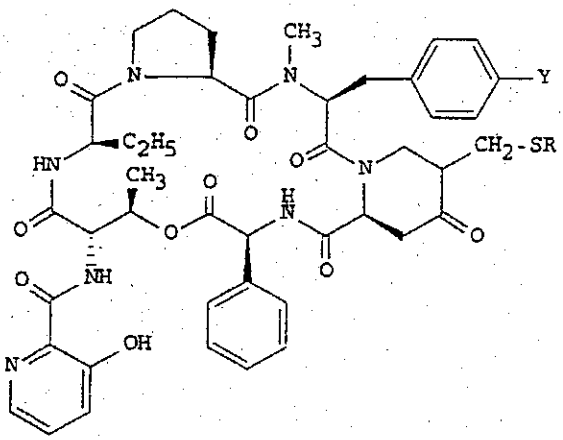
COMBINATIONS OF DALFOPRISTINE/QUINUPRISTINE WITH CEFPIROME

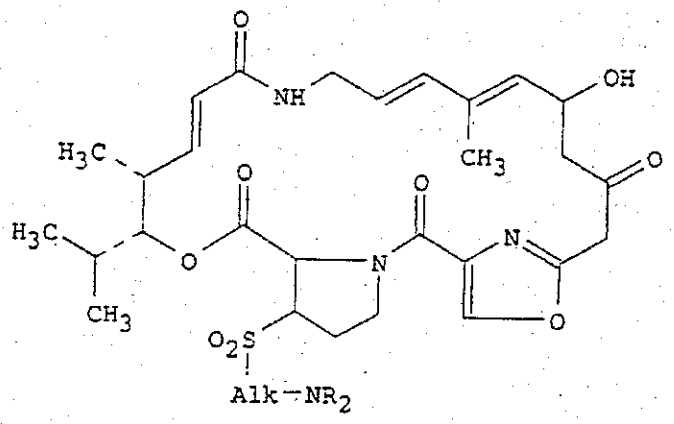
The present invention relates to combinations of quinupristine and dalfopristine with cefpirome which manifest a synergistic action which is both bacteriostatic and bactericidal.

The present invention also relates to injectable pharmaceutical compositions for the parenteral administration of quinupristine and dalfopristine, combined with cefpirome, and to kits for presenting the active principles which are the subject of the combination according to the invention.

European Patent EP 248,703 describes derivatives of group B streptogramins of general formula:

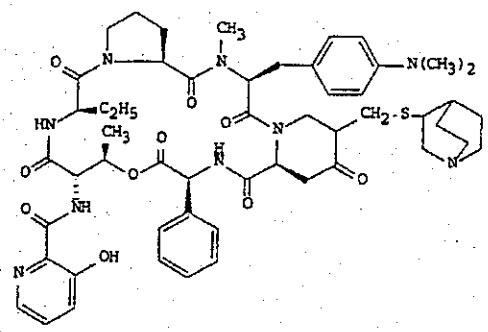


and their combinations with derivatives of group A streptogramins of structure:

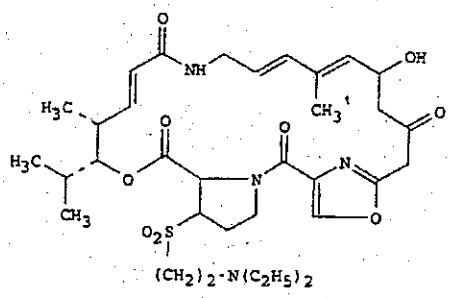


The derivatives of group A streptogramins are described in more detail in European Patent EP 191,662.

Quinupristine, which is a derivative of pristinamycin I (group B streptogramin), and dalfopristine, which is a derivative of pristinamycin II (group A streptogramin), are the constituents of Synercid®:



quinupristine



dalfopristine

Synercid® (quinupristine/dalfopristine) is an injectable 30/70 combination which is effective on most gram-positive microorganisms, in particular meticillin-resistant *Staphylococcus aureus* (MRSA), Macrolide-

Lincosamide-Streptogramin B-sensitive (MLS_B) *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* (VREF). Its antibacterial activity, in particular on vancomycin-resistant microorganisms, is cited in many publications [The Annals of Pharmacotherapy, 29, 1022-1026 (1995); Microbial Drug resistance, 1, 223-234 (1995); Antimicrobial Agents Chemother., 39, 1419-1424 (1995), etc.]. However, Synercid loses its bactericidal activity on staphylococci which are constitutively resistant to antibiotics of the Macrolide-Lincosamide-Streptogramin B group (C-MLS_B resistant).

International Application WO 98/22107 describes the preparation of stabilized pharmaceutical compositions comprising the quinupristine/dalfopristine combination, in the form of salt obtained by adding methanesulfonic acid or hydrochloric acid in amounts which are at least stoichiometric and at a pH included in the range [3.5; 5].

