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In vitro interaction between HAPA-gentamicin B and penicillins

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Summary

The inactivation kinetics of a new aminoglycoside shortly to be introduced in clinical practice, HAPA-gentamicin-B, by 3 commonly used penicillins (ampicillin, carbenicillin and ticarcillin) were studied in vitro considering the influence of the HAPA-B-penicillin concentrations ratio on the kinetics of the process. In all cases the kinetic process was of pseudo-first order. The degree of inactivation of HAPA-B depends on the type of penicillin it is combined with and on the percentage of the penicillin compound in the sample. The percentage of inactivated HAPA-B was maximum with ampicillin and minimum with ticarcillin. Comparison of the results obtained in the present study with those found in the literature concerning other aminoglycosides shows that HAPA-B is less susceptible to inactivation by penicillins than most of the antibiotics of this group.

Introduction

Several studies have pointed to the efficacy of combinations of aminoglycoside antibiotics with penicillins employed in the treatment of certain kinds of patients such as immunodepressed persons, patients with cancer, diabetics, etc., with severe infections produced by Gram-negative organisms which are difficult to treat. In vitro (Harwick et al., 1973; Kurtz et al., 1981; White et al., 1979) and in vivo (Winters et al., 1971; Comber et al., 1977) studies have demonstrated the synergistic effect of these combinations against microorganisms such as *Ps. aeruginosa*, numerous species of *Enterobacter* and other aminoglycoside- and penicillin-resistant microbes. In contrast, other research findings have revealed the existence of a physicochemical interaction between penicillins and aminoglycosides with a loss of activity of both (Pickering and Gearhart, 1979), the degree of inactivation depending on numerous factors of which the type of penicillin associated and the ratio of aminoglycoside–penicillin concentrations in the sample are the most striking (Flournoy, 1979; Thompson et al., 1982).

HAPA-gentamicin-B (HAPA-B), the generic name corresponding to the form: 1-*N*-(*S*-3-amino-2-hydroxypropionyl)-gentamicin-B, (Neu and Fu, 1978) is a semisynthetic aminoglycoside shortly to be introduced in clinical practice. It is currently in the research phase; up to now no data have been offered concerning its possible inactivation by penicillins. The aim of the present work was to determine the kinetics of in vitro inactivation of

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this antibiotic by 3 semisynthetic penicillins—ampicillin, ticarcillin and carbenicillin—and to compare the results with those appearing in the literature reporting on other aminoglycoside antibiotics.

Materials and Methods

The interaction of HAPA-B was studied with 3 penicillins—carbenicillin, ticarcillin and ampicillin—considering the influence of time and of the HAPA-B penicillin concentrations ratio on the inactivation process.

1-HAPA-B interaction with penicillin

The basis solution used consisted of HAPA-B in Sorensen phosphate buffer (pH = 7.4) at concentrations of 10 μg/ml; 4 aliquots of this were taken and to each of them a given amount of carbenicillin was added such that the HAPA-B carbenicillin ratio in the aliquots was 1 : 20, 1 : 40, 1 : 80, and 1 : 100. In suitably stoppered tubes, these solutions were placed in a thermostated bath at 37 °C. At previously programmed times (10, 24, 34, 48, 58 and 72 h) aliquots of 0.5 ml were taken from each of the solutions; these were placed in tubes containing penicillinase in sufficient amounts to inactivate the penicillin. Once the β-lactam had been inactivated, the aminoglycoside antibiotic was evaluated and the percentage of HAPA-B inactivated by the penicillin was calculated for the different proportions and the different times studied. All experiments were carried out 3 times.

From the same 10 μg/ml solution of HAPA-B in Sorensen phosphate buffer a further 4 aliquots were taken; to these were added increasing amounts of ampicillin to obtain 4 solutions in which the HAPA-B penicillin concentration ratios were the same as described above. The above procedure was also applied to these solutions, calculating the percentage of HAPA-B inactivated by ampicillin for the selected times and proportions.

An identical procedure was followed to determine the HAPA-B interaction with ticarcillin, similarly calculating the percentage of inactivated HAPA-B at the above times and proportions.

Analytical technique

In all cases, the determination of HAPA-B concentrations was performed by a microbiological plate diffusion method using *Bacillus subtilis* (ATCC 6633) as the assay organism (Sabath, 1972).

Standard curves of HAPA-B were prepared in a concentration range of 0.5–20 μg/ml in Sorensen phosphate buffer at pH = 7.4. The sensitivity limit of the analytical technique was 0.2 μg/ml and the variation coefficient was < 5%.

The inactivation of HAPA-B by the different penicillins assayed followed pseudo-first order kinetics according to the statistical evaluation made from the correlation coefficients and from the degrees of freedom. The evolution of the concentrations of the aminoglycoside as function of time during the inactivation process fitted the following monoexponential equation:

$$C = C_0 \cdot e^{-K_i t}$$

where C is the concentration of HAPA-B at time t; C_0 is the initial concentration of HAPA-B in the reaction medium; K_i is the pseudo-first order inactivation constant and e is the base of Neperian logarithms. The fitting of the experimental results to the inactivation model proposed was performed by the use of non-linear regression methods. For statistical analysis of the results Student's t-test was used after studying the variance of the data showing a normal distribution.

