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METHODS AND COMPOSITIONS FOR INDUCING RESISTANCE TO BACTERIAL INFECTIONS USING CERTAIN LYSINE DERIVATIVES

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ABSTRACT OF THE DISCLOSURE

A variety of substances are reported which alter host resistance to cocci and bacilli bacterial infections. Nevertheless, because of the extreme difficulty of total eradication, and the frequent reappearance of the same strains, even after their apparently successful elimination, there is a continuing need for drugs for the treatment of coccic infections. Certain lysine derivatives have been found effective in inducing resistance to infections due to cocci and bacilli.

BACKGROUND OF THE INVENTION

This invention pertains to antimicrobials. In a particular aspect this invention relates to antimicrobials effective in protecting against cocci and bacilli bacterial infections.

Bacteria such as cocci and bacilli are a unique group of organisms embodying within themselves an array of yet unanswered puzzles in biology, both fundamental and experimental. It is recongized that the significance of staphylococcal infections is not so much in severity, except in a few instances, as in the subleties of the infection due to the unpredictable vagaries of these organisms. The result is the disease continues to be a problem.

Treatment of staphylococcal diseases is complicated by the ability of the organisms to develop resistance. The magnitude of the problem is further amplified by the extreme difficulty of total eradication, and the frequent reappearance of the same strain even after apparently successful elimination. The inability to eliminate the carrier state by any of the currently known methods and the prevalence of the new antibiotic resistant hospital strains have added a new dimension to the frustrating situation.

Pencillin G (benzyl pencillin) is still the drug of choice for the treatment of infections caused by susceptible coccic strains. However numerous strains are known which elaborate an enzyme pencillinase in response to the drug and thus remain insensitive. This led to the development of semisynthetic pencillins which are not activated by pencillinase. Nevertheless recently, resistance of staphylococci to the newer penicillins has been reported. Hence there is a seemingly never ending demand for anticoccic agents.

A variety of substances are reported which alter host resistance to coccic infections. However, because of the ubiquitous nature of cocci and bacilli, and the diversification of their biological and bio chemical characteristics, there is a continuing need for drugs for the treatment of such infections. Thus the existence of multiple antibiotic-resistant strains of the organism suggests the desirability of investigating other drugs for combatting the infection. This invention provides an antimicrobial for the treatment of staphylococcic and bacillic infections.

SUMMARY OF THE INVENTION

In accordance with this invention it has been found that N^{α}-(ω-aminoacyl)-L-lysines afford desirable degrees of protection against cocci and bacilli infections. In fact these dipetides possess an anticococcic activity superior to that of their component omega-amino acids.

DETAILED DESCRIPTION OF THE INVENTION

The process of infection leading to disease is accepted to be a problem in the ecology of the parasite. It is being increasingly realized that the bacterial and host determinants are closely interrelated. Staphylococcal virulence derives from the combined action of several bacterial factors whose effectiveness is conditioned by the reactions of the host. Perhaps the most striking feature of host-parasite relationships in staphylococcal infections is the relatively atypical immunologic response. For this reason, additional antimicrobials are always in demand.

By the practice of this invention there is provided a method of protecting mammals against bacterial infections. In accordance with the invention an antibacterial amount of certain dipeptides of lysine is administered to mammals in need of an antimicrobial effective in protecting against cocci and bacilli. The dipeptides, administered for the inhibition of bacterial growth in vivo, are N^{α}-(ω-aminoacyl)-L-lysines having two to six carbon atoms in the omega aminoacyl group. Included are N^{α}-glycyl-L-lysine, N^{α}-(β-alanyl)-L-lysine, N^{α} - (γ - aminobutyryl)-L-lysine, N^{α}-(δ-aminovalyryl)-L-lysine, and N^{α}-(ϵ-aminocarproyl)-L-lysine. Whereas the dipeptide per se can be used, it can also be administered as an edible salt.

The antistaphylococcal activities of the N^{α}-(ω-aminoacyl)-L-lysines will be apparent from the following test results. Previously compounds such as homocarnosine were given mice subcutaneously for a period of 5 days before the infection, with an interval of 6 to 24 hours between the last drug injection and the organism challenge. However it was found that amino acids disappeared from the blood stream within 6 hours after their subcutaneous administration. Consequently in the technique employed herein a total of 5 mg. of each drug was given subcutaneously in equally divided doses 2 hours before and 4 hours after the injection of *Staphylococcus aureus.*

The strain of *S. aureus* used in the present investigations and termed "original" strain was isolated from an infected tonsil and has been maintained in our laboratory in the lyophilized state. It is pencillin-resistant, is highly chromogenic, ferments a number of sugars, including mannitol, mannose, maltose, lactose, galactose, glucose, and fructose, and produces coagulase, catalase, gelatinase, deoxyribonuclease, phosphatase, urease, and alpha-toxin.

