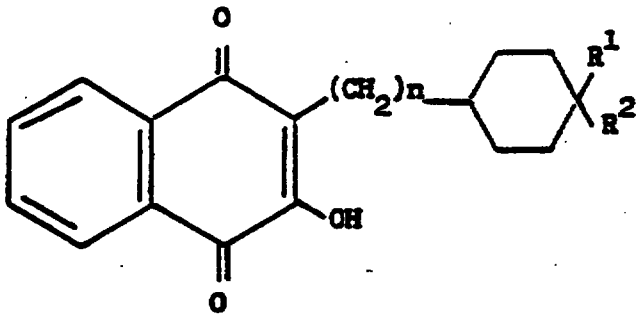
9604546-3
IPOS copy

ATOVAQUONE PHARMACEUTICAL COMPOSITIONS

The present invention relates to microfluidised particles of 2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone and to a method for preparing them. More particularly the invention is concerned with a pharmaceutical composition containing microfluidised particles of 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone ("atovaquone") and its use in therapy.

Atovaquone has previously been disclosed, for example in European Patent No. 0123238 and US Patent No. 5053432 (incorporated herein by reference) which relates to 2-substituted-3-hydroxy-1,4-naphthoquinones of formula (I):



(I)

wherein either R^1 is hydrogen and R^2 is selected from C_{1-6}alkoxy, aralkoxy, C_{1-6} alkyl-C_{1-6}alkoxy, phenyl substituted by one or two groups selected from halogen and C_{1-6}alkyl, halogen and perhalo-C_{1-6}alkyl or R^1 and R^2 are both C_{1-6}alkyl or phenyl, and n is zero or 1, and physiologically acceptable salts thereof. The compounds are said to have antiprotozoal activity. Specifically, compounds of formula (I) wherein n is zero are said to be active against the human malaria parasite Plasmodium falciparum and also against Eimeria species such as E.tenella and E.acervulina, which are causative organisms of coccidiosis and compounds of formula (I) where n is 1 are said to be active against protozoa of the genus Theileria, in particular T.annulata or T.parva. Amongst the compounds specifically named and exemplified is the compound of formula (I) wherein n is zero, R^1 is hydrogen and R^2 is 4-chlorophenyl, i.e. atovaquone.

EP 0362996 discloses the use of atovaquone in the treatment and/or prophylaxis of Pneumocystis carinii pneumonia.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

Further uses of atovaquone against Toxoplasmosis and Cryptosporidiosis are disclosed in European patent application nos. 0445141 and 0496729 respectively.

The efficacy of atovaquone as a therapeutic agent is limited by its bioavailability. Accordingly it is an object of the present invention to provide atovaquone in a more bioavailable form.

It has now been found that the bioavailability of atovaquone can be increased by ensuring that the particle size is within a certain defined range of small particles. However, conventional methods of reducing the particle size of atovaquone were found to be unsuccessful in producing particles of the size required to improve bioavailability.

The Microfluidiser has been marketed by the Microfluidics Corporation since 1985. The principle of its operation is based on a submerged jet technology. It was designed, primarily, as a homogenizing device for use in the food and pharmaceutical industries, for the preparation of e.g. emulsion and liposomal systems and has subsequently been used for cell-rupture purposes in biotechnology applications.

It has now surprisingly been found that microfluidised particles of atovaquone produced using a Microfluidiser have improved bioavailability of the compound. It is believed that this is due to the small size and narrow range of sizes of the microfluidised atovaquone particles.

During operation of the Microfluidiser, the feed stream is pumped into a specially designed chamber, in which fluid streams interact at very high velocities and pressures. Fixed microchannels within the interaction chamber provide an extremely focussed interaction zone of intense turbulence, causing the release of energy amid cavitation and shear forces. Without wishing to be bound by theory it is believed that since all product passes through a dimensionally fixed area of energy release, greater size uniformity and smaller sizes are achieved by using the Microfluidiser rather than conventional methods for producing fine particles.

Thus, in a first aspect, the present invention provides small particles of atovaquone. Preferably the particles are microfluidised particles. Suitably at least 90% of the

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

particles have a volume diameter in the range of 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0.1-2μm.

In a second aspect, the present invention provides a pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1-2μm. Preferably the particles are microfluidised particles.

The carriers must be acceptable in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof.

