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Modulation of prostanoid synthesis by antimicrobials

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To maintain cells or tissues in culture for extended periods of time often requires that antimicrobials be added to the culture medium. The influence of such agents, however, on the characteristics of the tissues must be considered. One characteristic of mammalian tissues which is of interest in many studies is the production of prostanoids (prostaglandins and thromboxanes). The modulation of prostaglandin synthesis by many drugs has been demonstrated [1–4], although antimicrobials have not been studied extensively in this respect. We have investigated, therefore, the effects on prostanoid synthesis of an antimicrobial mixture, containing penicillin, streptomycin, and amphotericin B, which is commonly used to inhibit microbial growth in culture media. The individual drugs in the mixture were tested and two other unrelated antimicrobials, tetracycline and metronidazole, were tested also.

The antibiotic–antimycotic mixture investigated in this study (No. 600-5240 Gibco Laboratories, Grand Island, NY) contained penicillin G (10,000 units/ml), streptomycin (10 mg/ml) and amphotericin B (25 μg/ml). The manufacturer recommends that this mixture be added to culture media in a concentration of 1% (v/v). Individual antimicrobial drugs were obtained from the Sigma Chemical Co., St. Louis, MO. The actions of the drugs on prostanoid biosynthesis were tested by use of a microsome-enriched preparation of bovine seminal vesicle (BSV) prostaglandin synthase that was obtained from Miles Laboratories, Elkhart, IN. The standard assay mixture contained 4 mg of BSV microsomal protein, sodium arachidonate (61 μM), and an appropriate amount of test drug dissolved in phosphate buffer (50 mM, pH 7.4) to give a final assay volume of 1.0 ml. The amphotericin B was soluble only to a small extent in aqueous media and was first dissolved in dimethyl sulfoxide (DMSO) (Baker Chemical, Phillipsburg, NJ) before being added to the assay mixture. Two concentrations of DMSO were used such that the final concentrations of DMSO in the test assay were 1% and 8.6% (w/v). DMSO at these concentrations, without amphotericin B, was also tested. After 20 min of incubation with gentle shaking at 37°, the reaction was stopped by adding 0.5 ml

Table 1. Effects of test drugs on the production of PGE_2, $PGF_{2\alpha}$, and TXB_2*

Test drug and concentration	Prostanoid		
	PGE_2	$PGF_{2\alpha}$	TXB_2
Antimicrobial mixture (%, v/v)			
3%	0.88 ± 0.03 (NS)†	0.88 ± 0.05 (NS)	0.79 ± 0.05‡
30%	0.60 ± 0.02§	0.88 ± 0.06 (NS)	0.37 ± 0.02§
Penicillin G (μM)			
1.4	0.76 ± 0.19 (NS)	1.08 ± 0.14 (NS)	NT‖
14.0	0.86 ± 0.07 (NS)	1.06 ± 0.08 (NS)	NT
140	0.81 ± 0.11 (NS)	0.99 ± 0.09 (NS)	NT
Streptomycin (μM)			
3.4	0.87 ± 0.09 (NS)	0.98 ± 0.06 (NS)	NT
34.0	0.69 ± 0.12¶	0.79 ± 0.05 (NS)	NT
340	0.68 ± 0.05¶	0.93 ± 0.06 (NS)	NT
DMSO (%, w/v)			
1%	1.08 ± 0.07 (NS)	1.11 ± 0.11 (NS)	1.27 ± 0.30 (NS)
8.6%	1.29 ± 0.05¶	1.08 ± 0.09 (NS)	1.23 ± 0.07 (NS)
Amphotericin (μM) in DMSO (8.6%, w/v) final concentration			
1.4	1.23 ± 0.08 (ND)**	0.85 ± 0.10 (NS)	1.19 ± 0.16 (NS)
14.0	1.32 ± 0.09 (ND)	1.03 ± 0.10 (NS)	1.27 ± 0.12 (NS)
140	1.27 ± 0.12 (ND)	0.91 ± 0.07 (NS)	1.23 ± 0.03 (NS)

* Each value is the mean ± S.E.M. (N = 3) of the ratio of prostanoid production in the test assays to prostanoid production in the control assays.
† Not significantly different from controls.
‡ Significantly different from controls, $P < 0.05$.
§ Significantly different from controls, $P < 0.001$.
‖ Not tested.
¶ Significantly different from controls, $P < 0.01$.
** Not significantly different from 8.6% DMSO.

of 0.4 M citric acid and 7.0 ml ethyl acetate. After mixing and centrifugation for 5 min at 600 *g*, the organic layer was removed, evaporated to dryness under nitrogen, and the residue dissolved in 1.0 ml of phosphate buffer. After a further dilution, which varied depending upon the prostanoid to be measured, prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and thromboxane B_2 (TXB_2) were quantified using specific radioimmunoassays [5, 6]. The drugs were tested at different concentrations in the assay mixture, and all experiments were conducted in triplicate with controls. Statistical significance was assessed by a one-way analysis of variance. Comparison of control values with those obtained with drugs in different concentrations was made by the Newman–Keuls method for multiple range comparisons [7]. A probability of $\alpha = 0.05$ was chosen as the level of statistical significance.