Results and Discussion

Table 1 shows the inactivation constants of HAPA-B in the presence of the 3 penicillins studied for the different values of R considered. Ampicillin proved to be the drug with the greatest inactivating capacity followed by carbenicillin and then ticarcillin ($P < 0.1$). In all cases, the value of the inactivation constant K_i decreased with the increase in the value of R; that is, the degree of inactivation increased with the concentration of penicillin in the sample. This situation was found in all 3 cases but of note was the greater influence of the HAPA-B penicillin concentrations ratio for ticarcillin compared with the other two penicillins

TABLE 1

Influence of the type of penicillin associated and the ratio of aminoglycoside:penicillin concentrations on the inactivation constant

R	K_i of HAPA-B			Mean value $\pm \sigma_{n-1}$
By carbenicillin				
1:100	0.014	0.009	0.011	$0.0113 \pm 2.50 \cdot 10^{-3}$
1:80	0.012	0.009	0.010	$0.0103 \pm 1.52 \cdot 10^{-3}$
1:40	0.007	0.006	0.008	$0.0072 \pm 1.00 \cdot 10^{-3}$
1:20	0.004	0.004	0.005	$0.0043 \pm 0.57 \cdot 10^{-3}$
By ticarcillin				
1:100	0.018	0.019	0.010	$0.0186 \pm 0.57 \cdot 10^{-3}$
1:80	0.011	0.010	0.008	$0.0086 \pm 1.52 \cdot 10^{-3}$
1:40	0.004	0.004	0.002	$0.0033 \pm 1.15 \cdot 10^{-3}$
1:20	0.004	0.003	0.005	$0.004 \pm 1.00 \cdot 10^{-3}$
By ampicillin				
1:100	0.016	0.019	0.017	$0.0173 \pm 1.52 \cdot 10^{-3}$
1:80	0.014	0.021	0.016	$0.0170 \pm 3.60 \cdot 10^{-3}$
1:40	0.008	0.010	0.012	$0.0107 \pm 1.15 \cdot 10^{-3}$
1:20	0.005	0.005	0.010	$0.0066 \pm 2.88 \cdot 10^{-3}$

employed, since a variation in R from 1:100 to 1:80 led to a 50% reduction in the K_i value obtained for ticarcillin; for carbenicillin and ampicillin, this variation was 11.3% and 2% respectively.

Fig. 1 shows the inactivation kinetics of HAPA-B in the presence of increasing amounts of carbenicillin in buffered medium (pH = 7.4); it may be seen that the inactivation process of the aminoglycoside under these conditions follows pseudo-first order kinetics. Likewise, Figs. 2 and 3 show the inactivation kinetics of HAPA-B by ampicillin and ticarcillin. In both cases, kinetics are again pseudo-first order and depend on the aminoglycoside:penicillin concentration ratio, as

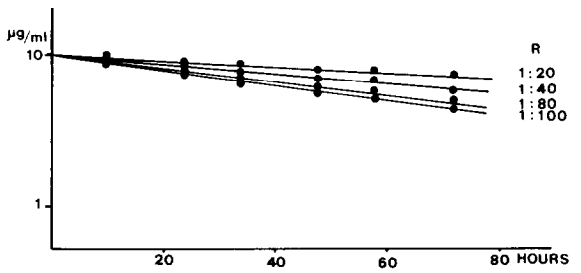

Fig. 1. Inactivation kinetics of HAPA-B by carbenicillin.

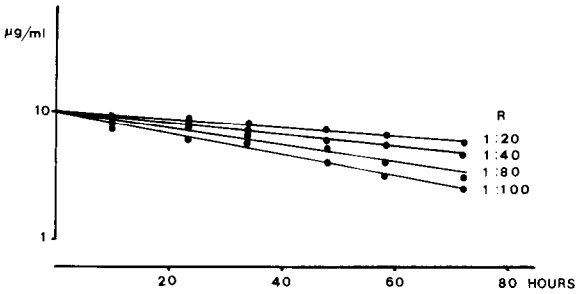

Fig. 2. Inactivation kinetics of HAPA-B by ampicillin.

was the case of the inactivation by carbenicillin.