Culture conditions were standardized, and the third subculture from the lyophilized mother culture was used. The subcultures were grown at 37° C. for 24 hours on Staphylococcus Medium 110 (Difco). The organisms from the third subculture were twice washed and suspended in TC Tyrode Solution (Difco), and the concentration was adjusted turbidimetrically, with a nephelometer, for injection into animals. The transmission levels on the scale of the instrument were taken as a reference of the density of the suspensions and were correlated with viable bacterial counts. Animals were inoculated subcutaneously with 0.5 ml. of a suspension having 70% transmission or 2×10^8 organisms by count. This dosage was approximately 1.5 times the LD_{50}.

Swiss albino female mice maintained on the Rockland diet, ranging in age from 8 to 10 weeks old and in weight from 20 to 25 gm., were used in all experiments. All mice were randomized for individual experiments. These mice were propagated in our laboratory from stock originally obtained from Texas Indred Mice Co., Houston, Tex.

The antistaphylococcal effects of the lysine dipeptides will now be given in tabular form, the mean values and upper and lower limits being those necessary to obtain a siginficance level of 95 percent, the data being based on a frequency distribution. In the table Gly-Lys, β-Ala-Lys, etc. have been employed as shortened form for

N^{α}-glycyl-L-lysine, N^{α}-(β-alanyl)-L-lysine, etc. Percent protection is (mortality control —mortality treated)×100/(mortality control) on the fourth day after infection with *S. aureus.*

Substance	Number of animals	Percent mortality at— Hours Post Challenge 24	48	72	96	Mean	Higher limit	Lower limit	Percent protection mean
Control	40	60	75	88	88	88	94	76	0
Lys	40	45	50	53	53	53	65	37	40
Gly-lys	39	28	41	41	41	41	53	29	53
β-Ala-lys	38	26	34	34	37	37	51	25	58
γ-Abu-lys	38	29	40	45	47	47	60	35	47
δ-Avl-lys	39	21	36	36	36	36	49	25	59
ϵ-Acp-lys	40	30	33	38	38	38	51	26	57

The desirable antistaphylococcal activities of the lysine dipeptides described herein are apparent from the table. Moreover, as can be seen from the following all of the dipeptides were more potent than their component omega-amino acids.

COMPARISON OF DIPEPTIDES WITH AMINO ACIDS

Compound:	Percent protection on 4th day mean
Glycine	12
Gly-Lys	53
Beta-alanine	12
β-Ala-Lys	58
Gaba	32
γ-Abu-Lys	47
Dava	51
δ-Avl-Lys	59
Eaca	33
ϵ-Acp-Lys	57

Gaba, gamma-aminobutyric acid; Dava, delta-aminovaleric acid; Eaca, epsilon-aminocaproic acid.

The dipeptides which are employed herein were prepared as follows, temperatures, where given, being degrees centigrade:

EXAMPLE (a)

N^{ϵ}-carbobenzoxy-L-lysine ethyl ester p-toluenesulfonate

In a flask placed in an oil bath and equipped with a water cooled condensing and collecting system containing an azeotropic mixture of water, ethanol, and carbon tetrachloride a mixture of 14.5 gm. of N^{ϵ}-carbobenzoxy-L-lysine, 10.5 gm. of p-toluenesulfonic acid monohydrate, 40 ml. of ethanol, and 200 ml. of carbon tetrachloride was boiled for 12 hrs. until a clear solution was obtained. The azeotropic mixture was approximately 13 ml. after 24 hrs. of continuous reaction. To the syrup, after concentrating the reaction mixture, was added 100 ml. of ether and 200 ml. of petroleum ether. Immediately N^{ϵ}-carbobenzoxy-L-lysine ethyl ester p-toluenesulfonate crystals were formed. After the recrystallization from hot acetone-ether, 24.0 gm. (approximately 100% yield) of product was obtained: M.P., 88–89°; $[\alpha]_D^{25}=+3.0°$, c. 2, H_2O. The product included a small amount of unreacted N^{ϵ}-carbobenzoxy-L-lysine, confirmed by thin layer chromatography.