The antimicrobial mixture decreased the production of TXB_2 by 21 and 63% at assay concentrations of 3 and 30% respectively (Table 1). PGE_2 production was not altered significantly by the mixture at a concentration of 3% but was reduced by 40% at the higher concentration. $PGF_{2\alpha}$ production was not affected by the antimicrobial mixture at either concentration tested.

Penicillin did not affect production of either PGE_2 or $PGF_{2\alpha}$. Streptomycin, however, inhibited the production of PGE_2 in a concentration-related manner, although $PGF_{2\alpha}$ production was unaffected.

The effect of amphotericin B in the assay was attributable to the effect of DMSO. DMSO at a 1% concentration (w/v) showed no effect but at 8.6% a small (29%) but significant increase in PGE_2 production was measured. This increase was indistinguishable from the effect of amphotericin B (in 8.6% DMSO) at all three drug concentrations. Neither DMSO alone nor amphotericin B in DMSO altered the production of $PGF_{2\alpha}$ or TXB_2.

Interestingly, tetracycline caused a concentration-related stimulation of all three prostanoids measured (Fig. 1). Metronidazole, however, caused a stimulation of TXB_2 only, no effect being noted on the production of either PGE_2 or $PGF_{2\alpha}$ (Fig. 2).

In summary, the results of this study are indicative that antibiotics can modify the synthesis of several prostanoids. A mixture of antimicrobials that is commonly used in culture media caused a decrease in the production of PGE_2 and TXB_2 (but not $PGF_{2\alpha}$). Testing of the individual component drugs produced results suggestive that the inhibitory activity resides in the streptomycin, penicillin and amphotericin being without effect in the concentrations tested. The prostaglandin synthase inhibitory effect of the streptomycin at concentrations used in tissue cultures is probably

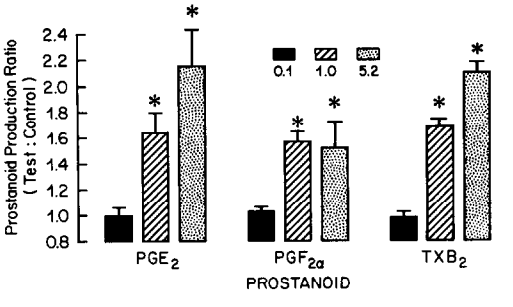

Fig. 1. Effect of tetracycline on the production of PGE_2, $PGF_{2\alpha}$, and TXB_2. Each value is the mean ± S.E. (N = 3) of the ratio of prostanoid production in the test assays to prostanoid production in the control assays. The key indicates test drug concentrations (mM). An asterisk (*) indicates significantly greater than control ($P < 0.05$).

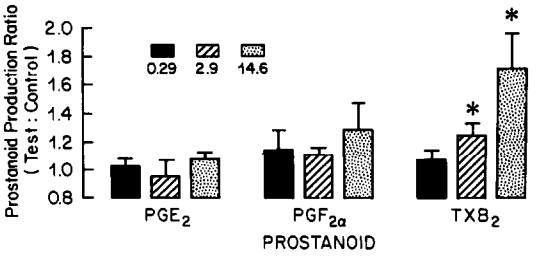

Fig. 2. Effect of metronidazole on the production of PGE_2, $PGF_{2\alpha}$, and TXB_2. Each value is the mean ± S.E. (N = 3) of the ratio of prostanoid production in the test assays to prostanoid production in the control assays. The key indicates test drug concentrations (mM). An asterisk (*) indicates significantly greater than control ($P < 0.05$).

not of sufficient magnitude by itself to alter significantly prostaglandin production rates. It has been reported, however, that aminoglycoside antibiotics can also inhibit phospholipase C activity [8]. Therefore, the possibility of a two-component inhibitory effect of aminoglycoside antibiotics on prostaglandin production should be considered in experiments conducted with intact cells in culture.

There is also considerable variation in the sensitivities of different tissues to prostaglandin synthase inhibitors [4]. Indeed, even in a single tissue type, the effects on prostaglandin production by a test agent may depend upon the experimental methods employed [3]. It is possible that, under different conditions, the effects of antimicrobials on prostaglandin synthesis may be reduced or even enhanced, compared to the effects reported here. Individual systems should, therefore, be evaluated in the context of such variations.

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