MODULATION OF HUMAN LYMPHOCYTE PROLIFERATION BY ANTIBACTERIAL DRUGS

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ABSTRACT
The aim of this work was to perform a systematic study of the interference of the different antibiotics with phytohaemaglutinin (PHA) stimulated human lymphocytes proliferation. Twelve antibiotics from different therapeutic classes were chosen: b-lactams (amoxicillin; ceftriaxone; imipenem), glycopeptides (vancomycin), aminoglycosides (gentamicin), macrolides (erythromycin), tetracyclines (tetracycline), rifampicin, quinolones (ciprofloxacin; nalidixic acid), sulfonamides (sulfamethoxazole) and nitrofuran (nitrofurantoin). Nitrofurantoin showed the strongest antiproliferative effect while tetracycline and rifampicin showed only moderate activities. All the other antibiotics were inactive even at the maximum concentration tested (100mg/ml).

KEYWORDS
Antibacterial agents; immunomodulation.

RESUMO
O objectivo do trabalho foi o estudo sistemático da interferência dos diferentes antibióticos com a proliferação de linfócitos humanos estimulados com fitohemaglutinina (PHA). Foram seleccionados doze antibióticos pertencentes a diferentes grupos terapêuticos: b-lactâmicos (amoxicilina; ceftriaxona; imipenemo), glicopéptidos (vancomicina), aminoglicosídeos (gentamicina), macrólidos (eritromicina), tetraciclinas (tetraciclina), rifampicina, quinolonas (ciprofloxacina; ácido nalidíxico), sulfonamidas (sulfametoxazole) e nitrofuranos (nitrofurantoína). A nitrofurantoína mostrou ser um potente inibidor da proliferação dos linfócitos enquanto a tetraciclina e a rifampicina apenas exibiram actividades moderadas. Nenhum dos outros antibióticos foi activo mesmo na concentração máxima testada (100 mg/ml).

PALAVRAS-CHAVE
Agentes antibacterianos; imunomodulação.

1. **INTRODUCTION**

Immunomodulation emerged in 1796 when Jenner performed the first vaccination and was firstly thought of as an induction of immunity to pathogens. Several methods were developed to counteract infectious agents as vaccination or transfer of humoral factors (Labro).

But, when an infectious disease was already in course, antibiotherapy, directly targeted to the bacteria, was one of the most important medical discoveries. With the beginning of chemotherapy, the development of resistances and the search for new antimicrobial drugs, we were forced to look to the molecules by another perspective. Their capacity to modulate the immune system and reinforce their activity fighting against microbial agents or stimulating immunossupressed hosts is now being explored (Labro).

The effect of antibacterial drugs on the immune system is being studied from a long time (Banck and Forsgren; Butler et al.; Chaperon and Sanders) respecting either in lymphocyte activation/proliferation, monocyte functions (Takahashi et al.) or inflammation (Shinkai et al.). Those effects were dependent of interference with cell cycle progression, nitric oxide synthesis and inhibition of cytokines production/activity (Bamaias et al.; Brooks et al.; Buijis et al.; Dalhoff and Shalit; Takahashi et al.; Weiss et al.; Werber et al.; Zhang and Ward). Few clinical studies also report the immunomodulatory activity of antibacterial drugs in cases of infectious diseases and subsequent sepsis (Calbo et al.). However, there are no systematic studies on their effect on the immune system, either *in vitro* or *in vivo*. The present work represents a systematic study of the interference of different classes of antibiotics with human lymphocyte *in vitro*, allowing the direct comparison of their activities.

2. **MATERIAL AND METHODS**

2.1. **REAGENTS**

Amoxicilin and clavulanic acid (CLAVEPEN®), ceftriaxone and gentamicin were from Labesfal, imipenen from Merck Sharp & Dohme and ciprofloxacin from Bayer.

Unless otherwise indicated all reagents were obtained from Sigma.

2.2. **ANTIBACTERIAL SOLUTIONS**

Stocks solutions of the antibacterial agents were prepared in DMSO and stored at -20 ºC unless otherwise stated. Stock solution of vancomycin, amoxicillin and nalidixic acid, ceftriaxone, imipenem were prepared in RPMI-1640 while rifampicin was ressuspended in methanol and tetracycline in water. Commercially available intra-venous solutions of gentamicin and ciprofloxacin were used.