In the clinical environment, certain bacteria (C-MLS_B-resistant MRSA for example) may compromise the effectiveness of the quinupristine/dalfopristine combination if appropriate concentrations of dalfopristine are not present at the site of infection. One of the means for resolving the problem has been the use of an increasing number of quinupristine/dalfopristine doses in a 24 hour period, or to use a system of continuous infusion.

Cefpirome: (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetoamido]-3-(1-pyridinomethyl)-3-cephem-4-carboxylic acid sulfate [Martindale, 32nd edition, Kathleen Parfitt Ed.], is a ß-lactam of the class of 4th generation cephalosporins which is blessed with good activity on meticillin-resistant *Staphylococcus aureus* (MRSA) strains, in comparison with other products of this class. It is conventionally administered parenterally by i.v. injection in 3 to 5 minutes, or by infusion over a period of 20 to 30 minutes, at cefpirome sulfate doses of 1 to 2 g per 12 hours depending on the severity of the infection. It is particularly indicated in the treatment of urinary tract and respiratory tract infections and skin infections, and also in the treatment of septicemias and of infections in immunodepressed individuals.

It has now been found, which is the subject of the present invention, that the combinations of quinupristine/dalfopristine with cefpirome can have a particular advantage in the treatment of infections which are very difficult to treat or which put the life of the patient at risk, and which require rapid and effective bactericidal activity, as can be the case of combinations manifesting synergistic action against bacteria.

In one aspect of the present invention, there is provided combination with a synergistic action, characterized in that it consists of a combination of quinupristine/dalfopristine with cefpirome.

In another aspect of the present invention, there is provided injectable pharmaceutical compositions for the parenteral administration of quinupristine and dalfopristine, combined with cefpirome.

In yet another aspect of the present invention, there is provided presentation kit for the quinupristine/dalfopristine combination with cefpirome.

The synergism produces a much higher potency, and can, for example, make it possible to decrease the quinupristine/dalfopristine or cefpirome concentration,

or to increase the time between administration of the doses required to inhibit or eradicate said bacterium (more particularly with respect to multiresistant staphylococci such as, in particular, the meticillin-resistant strains). The synergism can also make it possible to treat infections on which each of the active principles, administered in monotherapy, would not have been effective.

Synercid® (quinupristine/dalfopristine- hereafter Q-D) and cefpirome were tested separately in rats, on experimental endocardites due to C-MLS_B-resistant MRSA strains.

None of the products showed any effectiveness in vivo in monotherapy on the microorganisms studied. Conversely, the quinupristine/dalfopristine combination with cefpirome is effective in more than 90% of the rats treated.

The quinupristine/dalfopristine combination with cefpirome is particularly advantageous due to the fact that it is set to become also a treatment of choice for patients requiring a simultaneous treatment for gram-positive and gram-negative microorganisms.

EXPERIMENTAL DETERMINATION

Materials and methods

The studies carried out in vitro, and in vivo in animals, are described below.

The microorganisms tested are 2 clinical isolates of C-MLS_B-resistant MRSA: strains AW7 and P8,

previously characterized in vitro, and in vivo in rat experimental endocarditis.

The study in vitro and the determination of the MICs (minimum inhibitory concentrations) is carried out using cultures on agar plates [Antimicrob. Agents Chemother., 39, 1419-1424 (1995)].

For the experimental endocarditis study, Wistar female rats weighing 200 g with aortic vegetation [produced according to the method described by Heraief et al., Infect Immunol., 37, 127-31 (1982)] were infected with an inoculum of 10^5 CFU/ml of the organism to be tested, which corresponds to 10 times the minimum inoculum to produce endocarditis in at least 90% of the untreated controls.

The treatment is started 12 hours after the bacterial infection; it is continued for 5 days. The antibiotics are administered by means of a permanent venous catheter with a programable pump (pump 44; Harvard Apparatus Inc.) according to the method described in Antimicrobial Agents Chemother., 39, 1419-1424 (1995). The treatments carried out simulate kinetics in humans, which are produced by intravenous administration of 7 mg/kg of quinupristine/dalfopristine every 12 hours, or of 2 g of cefpirome via the i.v. route every 12 hours. The relatively low dose of quinupristine/dalfopristine was chosen so as to demonstrate, where appropriate, a synergism with the cefpirome.

Prior to the treatment experiments, the setting up of the simulation of the human kinetics of the quinupristine/dalfopristine combination with cefpirome, in rats, is carried out on animals which are catheterized but not infected. In these preliminary experiments, each point of the pharmacokinetics curve is the mean (± standard deviation) of the concentrations measured in 6 different rats. The concentrations of product in the serum are measured in a bioassay (using *Micrococcus luteus* for quinupristine/dalfopristine, and *Bacillus subtilis* ATCC 6633 for cefpirome).

