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Experimental Investigations on Interactions between Gentamicin and Betamethasone Dipropionate

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SUMMARY

As a model for recommendable investigations in combined drug therapy, experiments on possible interactions between a modern glucocorticoid (betamethasone 17,21 dipropionate) and an antibacterial antibiotic (gentamicin) were performed. On resting and proliferating bacteria (staphylococci, pseudomonas), the antibacterial efficacy of gentamicin was not impaired in the presence of betamethasone dipropionate in "activating" concentrations, which means that the glucocorticoid itself, in the concentration used for the experiments, enhanced bacterial metabolism. The topical potency of the glucocorticoid was measured by vasoconstrictor assay; in low concentrations (0·001% to 0·00001%), the percentage of blanching reactions elicited by the steroid remained practically unchanged in the presence of gentamicin in a twenty-fold concentration as applied clinically. It was concluded from these experimental in vitro and in vivo studies that betamethasone dipropionate and gentamicin may safely be used together, without the risk of unwanted interactions.

INTRODUCTION

In the development of combined drug preparations, the possibility of interactions between the single components deserves attention. In local therapy of skin diseases, not infrequently ointments or creams are used which contain a glucocorticoid and an antimicrobial substance (antibiotic). Interactions might occur which impair either the antimicrobial activity of the antibiotic or the antiphlogistic potency of the glucocorticoid. From the viewpoint of the clinical pharmacologist, it is necessary that only such drug combinations are developed and used where the occurrence of interactions between the single components has been ruled out.

This paper deals with investigations which were performed to probe the simultaneous applicability of betamethasone dipropionate and gentamicin, as an example for numerous similar drug combinations for topical use. Bactericidal and bacteriostatic activity of gentamicin was measured in the absence and presence of the glucocorticoid and the vasoconstrictor potency of betamethasone dipropionate was estimated with and without the antibiotic.

MATERIAL AND METHODS

Activity of Gentamicin (Experiments in vitro)

The experiments were performed on four bacterial strains : *Staph. albus*, *Staph. aureus haemolyticus* and two different strains of *Pseudomonas aeruginosa*. All strains had been isolated from dermatological lesions. The bacteria were grown on solid agar plates and inoculated into liquid broth (Merck standard broth, pH adjusted to 7·4).

After an incubation period of 18 hours at 37° C., the broth was centrifuged (5 min. at 4,500 rev.) and the sediment was washed three times, either with Ringer's solution pH 7·4 (experiments with resting bacteria) or with standard broth (experiments with proliferating bacteria). Finally, for the experiments with resting bacteria, a suspension in Ringer's solution was prepared which contained approximately 5×10^7 bacteria per millilitre ; and for the experiments

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with proliferating bacteria, a suspension in broth was prepared which contained approximately 10^7 bacteria per millilitre.

Gentamicin. Gentamicin (Batch GMC–2M–203, potency 566 μg./mg., obtained by courtesy of Schering Corp., U.S.A.) was dissolved in Ringer's solution (experiments on resting bacteria, final concentrations 300 and 1,000 μg./ml.) or broth (experiments on proliferating bacteria, final concentrations 1 and 2 μg./ml.).

Betamethasone Dipropionate. Betamethasone dipropionate (Batch 3971–39, obtained by courtesy of Schering Corp., U.S.A.) was dissolved in ethanol. On resting and on proliferating bacteria this glucocorticoid ester was used in final concentrations of 33 μg./ml.; in preliminary experiments this concentration had been evaluated as lying within the activating range. Due to the solubility properties of betamethasone dipropionate, all the experiments had to be run in media containing 3·3% ethanol.

Experiments. In all series experiments were performed with gentamicin and betamethasone dipropionate separately, and with both drugs together. The data obtained were compared with the controls.

Controls. In all the experiments double controls were run with the respiratory media (Ringer's solution or broth), substrates (glucose in the case of resting bacteria) and ethanol (0·1 ml. per 3·0 ml. medium).