According to a third aspect, the present invention provides a method for the preparation of microfluidised particles of atovaquone which comprises mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser in order to provide the atovaquone in the form of particles wherein at least 90% of the particles have a volume diameter in the range 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0-1-2μm.

In a further aspect the present invention provides a method for the preparation of a pharmaceutical composition comprising the steps of:-

a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL.

b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range 0.1-2μm.

c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

Suitably, the mixture is subjected to 10-50 passes through the Microfluidiser, e.g. 25-30 passes. Preferably the mixture is subjected to 15-25 passes through the Microfluidiser.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

In one embodiment, the liquid vehicle is a surfactant. Preferably, the liquid vehicle is a surfactant solution. In a particularly preferred embodiment the surfactant is Poloxamer 188 solution. In another preferred embodiment the pharmaceutically acceptable carriers include a suspending agent. Suitable suspending agents include methyl cellulose and xanthan gum. Preferably the suspending agent is xanthan gum.

Pharmaceutical formulations include those suitable for oral and parenteral (including subcutaneous, intradermal, intramuscular and intravenous) administration as well as administration by naso-gastric tube. Suitable formulations within the scope of the present invention include, for example, solid dosage forms such as tablets and liquid dosage forms, such as suspensions, which are preferred formulations. The formulation may, where appropriate, be conveniently presented in discrete dosage units and may be prepared from the microfluidised particles using methods known in the art of pharmacy.

Tests to measure the bioavailability of atovaquone in vivo indicate that formulations of microfluidised atovaquone have improved bioavailability compared to prior art formulations. The invention therefore provides, in a further aspect, formulations comprising microfluidised atovaquone for use in therapy, in particular in the treatment and prophylaxis of protozoal parasitic infections, e.g. malaria and toxoplasmosis, and infections caused by P.carinii.

The invention will now be further illustrated by the following non-limiting examples:-

Example 1

Preparation of Microfluidised particles of atovaquone

Atovaquone was prepared by methods according to the prior art, e.g. US patent no. 5053432 (incorporated herein by reference). 600mL of a mixture consisting of 2.5% w/v atovaquone in 0.25% w/v aqueous Celacol M2500 was prepared and 100mL were retained in a glass jar as a control. A laboratory scale model 120B Microfluidiser was connected to a 90 psi pneumatic supply and adjusted to produce a fluid pressure of 15000 psi. The machine base, interaction chamber and pipework of the Microfluidiser

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

were immersed in a bath of cold water. 500mL of the mixture were loaded into the Microfluidiser's bulk vessel and passed through the Microfluidiser interaction chamber before being returned to the top, and side, of the bulk chamber. The mixture was recirculated continuously through the interaction chamber, and samples were taken at 10, 20, 30, 45 and 60 minutes. The number of passes to which each of these samples had been subjected was calculated and is shown in Table 1 below.

TABLE 1

Sample	Microfluidisation time (minutes)	Sample Volume (ml)	Number of passes
Control	0	100	0
1	10	105	8
2	20	105	9-19
3	30	110	31-35
4	45	105	65-77
5	60	35	142-244

Microscopic observations of the control and samples at 40x magnification were made and the results were as follows:-

Control - Varied shapes, plates, rods and spheroids, around 5x5μm generally and up to 7.5x10μm, loosely aggregated.

Sample 1 - More rounded smaller shapes, some "large" crystals, lots of small fragments 2.5x2.5μm, more dispersed.

Sample 2 - More rounded, smaller shapes, more fragments.

Sample 3 - Still more rounded, smaller shapes, more fragments.

Sample 4 - Yet more rounded, smaller shapes, more fragments.

THIS SHEET HAS BEEN REPLACED

Sample 5 - Very small particles, all under 2.5μm, all rounded, monodisperse.

Example 2

Pharmaceutical Formulation

An oral suspension formulation was prepared by mixing the following ingredients:-

Microfluidised particles of atovaquone		150.0mg
Poloxamer 188		5.0mg
Benzyl alcohol		10.0mg
Xanthan gum		7.5mg
Purified water	to make	1.0mL

Example 3

Biological Test

Nine healthy fasted male volunteers received single doses of 5mg/mL suspensions containing 250mg atovaquone as a 3μm mean particle size suspension and 1μm Microfluidised suspension in a randomised crossover study. Plasma samples were taken at intervals up to two weeks after the last dose and assayed by HPLC. The results are given in table 2 below:

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

TABLE 2

	3μm suspension	1μm suspension
mean(SD)AUC	95 (62)μg/mL.h	247(85)μg/mL.h
mean(SD)C_{max}	1.2(0.7)μg/mL	5.0(1.6)μg/mL
median T_{max}	5 hours	1 hour

The mean (95% CI) increase for the AUC of the 1μm suspension relative to the 3μm suspension was 2.6-fold (1.9-3.5) and for C_{max} was 4.1-fold (2.5-6.6).