These results show that HAPA-B is inactivated in the presence of penicillins like the rest of the antibiotics of this group; however, there are important quantitative differences when the results obtained for HAPA-B in this study are compared with those reported for other aminoglycoside antibiotics in similar studies. Up to now, the studies conducted (Blair et al., 1982; Farchione, 1981; Glew and Pavuk, 1983, among others) show that amikacin is the most stable antibiotic in the presence of penicillins. In a study carried out under similar experimental conditions to our own, Farchione (1981) found that amikacin is inactivated to a much smaller extent than the other aminoglycosides included in the same study (gentamicin, tobramycin). On comparing the results obtained by this author with these observed in our laboratory for HAPA-B, it may be concluded that this antibiotic has a stability in the presence of penicillins, which is equal to or greater than that of amikacin and much greater than that of the rest of the aminoglycosides included in that

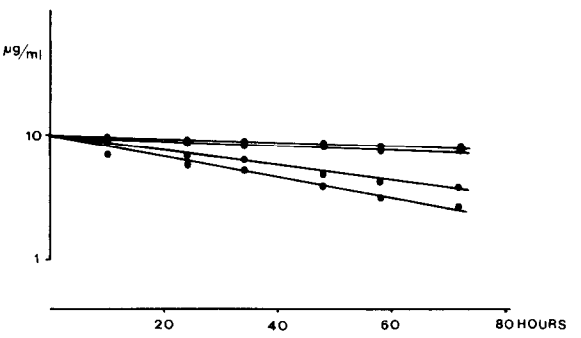

Fig. 3. Inactivation kinetics of HAPA-B by ticarcillin.

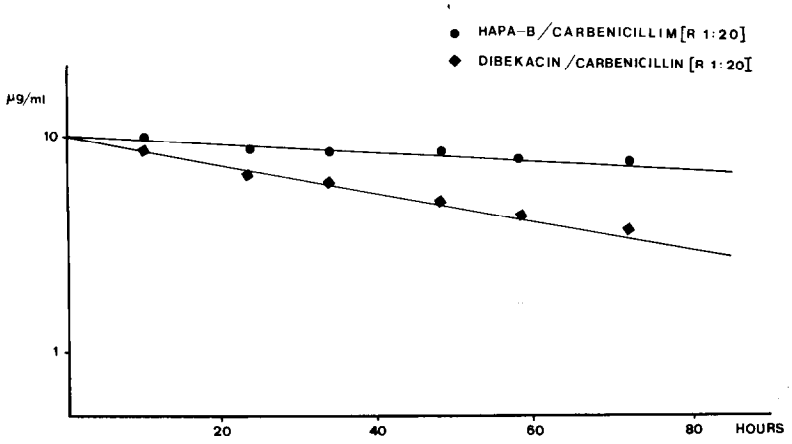

Fig. 4. Inactivation kinetics of HAPA-B and dibekacin by carbenicillin.

study. Similar results have been reported for the inactivation of these aminoglycosides with carbenicillin and ampicillin.

Fig. 4 shows the inactivation kinetics of HAPA-B compared with the findings obtained for dibekacin by Navarro et al. (1986) in the presence of carbenicillin when the concentration solution was 1 : 20. In this figure, a significant difference may be observed between the degree of inactivation of both aminoglycosides by the same penicillin. In view of such findings, HAPA-B may be said to be inactivated to a lesser extent than the rest of the aminoglycoside group and its stability in the presence of penicillins is similar to that of amikacin.

From the comparison of the results obtained with 3 semisynthetic penicillins it may be inferred that, for the same conditions, ampicillin has a greater inactivation capacity. These results contrast with those reported by other authors for several different aminoglycosides. In this sense, McLaughlin and Reeves (1971) and Pickering et al. (1979) found that in the case of gentamicin the highest degree of inactivation took place in the presence of carbenicillin; however, Navarro et al. (1986) showed that for dibekacin ticarcillin had the highest inactivation capacity in the same working conditions.

Table 2 shows the equations established for the 3 penicillins included in this study, defining the linear relationship between the inactivation constants and the penicillin concentrations (C) used in each experiment.

TABLE 2

Linear relationship established between the inactivation constants and the penicillin concentrations (C) used in the experiment

$K_i(h^{-1}) = 3.035 + 0.086\ C\ (\mu g/ml)$;	$r = 0.988$
$K_i(h^{-1}) = 1.775 + 0.177\ C\ (\mu g/ml)$;	$r = 0.917$
$K_i(h^{-1}) = 4.535 + 0.139\ C\ (\mu g/ml)$;	$r = 0.977$

These relationships allow us to deduce the inactivation constants of HAPA-B for different concentrations of each of the penicillins considered and to predict the fraction of aminoglycoside inactivated during the dosage regimen.

Taking into account that the dosage interval of the aminoglycoside antibiotics is usually 8 h and that the HAPA-B penicillin concentration ratio used in this study are representative of what is used in clinical practice, these results allow one to predict a 0.5% inactivation of HAPA-B by carbenicillin during a dosage interval for a concentration ratio of 1 : 100. In a clinical setting where the use of dosage intervals above 8 h is required, such as in the case of patients with renal impairment, the degree of inactivation of the aminoglycoside would presumably be greater, parallelling the increase occurring in the dosage interval. In any case, the low inactivation capacity of HAPA-B obtained in this study does not pre-

suppose the existence of a significant interaction of clinical transcendence between both antibiotics. Taking into account the frequent association in clinical practice of aminoglycosides with carbenicillin, the low degree of HAPA-B inactivation observed makes this drug an ideal candidate for use when this kind of combination is proposed.

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