Performance of the Experiments: Resting Bacteria: 0·2 ml. of the bacterial suspensions (approximately 10^7 organisms), 2·3 ml. Ringer's solution, 0·2 ml. of a 5% solution of glucose, 0·2 ml. of the gentamicin solution in Ringer's solution (or an aliquot of Ringer's solution in the betamethasone dipropionate and control experiments) and 0·1 ml. of the alcoholic solution of betamethasone dipropionate (or an aliquot of ethanol in the gentamicin and control experiments) were brought into the main compartments of sterile Warburg flasks. The central compartments of these flasks contained 0·2 ml. of a 20% KOH solution for absorption of carbon dioxide. The flasks were fixed to the manometers, hung into a water bath (37° C.), and shaken. After an adaption period of 10 minutes, flasks and manometers were closed and registration of oxygen consumption started (readings every 15 minutes, gas phase air). The experiments were run over a period of 90 minutes. Altogether 15 experimental series with 12 flasks each were run: two with *Staph. albus*, five with *Staph. aureus haemolyticus*, two with a gentamicin-insensitive strain of *Pseudomonas aeruginosa*, and six with the second strain of *Pseudomonas aeruginosa.*

Proliferating Bacteria (Log Phase): Into the main compartments of sterile Warburg flasks, 2·4 ml. of standard broth and 0·1 ml. of the bacterial suspensions (approximately 10^6 organisms) were given. The central compartments of these flasks, again, contained 0·2 ml. of a 20% solution of KOH for absorption of carbon dioxide. The side compartments of the Warburg flasks were filled with 0·4 ml. of the gentamicin solution in broth (or an aliquot of broth in the betamethasone dipropionate and control experiments) and 0·1 ml. of the alcoholic solution of betamethasone dipropionate (or 0·1 ml. of ethanol in the gentamicin and control experiments). Flasks were fixed to the manometers, hung into the water bath (37° C.), and shaken. After an adaption period of 10 minutes flasks and manometers were closed.

The occurrence of the proliferative phase was confirmed by reading the oxygen consumption from gas phase air every 10 minutes (logarithmic increase). After 30 minutes the contents of the side compartments were dumped into the main compartments without opening the flasks. From this point (start), oxygen consumption was read every 30 minutes over a period of 210 minutes (end of proliferation in the control flasks). Altogether, 38 experiments with 12 flasks each were performed: 10 with *Staph. albus*, 10 with *Staph. aureus haemolyticus*, four with *Pseudomonas aeruginosa* (insensitive) and 14 with the second strain of *Pseudomonas aeruginosa.*

Vasoconstrictor Potency of Betamethasone Dipropionate (Experiments in vivo)

Betamethasone dipropionate (batch 3971–39) was dissolved in ethanol to form solutions of 0·001% (=approx. 2×10^{-5} M), 0·0001% (=approx. 2×10^{-6} M) and 0·00001% (=approx. 2×10^{-7} M). Into one part of these solutions gentamicin (batch GMC–2M–203) was suspended in a twenty-fold concentration of the glucocorticoid. (It must be mentioned that no dermatologically acceptable solvent could be found in which both the steroid and the antibiotic could be dissolved.)

In 12 healthy volunteers 0·05 ml. of the solutions/suspensions were applied to the forearm skin, according to the standard vasoconstrictor assay. The whole test area was occluded with plastic, which was fixed with adhesive tape at the edges. This occlusive bandage remained for 18 hours. After removal of the bandage the occurrence or absence of vasoconstriction was read (blanching phenomenon).

RESULTS

Influence of Betamethasone Dipropionate on the Activity of Gentamicin

Staph. albus. In concentrations of 300 and 1,000 μg./ml., gentamicin remained without significant effect on oxygen consumption of this strain of