The concentrations of quinupristine/dalfopristine in the serum of the rats are 5 mg/l, 1 hour after the start of treatment, and gradually decrease to undetectable concentrations after 6 hours. The concentrations of cefpirome are 160.6 ± 19.7 mg/l, 5 minutes after the start of treatment, and gradually decrease to 2.2 ± 1.4 mg/l after 12 hours. These values are in accordance with the therapeutic levels observed in humans for these active principles. In addition, during each treatment experiment, these concentrations are controlled in 3 rats taken randomly, for each reference time of the pharmacokinetics.

The control rats are sacrificed at the start of the treatment, and the treated rats are sacrificed 24 hours after the final antibiotic administration. The bacterial density in the vegetations is determined

after dissecting the vegetations under sterile conditions, weighing, homogenizing in 1 ml of saline solution, and then diluting the solution (series of dilutions) before placing it onto plates for counting of the colonies. The number of colonies growing on the plates is determined after 48 hours of incubation at 35°C. The bacterial densities in the vegetations are expressed as $\log_{10}$ CFU/g of tissue. The minimum detection level is ≥ 2 $\log_{10}$ CFU/g of vegetation. The possibility of an emergence of resistance is also verified. The mean bacterial densities in the vegetations of the various groups are compared by the Kruskal-Wallis method complemented with the Dunn method. The differences were considered to be significant when P is ≤ 0.05.

Results

The minimum inhibitory concentrations (MICs) of quinupristine/dalfopristine on the MRSA strains AW7 and P8 are 0.5 mg/l for each of the strains, and the MICs for cefpirome are, respectively, 4 mg/l on AW7 and 2 mg/l on P8.

Bacterial populations of the MRSA strains AW7 and P8 can grow on plates containing up to 60 mg/l of cefpirome. It is observed, after addition to the plates of subinhibitory concentrations (¼MIC - noneffective concentration) of the [lacuna] cannot grow on plates containing concentrations ≥ 15 mg/l of cefpirome.

Figure I, attached below, illustrates this synergistic action.

The study in vivo in rat experimental endocarditis also made it possible to demonstrate a synergism.

Figure II, attached below, shows that neither quinupristine/dalfopristine, nor cefpirome in monotherapy are significantly effective on the aortic endocarditis due to the MRSA strains AW7 and P8 ($P > 0.05$); conversely, the combination of quinupristine/dalfopristine with cefpirome was shown to be significantly effective ($P < 0.05$) on each of them (log_{10} CFU/g of vegetation ≤ 2 in more than 80% of the cases), as also shown in the tables below.

	MRSA AW7			
	Control	Cefpirome	Synercid	Synercid/ cefpirome
log_{10} CFU/g of vegetation	from 7.2 to 8.6 in 100% of the cases	> 8.7 in # 38% of the cases approximately 3.5 in 25% of the cases, and ≤ 2 in 25% of the cases	> 7.2 in 100% of the cases of which ≥ 9 in # 70% of the cases	≤ 2 in 80% of the cases, and 3.5 to 4.6 in 20% of the cases

	MRSA P8			
	Control	Cefpirome	Synercid	Synercid/ cefpirome
log_{10} CFU/g of vegetation	> 7.5 in 100% of the cases	8.7 to 9 in # 22% of the cases 5.4 to 6.3 in # 22% of the cases, and ≤ 2 (not significant) in # 55% of the cases	> 8.4 in 100% of the cases	≤ 2 in # 80% of the cases, and 3.6 to 4.4 in # 18% of the cases

As a result, treatment with such a combination on the one hand makes it possible to attain an effectiveness which was not obtained in monotherapy and/or, on the other hand, allows the use of lower doses of the products combined or the choice of longer intervals between administrations, with a greater effectiveness (bacteriostatic and bactericidal) than that which was attained in monotherapy. In particular in the case of severe infections (such as, for example, endocarditis), the synergism makes it possible to use the cefpirome at usual doses, whereas in the absence of the synergism-producing combination, it would have been necessary, in order to obtain effectiveness, to increase the doses to such a level that cefpirome could not have been used.

The doses of active principle (Synercid®: quinupristine + dalfopristine combination) administered to patients are between 5 and 15 mg/kg every 12 hours, but are in general, and preferably, 5 to 7.5 mg/kg every 8 hours.

The dose of cefpirome conventionally administered to patients is 2 to 4 g per day in 2 infusions or in 2 direct intravenous injections, or 1 to 2 g every 12 hours depending on the severity of the infection.

In the combinations above, the quinupristine/dalfopristine combinations with cefpirome can be advantageously used, for treating patients, as a continuous injection or in several administrations. The doses chosen depend on the bacterial infection to be treated and on the synergistic effect desired.