The frozen samples were freshly diluted to the desired final concentrations with culture medium prior to the different assays. Final concentrations of DMSO or methanol did not interfere with any of the biological activities tested.

2.3. **HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS ISOLATION**

Human mononuclear cells were isolated from heparinized peripheral blood of healthy volunteers by Histopaque-1077 density centrifugation. Human mononuclear cells were adjusted to 2–3 x 10^6 cells/ml in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine and 50 mg/ml of gentamicin (designated thereafter as culture medium).

2.4. **MTT-PROLIFERATION ASSAY**

The effect of antibacterial compounds on the mitogenic response of human lymphocytes to PHA (10 mg/ml) was evaluated using a modified version of the colorimetric MTT-assay (Mosmann), which was previously described by us (Cerqueira et al.).

2.5. **STATISTICS**

Results are expressed as mean values ± SEM (standard error of the mean).

3. **RESULTS AND DISCUSSION**

The effect of twelve antibacterial drugs on the mitogenic response of peripheral human lymphocytes to PHA was evaluated and the results, given in concentrations that cause 50% inhibition of proliferation (IC_{50}), are summarized in Table 1.

Antimicrobial agent	IC_{50} (µg/ml)	Antimicrobial agent	IC_{50}
Vancomycin	>100	Tetracycline	40,97 ± 7,13
Amoxicillin	>100	Rifampicin	74,76 ± 17,51
Ceftriaxone	>100	Ciprofloxacin	>100
Imipenem	>100	Nalidixic acid	>100
Gentamicin	>100	Sulfamethoxazole	>100
Erythromycin	>100	Nitrofurantoin	13,13 ± 2,20

TABLE 1 - Effect of antibacterial drugs on the PHA-induced proliferation of human lymphocytes.

The effect was evaluated by MTT assay after 96 h of culture. Results are the mean ± SEM of 3 or 4 independent experiments.

Results showed that nitrofurantoin showed the strongest antiproliferative effect while tetracycline and rifampicin showed moderate activities. However, those IC_{50} values were above the serum concentrations in therapeutics (Sousa). None of the other antibiotic drugs showed capacity to inhibit PHA-induced lymphocyte proliferation even when tested at 100 mg/ml.

Although the IC_{50} of nitrofurantoin is higher than the serum concentration usually obtained this results suggests a possible immunomodulation activity of the drug that could be exploited in therapeutics. Our results are in agreement with previous studies showing that at a concentration of 10 mg/ml the drug profoundly affected the B- and T-lymphocyte responses to mitogens, antibody production, and protein synthesis in unstimulated lymphocytes

(Banck and Forsgren). The same study showed that rifampicin significantly depressed the mitogenic responses of B and T lymphocytes at a concentration of 50mg/ml, and this is in accordance with our report (Banck and Forsgren).

Results for tetracycline include either the stimulation or suppression of lymphocytes, depending on the doses and the study (Ingham et al.). We demonstrated that tetracycline was able to inhibit PHA-induced lymphocyte stimulation.

Ciprofloxacin effects on the immune system have been object of several studies. Ciprofloxacin did not influence the proliferation of mononuclear cell proliferation at concentrations as high as 100mg/ml, which is in accordance with other studies (Gollapudi et al.). This and other quinolone compounds are referred as inhibitors peripheral blood lymphocytes cell (PBLs) growth due to the arrest of cell cycle (Forsgreen et al.). Despite this results, ciprofloxacin as shown to increase [3H]thymidine incorporation of PHA stimulated peripheral blood lymphocytes, potentiate IL-2 synthesis and altering the expression of IL-4 and IFN-g influencing Th1/Th2 ratios (Riesbeck et al.).

Although some cefalosporins are known to inhibit the mitogenic proliferation of lymphocytes (Chaperon and Sanders), ceftriaxone (third class cephalosporin) showed no activity at the concentrations tested.

For erythromycin literature refers that it only inhibited lymphocyte function at high concentrations (Banck and Forsgren).

4. **CONCLUSION**

Many current antibacterial agents have not revealed all their facets.

Besides their antimicrobial activity, some antibacterial drugs, such as nitrofurantoin, tetracycline and rifampicin, are able to inhibit PHA-induced lymphocyte proliferation, revealing an immunomodulatory activity that must be explored.

The immunomodulatory activity of antimicrobial drugs should be systematically evaluated either on innate or acquired immunity.

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