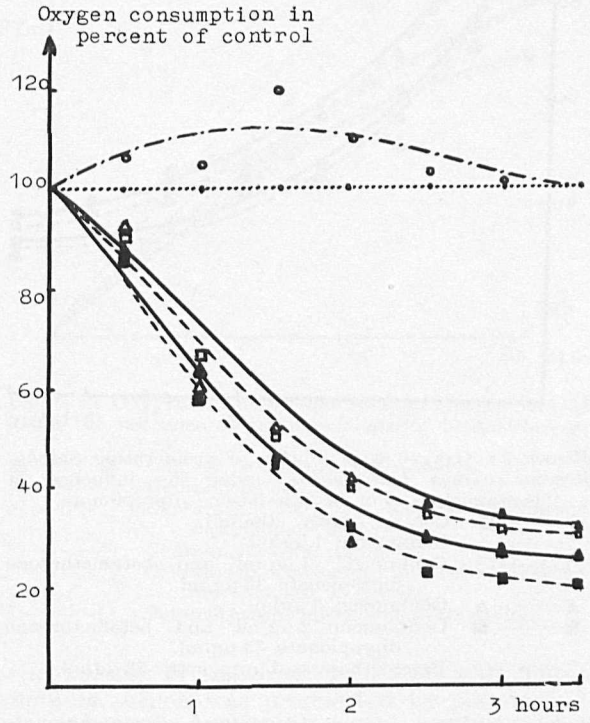

Figure 1: Oxygen consumption of proliferating *Staphylococcus albus under* the influence of gentamicin and/or betamethasone dipropionate.

●·····● Control (broth and ethanol).
△——△ Gentamicin 1 μg/ml.
□- - -□ Gentamicin 1 μg/ml and betamethasone dipropionate 33 μg/ml.
▲——▲ Gentamicin 2 μg/ml.
■- - -■ Gentamicin 2 μg/ml and betamethasone dipropionate 33 μg/ml.
○-·—·-○ Betamethasone dipropionate 33 μg/ml.

Staph. albus in the resting phase. Betamethasone dipropionate provoked a significant increase in oxygen consumption during the first half hour of the experiments.

On proliferating *Staph. albus*, gentamicin in concentrations of 1 and 2 µg./ml. exerted marked bacteriostatic activity. The presence of betamethasone dipropionate (33 µg./ml.) which, by itself, provoked an increase in oxygen consumption failed to lower the antibacterial action of gentamicin. Oxygen consumption of proliferating *Staph. albus* under the influence of gentamicin and/or betamethasone dipropionate is depicted in Figure 1.

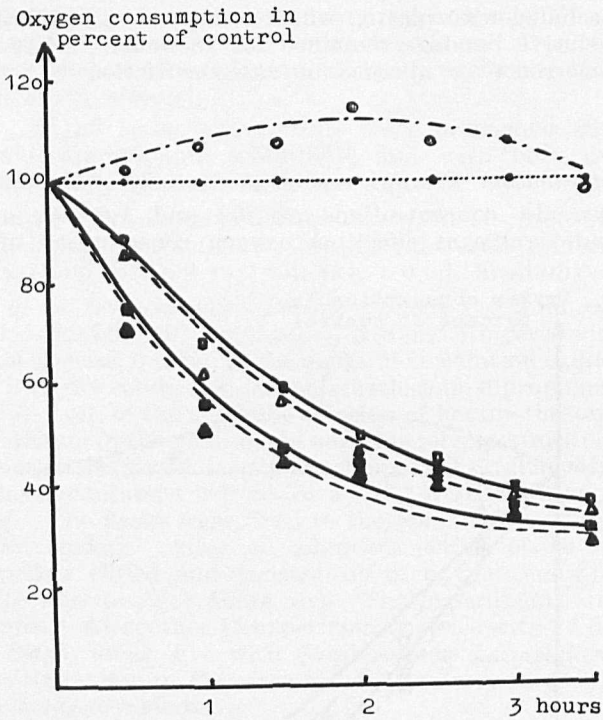

Figure 2: Oxygen consumption of proliferating *Staphylococcus aureus haemolyticus* under the influence of gentamicin and/or betamethasone dipropionate.

●·····● Control (broth, ethanol).
△- - - -△ Gentamicin 1 µg/ml.
□————□ Gentamicin 1 µg/ml and betamethasone dipropionate 33 µg/ml.
▲- - - -▲ Gentamicin 2 µg/ml.
■————■ Gentamicin 2 µg/ml and betamethasone dipropionate 33 µg/ml.
○-·—·-○ Betamethasone dipropionate 33 µg/ml.