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

CLAIMS

1. Atovaquone in the form of particles wherein at least 95% of the particles have a volume diameter in the range 0.1-2μm.

2. Microfluidised particles of atovaquone.

3. Microfluidised particles of atovaquone wherein at least 95% of the particles have a volume diameter in the range 0.1-2 μm.

4. A pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1 - 2 μm.

5. A pharmaceutical composition according to claim 4 wherein the particles are microfluidised particles.

6. A pharmaceutical composition according to claim 4 or claim 5 in suspension form.

7. A pharmaceutical composition according to any of claims 4 to 6 wherein the pharmaceutically acceptable carriers include a suspending agent.

8. A pharmaceutical composition according to claim 7 wherein the suspending agent is xanthan gum.

9. A pharmaceutical composition according to any of claims 4 to 8 for use in therapy.

10. A pharmaceutical composition according to any of claims 4 to 8 for use in the treatment and/or prophylaxis of protozoal parasitic infections and infections caused by P.carinii.

11. A method for the preparation of microfluidised particles of atovaquone according to claim 2 or claim 3 which comprises mixing atovaquone with a

THIS SHEET HAS BEEN REPLACED

liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser.

12. A method for the preparation of a pharmaceutical composition which method comprises the steps of:-

(a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL

(b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range of 0.1 - 2 μm.

(c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

13. A method according to claim 11 or claim 12 wherein the mixture is subjected to 20 to 50 passes through the Microfluidiser.

14. A method according to claim 13 wherein the mixture is subjected to 15 - 25 passes through the Microfluidiser

15. A method according to any of the claims 11 to 14 wherein the liquid vehicle is a surfactant solution.

16. A method according to claim 15 wherein the surfactant solution is Poloxamer 188 solution.

17. A method according to claim 12 wherein the pharmaceutically acceptable carriers include a suspending agent.

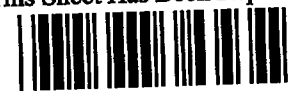
18. A method according to claim 17 wherein the suspending agent is xanthan gum.

THIS SHEET HAS BEEN REPLACED

* C L M S *

19. A pharmaceutical composition produced by a process according to any of claims 12 to 18.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/12, 9/14 — A1

(11) International Publication Number: WO 94/14426

(43) International Publication Date: 7 July 1994 (07.07.94)

(21) International Application Number: PCT/GB93/02646

(22) International Filing Date: 23 December 1993 (23.12.93)

(30) Priority Data:
9226905.9 24 December 1992 (24.12.92) GB

(71) Applicant *(for all designated States except US)*: THE WELLCOME FOUNDATION LIMITED [GB/GB]; Unicorn House, 160 Euston Road, London NW1 2BP (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DEARN, Alan, Roy [GB/GB]; Temple Hill, Dartford, Kent DA1 5AH (GB).

(74) Agent: ROLLINS, Anthony, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB).

(81) Designated States: AU, BG, BR, BY, CA, CZ, FI, HU, JP, KR, KZ, LV, NO, NZ, PL, RO, RU, SK, UA, US, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ATOVAQUONE PHARMACEUTICAL COMPOSITIONS

(57) Abstract

The invention relates to microfluidised particles of atovaquone and to a method of preparing them. More particularly, the invention is concerned with a pharmaceutical composition containing microfluidised particles of atovaquone which has improved bioavailability and its use in therapy.

