardii*. Pharmacol Res 97: 48-63.

Guchhait G, Altieri A, Gorityala B, Yang X, Findlay B, Zhanel G G, MookherjeeN, Schweizer F (2015) Amphiphilic tobramycins with immunomodulatory properties. Angew Chem Int Ed Engl 54: 6278-6282.

Ha Y E, Kong K H, Cho M H, Kim O H, Song Y S, Yoon S Y (2015) Vancomycin blocks autophagy and induces interleukin-1β release in macrophages. J Antibiot (Tokyo) 68: 76-80.

Kanoh S, Rubin B K (2010) Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin Microbiol Rev 23: 590-615.

Lahat G, Halperin D, Barazovsky E, Shalit I, Rabau M, Klausner J, Fabian I (2007) Immunomodulatory effects of ciprofloxacin in TNBS-induced colitis in mice. Inflamm Bowel Dis 13: 557-565.

Lamb R, Ozsvari B, Lisanti C L, Tanowitz H B, Howell A, Martinez-Outschoorn U E, Sotgia F, Lisanti M P (2015) Antibiotics that target mitochondria effectively eradicate cancer stem cells, across multiple tumor types: treating cancer like an infectious disease. Oncotarget 6: 4569-4584.

Marczak A, Denel-Bobrowska M, Łukawska M, Oszczapowicz I (2015) Formamidinodoxorubicins are more potent than doxorubicin as apoptosis inducers in human breast cancer cells. Anticancer Res 35: 1935-1940.

Ren G, Hu J, Wang R, Han W, Zhao M, Zhou G, Zhang C, Zhang Z (2014) Rapamycin inhibits Toll-like receptor 4-induced pro-oncogenic function in head and neck squamous cell carcinoma. Oncol Rep 31: 2804-2810.

Schmall A, Al-Tamari H M, Herold S, Kampschulte M, Weigert A, Wietelmann A, Vipotnik N, Grimminger F, Seeger W, Pullamsetti S S, Savai R (2015) Macrophage and cancer cell cross-talk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer. Am J Respir Crit Care Med 191: 437-447.

Sharma PC, Chaudhary M, Sharma A, Piplani M, Rajak H, Prakash O (2013) Insight view on possible role of fluoroquinolones in cancer therapy. Curr Top Med Chem 13: 2076-2096.

Siedlar M, Szczepanic A, Wieckiewicz J, Pituch-Novoroiska A, Zenbala M (1997) Vancomycin down-regulates lipopolysaccharide-induced tumor necrosis factor alpha (TNF alpha) production and TNF alpha-mRNA accumulation in human blood monocytes. Immunopharmacology 35: 265-271.

Zwolinska-Wcislo M, Krzysiek-Maczka G, Ptak-Belowska A, Karczewska E, Pajdo R, Sliwowski Z, Urbanczyk K, Drozdowicz D, Konturek SJ, Pawlik WW, Brzozowski T (2014) Antibiotic treatment with ampicillin accelerates the healing of colonic damage impaired by aspirin and coxib in the experimental colitis. Importance of intestinal bacteria, colonic microcirculation and proinflammatory cytokines. J Physiol Pharmacol 62: 357-368.