Staph. aureus haemolyticus. On resting *Staph. aureus haemolyticus* gentamicin in concentrations of 300 and 1,000 µg./ml. exerted a pronounced antibacterial action. However, oxygen consumption was only lowered to about 80% of control values. Betamethasone dipropionate (33 µg./ml.) provoked an increase in oxygen consumption. In the presence of betamethasone dipropionate no significant changes in antibacterial efficacy of gentamicin could be detected.

On proliferating *Staph. aureus haemolyticus*, gentamicin (1 and 2 µg./ml.) revealed a marked bacteriostatic action. Betamethasone dipropionate (33 µg./ml.) increased oxygen consumption but did not alter the bacteriostatic action of gentamicin (Figure 2).

Pseudomonas aeruginosa I (*insensitive*). In the resting phase this strain of *Pseudomonas aeruginosa* taken at random from the routine laboratory, exhibited no change in oxygen consumption in the presence of gentamicin in concentrations of 3–0 and 1,000 μg./ml. Betamethasone dipropionate provoked an increase in oxygen consumption, as was the case with all the other bacteria. It should be mentioned that by standard microbiological assay the insensitivity of this strain against gentamicin was confirmed.

In the proliferating phase, this strain of *Pseudomonas aeruginosa* was slightly depressed in its oxygen consumption by the presence of gentamicin. This effect remained unchanged when betamethasone dipropionate was added despite the fact that the steroid by itself increased oxygen consumption.

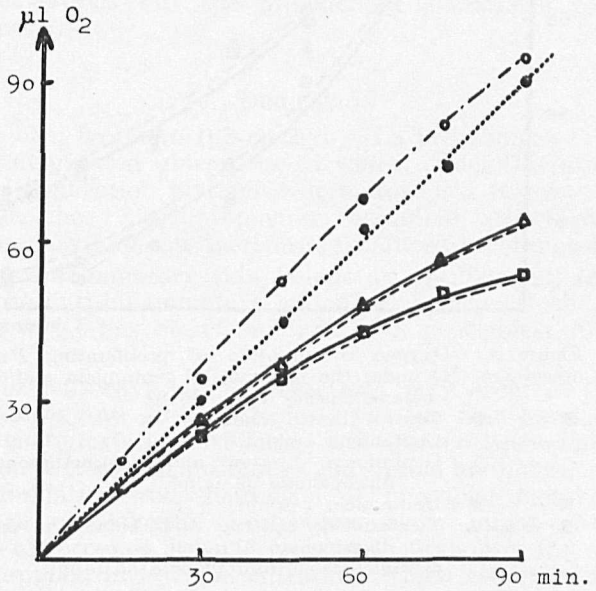

Figure 3 : Oxygen consumption of resting *Ps. aeruginosa* (II) under the influence of gentamicin and/or betamethasone dipropionate.

●·····● Control (Ringer/glucose/ethanol).
△———△ Gentamicin 300 μg/ml.
△– – – –△ Gentamicin 300 μg/ml and betmeathasone dipropionate 33 μg/ml.
□———□ Gentamicin 1,000 μg/ml.
□– – – –□ Gentamicin 1,000 μg/ml and betamethasone dipropionate 33 μg/ml.
○–·—·–○ Betamethasone dipropionate 33 μg/ml.

Pseudomonas aeruginosa II (*sensitive to gentamicin*). This second strain of *Pseudomonas aeruginosa*, which was preselected by standard determination of bacterial resistance, exhibited a marked decrease in oxygen consumption in the resting phase when gentamicin was present. However, only a non-significant difference was found between the effect of the 300 μg./ml. and the 1,000 μg./ml. concentrations (Figure 3). Betamethasone dipropionate, which by itself increased oxygen consumption, failed to influence these effects of gentamicin.

In the proliferating phase a marked reduction in metabolic activity was encountered when gentamicin was added ; oxygen consumption dropped to values below 10% of controls. Betamethasone dipropionate (33 μg./ml.) provoked a significant increase in oxygen consumption (maximal effect in the second hour after dumping the test substances into the main compartments).