THIS SHEET HAS BEEN REPLACED

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced



9604546-3

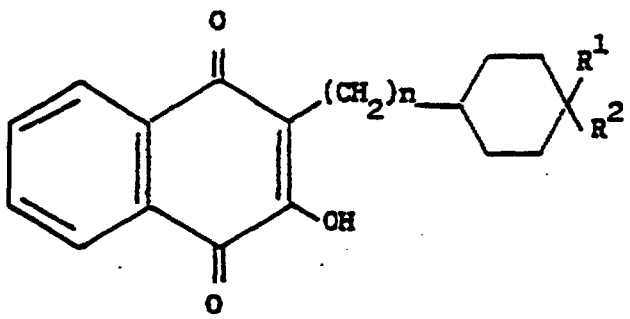
REPLACEMENT SHEET

17 OCT 1996

ATOVAQUONE PHARMACEUTICAL COMPOSITIONS

The present invention relates to microfluidised particles of 2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone and to a method for preparing them. More particularly the invention is concerned with a pharmaceutical composition containing microfluidised particles of 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone ("atovaquone") and its use in therapy.

Atovaquone has previously been disclosed, for example in European Patent No. 0123238 and US Patent No. 5053432 (incorporated herein by reference) which relates to 2-substituted-3-hydroxy-1,4-naphthoquinones of formula (I):



(I)

wherein either R^1 is hydrogen and R^2 is selected from C_{1-6}alkoxy, aralkoxy, C_{1-6} alkyl-C_{1-6}alkoxy, phenyl substituted by one or two groups selected from halogen and C_{1-6}alkyl, halogen and perhalo-C_{1-6}alkyl or R^1 and R^2 are both C_{1-6}alkyl or phenyl, and n is zero or 1, and physiologically acceptable salts thereof. The compounds are said to have antiprotozoal activity. Specifically, compounds of formula (I) wherein n is zero are said to be active against the human malaria parasite Plasmodium falciparum and also against Eimeria species such as E.tenella and E.acervulina, which are causative organisms of coccidiosis and compounds of formula (I) where n is 1 are said to be active against protozoa of the genus Theileria, in particular T.annulata or T.parva. Amongst the compounds specifically named and exemplified is the compound of formula (I) wherein n is zero, R^1 is hydrogen and R^2 is 4-chlorophenyl, i.e. atovaquone.

EP 0362996 discloses the use of atovaquone in the treatment and/or prophylaxis of Pneumocystis carinii pneumonia.

This Sheet Has Been Replaced

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
17 OCT 1996

Further uses of atovaquone against Toxoplasmosis and Cryptosporidiosis are disclosed in European patent application nos. 0445141 and 0496729 respectively.

The efficacy of atovaquone as a therapeutic agent is limited by its bioavailability. Accordingly it is an object of the present invention to provide atovaquone in a more bioavailable form.

It has now been found that the bioavailability of atovaquone can be increased by ensuring that the particle size is within a certain defined range of small particles. However, conventional methods of reducing the particle size of atovaquone were found to be unsuccessful in producing particles of the size required to improve bioavailability.

The Microfluidiser has been marketed by the Microfluidics Corporation since 1985. The principle of its operation is based on a submerged jet technology. It was designed, primarily, as a homogenizing device for use in the food and pharmaceutical industries, for the preparation of e.g. emulsion and liposomal systems and has subsequently been used for cell-rupture purposes in biotechnology applications.

It has now surprisingly been found that microfluidised particles of atovaquone produced using a Microfluidiser have improved bioavailability of the compound. It is believed that this is due to the small size and narrow range of sizes of the microfluidised atovaquone particles.

During operation of the Microfluidiser, the feed stream is pumped into a specially designed chamber, in which fluid streams interact at very high velocities and pressures. Fixed microchannels within the interaction chamber provide an extremely focussed interaction zone of intense turbulence, causing the release of energy amid cavitation and shear forces. Without wishing to be bound by theory it is believed that since all product passes through a dimensionally fixed area of energy release, greater size uniformity and smaller sizes are achieved by using the Microfluidiser rather than conventional methods for producing fine particles.

Thus, in a first aspect, the present invention provides small particles of atovaquone. Preferably the particles are microfluidised particles. Suitably at least 90% of the

THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
17 OCT 1996

particles have a volume diameter in the range of 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0.1-2μm.

In a second aspect, the present invention provides a pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1-2μm. Preferably the particles are microfluidised particles.

The carriers must be acceptable in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof.

According to a third aspect, the present invention provides a method for the preparation of microfluidised particles of atovaquone which comprises mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser in order to provide the atovaquone in the form of particles wherein at least 90% of the particles have a volume diameter in the range 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0-1-2μm.