When synergism makes it possible, due to the combination, to obtain an effect on microorganisms which could not have been treated in monotherapy with one or other of the active principles, the doses combined can be chosen within the range of doses conventionally used in monotherapy or, where appropriate, can be lower doses depending on the nature of the infection; namely, daily doses of quinupristine/dalfopristine within the range 15 to 30 or 60 mg/kg, or within the range 10 to 30 or 60 mg/kg, in fractionated doses or as a continuous injection, and

daily doses of cefpirome of 2 to 4 g in 2 infusions or in 2 direct intravenous injections.

When synergism makes it possible, due to the combination, to treat bacterial infections at lower doses of quinupristine/dalfopristine and/or of cefpirome, thus with fewer risks of side effects (for example in the case of microorganisms showing less resistance), the doses of quinupristine/dalfopristine and of cefpirome combined can be chosen within a range which is smaller with respect to the maximum usable dose in monotherapy; namely, doses of quinupristine/dalfopristine of between 10 and 22.5 to 30 mg/kg maximum in fractionated doses or as a continuous injection, and doses of cefpirome of between 2 and 4 g in 2 infusions or in 2 direct intravenous injections.

It is understood that the combinations can include several types of administration, such as simultaneous coadministration, successive administration at delayed times or administration via multilumen (multichannel) catheters.

For treatment, the quinupristine/dalfopristine formulations are in liquid, lyophilized or frozen form.

The lyophilized compositions can be taken up, at the time of use, in water for injection (wfi) or in any other compatible injectable medium, in particular in glucose medium (aqueous solution of 5% glucose for

example), or, without any limitation being implied, in dextran solutions, polyvinylpyrrolidone solutions, or polysorbate 80 solutions. According to one preferred embodiment of the invention, the formulations are redissolved by intermediate passage via a concentrated solution (of 50 to 250 mg/ml, preferably approximately 100 mg/ml) hereafter called "concentrate"; this solution is diluted at the time of use in a medium for injection as described above, for administration in the form of infusion; it is also possible to take the lyophilizate up in wfi, and then to dilute the concentrate thus obtained in the chosen injection medium.

The pharmaceutical compositions can be frozen from the solutions initially prepared (5 to 250 mg/ml) or from prediluted solutions (for the preparation of frozen bags for example). They are thawed at the time of use, and then diluted if necessary. The solutions provided in liquid form contain 5 to 250 mg/ml of active principle. They are diluted at the time of use, to concentrations of between 0.5 and 10 mg/ml.

According to the invention, the quinupristine/dalfopristine formulations, which are optionally in concentrated solution form or diluted solution form, can be combined, for coadministration, with a solution of cefpirome at the time of injection. The combination can be carried out in the form of 2 infusion bags, one containing the quinupristine/

dalfopristine in its injection medium and the other containing the solution of cefpirome, or by using 2 syringes, one containing quinupristine/dalfopristine and the other containing the solution of cefpirome, or alternatively, the combination can be carried out by using one of the active principles in an infusion bag and the other in a syringe.

It is understood that the presentation as kits for the formulation of quinupristine/dalfopristine and of cefpirome also falls within the context of the present invention.

Presentation kits of any form can be suitable, in particular, for example, presentations in the form of a twin-bottle, presentations in the form of infusion bags containing the active principles, and presentations in the form of an infusion bag containing one of the active principles and of a bottle or of a vial containing the other active principle, of one or more bottles comprising one of the active principles (for example comprising the quinupristine/dalfopristine lyophilizate) and a bottle or vial containing the other active principle. Devices such as a double-compartment syringe may also prove to be particularly suitable.

It is understood that the present invention can also apply to other derivatives of pristinamycin, such as for example to the derivatives described in European Patents EP 133,097, EP 135,410, EP 191,662, EP 248,703, WO 99/43699 and WO 99/05165.

CLAIMS

1. Combination with a synergistic action, characterized in that it consists of a combination of quinupristine/dalfopristine with cefpirome.

2. Injectable pharmaceutical compositions for the parenteral administration of quinupristine and dalfopristine, combined with cefpirome.

3. Injectable pharmaceutical compositions according to Claim 2, characterized in that they are intended for intravenous administration.

4. Combinations according to Claim 1, characterized in that quinupristine/dalfopristine and cefpirome are administered simultaneously or successively.

5. Presentation kit for the quinupristine/dalfopristine combination with cefpirome.

6. Presentation kit according to Claim 5, characterized in that it is intended for parenteral administration.

7. Presentation kit according to Claim 6, characterized in that it is a twin-bottle, infusion bags containing the active principles, an infusion bag containing one of the active principles and a bottle or vial containing the other active principle, one or more bottles containing one of the active principles and a bottle or vial containing the other, or alternatively a double-compartment syringe.