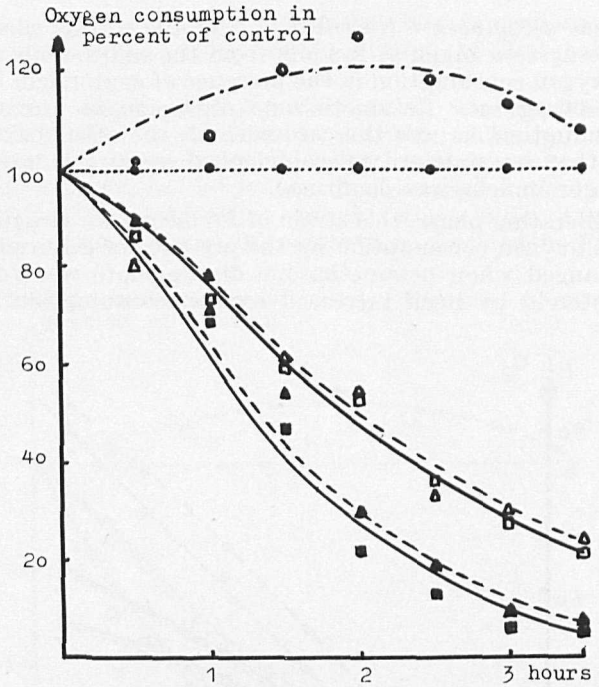

Figure 4: Oxygen consumption of proliferating *Ps. aeruginosa* (II) under the influence of gentamicin and/or betamethasone dipropionate.

●·····● Control (broth, ethanol).
□———□ Gentamicin 1 µg/ml.
△- - - -△ Gentamicin 1 µg/ml and betamethasone dipropionate 33 µg/ml.
■———■ Gentamicin 2 µg/ml.
▲- - - -▲ Gentamicin 2 µg/ml and betamethasone dipropionate 33 µg/ml.
○-·—·-○ Betamethasone dipropionate 33 µg/ml.

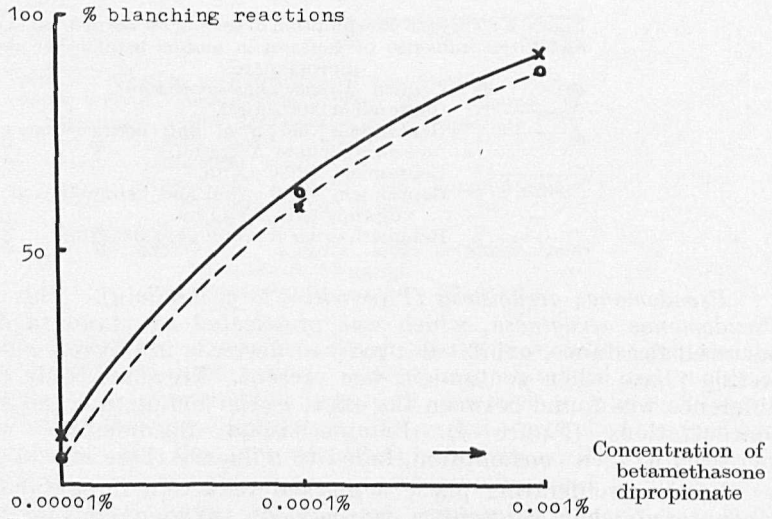

Figure 5: Percentage of positive blanching tests following occlusion with betamethasone dipropionate in three different concentrations, with and without a twenty-fold concentration of gentamicin.

×———× Betamethasone dipropionate with gentamicin.
○- - - -○ Betamethasone diproprionate without gentamicin.

Bacteriostatic activity of gentamicin (1 and 2 μg./ml.) remained unchanged in the presence of betamethasone dipropionate (Figure 4).

Vasoconstrictor Assay

The comparative evaluation of betamethasone dipropionate with and without gentamicin failed to show any significant differences due to the presence of the antibiotic in regard to the vasoconstrictor potency of the steroid. Betamethasone dipropionate 0·001% provoked blanching in 28 of 32 tested sites; with gentamicin 0·02% suspended in this solution of betamethasone dipropionate the number of reacting sites stayed about the same (29 out of 32). Similar results were obtained with the lower concentrations of steroid and antibiotic. The percentage of blanching sites gradually decreases with the lowered glucocorticoid concentrations but the presence of gentamicin remained without influence (Figure 5).