EXAMPLE (a)

N^{α}-(δ-aminovaleryl)-L-lysine

N^{δ}-carbobenzoxy-δ-aminovaleric acid was prepared by the known reaction with benzylchloroformate using NaOH and a low temperature. To a solution of 6.3 gm. of N^{δ}-carbobenzoxy-δ-aminovaleric acid in 125 ml. of methylenechloride was added 3.5 ml. of triethylamine. After the resulting solution had been chilled to —5°, 2.4 ml. of ethylchloroformate was added and the mixture was kept at —5° for 10 min. To this product was added rapidly a solution of N^{ϵ}-carbobenzoxy-L-lysine ethyl ester p-toluenesulfonate prepared by the addition of 10 ml. of triethylamine to a solution of 12.0 gm. of N^{ϵ}-carbobenzoxy-L-lysine ethyl ester p-toluenesulfonate in 125 ml. of methylenechloride which had been chilled at 0°. The resulting mixture was stored at 25° for 2 days. It was then washed with 200 ml. of water and 200 ml. of 1 N $NaHCO_3$, dried over Na_2SO_4, and concentrated to a syrupy consistency. This product was dissolved in 50 ml. of ethanol and then 50 ml. of 1 N NaOH was added. After 3 hrs. at 25° the solution was adjusted to pH 5.0 with 2 N H_2SO_4 and concentrated to dryness in vacuo. The dried residue was extracted with two portions of 50 ml. each of hot ethanol, followed by 50 ml. of water. After the addition of 0.5 gm. of 10% palladium charcoal, the mixture was hydrogenated in an apparatus with an outlet for excess hydrogen gas. The formation of CO_2 gas was chemically checked occasionally. After 8 hrs. the evolution of CO_2 had ceased. The solution was filtered and then concentrated in vacuo. The remaining syrup was dissolved in 20 ml. of water and 2 N HOl was added to make the solution more acid than pH 5.0. For the separation on Amberlite CG–120 resin (which had been equilibrated with 2 N NH_4OH and washed with water), water, 0.1 M NH_4OH, and 0.3 M NH_4OH effluent solution were used. The product was purified by ion-exchange chromatography. The pure N^{α}-(δ-aminovaleryl)-L-lysine fractions, No. 115–170, were pooled and concentrated in vacuo. The syrupy residue was treated with 1 N HOl to adjust the pH to approximately 5.0, and upon the addition of ethanol, fine white crystals formed: Yield, 83.5%; M.P. 183–184° (dec.); $[\alpha]_D^{25}=+0.00$, c. 1, H_2O.

Analysis.—Calcd. for $C_{11}H_{23}N_3O_3 \cdot 2HCl$ (percent): C, 41.50; H, 7.92; N, 13.21. Found (percent): C, 43.84; H, 8.54; N, 12.21 (analyzed by Crobaugh Labs.).

EXAMPLE (b)

N^{α}-(ϵ-aminocaproyl-L-lysine

N^{ϵ}-carbobenzoxy-ϵ-aminocaproic acid (6.7 gm.) and 12.0 gm. of N^{ϵ}-carbobenzoxy-L-lysine ethyl ester p-toluenesulfonate were used as the starting materials. The conditions for the reaction were the same as previously described. The product was purified by ion-exchange chromatography. The pure N^{α}-(ϵ-aminocaproyl)-L-lysine fractions, No. 135–185, were pooled and concentrated in vacuo. The dry residue was crystallized from hot water-ethanol to form fine white crystals: Yield, 92.7%; M.P., 162–163°; $[\alpha]_D^{25}=1.0$, c. 2, H_2O.

Analysis.—Calcd. for $C_{12}H_{25}N_3O_3$ (percent): C, 55.56; H, 9.72; N, 16.21. Found (percent): C, 55.12: H, 9.68; N, 15.78 (analyzed by Crobaugh Labs.).

EXAMPLE (c)

To prepare N^{α}-glycyl-L-lysine a mixture of 5.1 gm. of N^{α}-carbobenzoxyglycine and 12.0 gm. of N^{ϵ}-carbobenzoxyl-L-lysine ethyl ester p-toluenesulfonate was used as the starting material. The conditions were the same as described before. The pure fractions of N^{α}-glycyl-L-lysine, No. 35–55, were pooled and concentrated in vacuo. The dry residue was crystallized from hot water-ethanol to form fine white crystals: Yield, 62.0%; M.P. 193–194°.

Analysis.—Calcd. for $C_8H_{17}N_3O_3$ (percent): C, 47.26; H, 8.43; N, 20.68. Found (percent): C, 47.52; H, 8.23; N, 20.21 (analyzed by Crobaugh Labs.).

EXAMPLE (d)

To prepare N^{α}-(β-alanyl)-L-lysine a mixture of 5.5 gm. of N^{β}-carbobenzoxy-beta-alanine and 12.0 gm. of N-car-