DISCUSSION

Betamethasone, 9α-fluoro-16β-methyl-△-1,4-pregnadiene-11β, 17α, 21-triol-3,20-dione, is a fluorinated glucocorticoid which, under the form of its valerate, has found wide application in topical dermatological therapy. Another betamethasone ester, the 17,21-dipropionate, exhibited still higher topical anti-inflammatory potency and was therefore introduced in dermatological therapy.

As in many inflammatory skin lesions an antibacterial effect is desirable together with the anti-inflammatory action of a glucocorticoid, betamethasone dipropionate (0·01%) has been combined with gentamicin (0·2%) in topical preparations.

Over various pathways, drug interactions may occur in the case of antimicrobial substances and glucocorticoids. On the first hand, direct chemical reactions (e.g. bacteriostatic antibiotics and surface active substances), competitive inhibition (e.g. steroid antibiotics and steroid hormones) and physiological antagonism (stimulation and depression of microbial metabolism) must be mentioned. Such physiological antagonism easily might occur with glucocorticoids, as these hormones enhance microbial metabolism and microbial growth when they are applied in low concentrations which are encountered in deeper skin layers. Glucocorticoids in high concentrations as they are used in topical applications directly exert an antimetabolic effect on microbes by themselves; here, an additive effect with antibiotics was encountered. It must be mentioned, however, that—as an exception of the rule—the metabolism of *Pseudomonas aeruginosa* is not susceptible to the antimetabolic effect of glucocorticoids. In comparison to betamethasone valerate, the dipropionate reveals a slightly more pronounced " activating " effect. This observation might be explained by the liberation of two molecules of propionate under the influence of bacterial esterases; these propionates were used as substrates for the bacterial metabolism (increase in oxygen consumption). The one molecule of valerate has less influence in this respect.

The influence of betamethasone dipropionate on gentamicin was investigated on bacteria under two metabolic conditions: resting bacteria as a model for saprophytes on the skin and bacteria in proliferation (log. phase) as a model for true pathogenic germs. In no instance could a decrease in antibacterial activity of gentamicin be demonstrated, although the glucocorticoid, by itself, enhanced bacterial metabolism in the concentration range used.

It was interesting to note that one bacterial strain (*Staph. albus*) which—according to common terms—was sensitive to gentamicin, failed to show such sensitivity under the conditions of resting phase. On the other hand one strain of *Pseudomonas aeruginosa* which had to be considered as insensitive to gentamicin exhibited decreased oxygen consumption in the proliferative phase when gentamicin (1 μg./ml.) was present. It should be mentioned in this connection

that the percentage of pseudomonas strains insensitive to gentamicin has not changed significantly since the introduction of gentamicin in Austria.

The evaluation of a glucocorticoid in the presence of an antibiotic seems more difficult than the evaluation of an antibiotic in the presence of a steroid ; the best way to probe topical glucocorticoid actions is still under discussion. So far, the vasoconstrictor assay seems to be the most reliable and valuable method. It has been demonstrated that there is an approximate correlation between the potency of glucocorticoids to produce vasoconstriction after epicutaneous application on normal skin and the relative clinical efficacy of a topical preparation of this steroid in skin lesions.

The experiments reported here were undertaken with low concentrations of betamethasone dipropionate (0·001% to 0·00001%) which, in this test system, were already at the borderline of effectiveness. Only the presence or absence of a vasoconstriction was read, without graduation. The data collected with betamethasone dipropionate alone corresponded roughly with the data of other investigators who tested betamethasone valerate. The solutions of betamethasone dipropionate with gentamicin in a twenty-fold concentration of the steroid were found as active as the solutions without the antibiotic. It must be mentioned, however, that these studies were performed with pure chemicals dissolved or suspended in ethanol and not with the preparation (emulsion of two phases) actually meant for dermatological therapy. Still, the absence of an interaction of gentamicin with the betamethasone dipropionate in regard to vasoconstrictor potency which depends on pharmacological activity and permeation permits the assumption that gentamicin and betamethasone dipropionate may safely be used together, without interactions of any kind.

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ADDENDUM

In the meanwhile, additional experiments were performed with two-phase media, mimicking the practical therapeutic conditions. In those experiments, no interaction could be detected, neither.