In a further aspect the present invention provides a method for the preparation of a pharmaceutical composition comprising the steps of:-

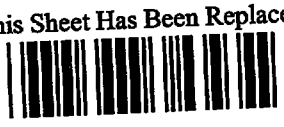
a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL.

b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range 0.1-2μm.

c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

Suitably, the mixture is subjected to 10-50 passes through the Microfluidiser, e.g. 25-30 passes. Preferably the mixture is subjected to 15-25 passes through the Microfluidiser.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
17 OCT 1996

In one embodiment, the liquid vehicle is a surfactant. Preferably, the liquid vehicle is a surfactant solution. In a particularly preferred embodiment the surfactant is Poloxamer 188 solution. In another preferred embodiment the pharmaceutically acceptable carriers include a suspending agent. Suitable suspending agents include methyl cellulose and xanthan gum. Preferably the suspending agent is xanthan gum.

Pharmaceutical formulations include those suitable for oral and parenteral (including subcutaneous, intradermal, intramuscular and intravenous) administration as well as administration by naso-gastric tube. Suitable formulations within the scope of the present invention include, for example, solid dosage forms such as tablets and liquid dosage forms, such as suspensions, which are preferred formulations. The formulation may, where appropriate, be conveniently presented in discrete dosage units and may be prepared from the microfluidised particles using methods known in the art of pharmacy.

Tests to measure the bioavailability of atovaquone in vivo indicate that formulations of microfluidised atovaquone have improved bioavailability compared to prior art formulations. The invention therefore provides, in a further aspect, formulations comprising microfluidised atovaquone for use in therapy, in particular in the treatment and prophylaxis of protozoal parasitic infections, e.g. malaria and toxoplasmosis, and infections caused by P.carinii.

The invention will now be further illustrated by the following non-limiting examples:-

Example 1

Preparation of Microfluidised particles of atovaquone

Atovaquone was prepared by methods according to the prior art, e.g. US patent no. 5053432 (incorporated herein by reference). 600mL of a mixture consisting of 2.5% w/v atovaquone in 0.25% w/v aqueous Celacol M2500 was prepared and 100mL were retained in a glass jar as a control. A laboratory scale model 120B Microfluidiser was connected to a 90 psi pneumatic supply and adjusted to produce a fluid pressure of 15000 psi. The machine base, interaction chamber and pipework of the Microfluidiser

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced



REPLACEMENT SHEET
17 OCT 1996

were immersed in a bath of cold water. 500mL of the mixture were loaded into the Microfluidiser's bulk vessel and passed through the Microfluidiser interaction chamber before being returned to the top, and side, of the bulk chamber. The mixture was recirculated continuously through the interaction chamber, and samples were taken at 10, 20, 30, 45 and 60 minutes. The number of passes to which each of these samples had been subjected was calculated and is shown in Table 1 below.

TABLE 1

Sample	Microfluidisation time (minutes)	Sample Volume (ml)	Number of passes
Control	0	100	0
1	10	105	8
2	20	105	9-19
3	30	110	31-35
4	45	105	65-77
5	60	35	142-244

Microscopic observations of the control and samples at 40x magnification were made and the results were as follows:-

Control - Varied shapes, plates, rods and spheroids, around 5x5µm generally and up to 7.5x10µm, loosely aggregated.

Sample 1 - More rounded smaller shapes, some "large" crystals, lots of small fragments 2.5x2.5µm, more dispersed.

Sample 2 - More rounded, smaller shapes, more fragments.

Sample 3 - Still more rounded, smaller shapes, more fragments.

Sample 4 - Yet more rounded, smaller shapes, more fragments.

THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
17 OCT 1996

Sample 5 - Very small particles, all under 2.5μm, all rounded, monodisperse.

Example 2

Pharmaceutical Formulation

An oral suspension formulation was prepared by mixing the following ingredients:-

Microfluidised particles of atovaquone		150.0mg
Poloxamer 188		5.0mg
Benzyl alcohol		10.0mg
Xanthan gum		7.5mg
Purified water	to make	1.0mL

Example 3

Biological Test

Nine healthy fasted male volunteers received single doses of 5mg/mL suspensions containing 250mg atovaquone as a 3μm mean particle size suspension and 1μm Microfluidised suspension in a randomised crossover study. Plasma samples were taken at intervals up to two weeks after the last dose and assayed by HPLC. The results are given in table 2 below:

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

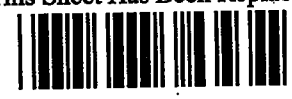
REPLACEMENT SHEET
17 OCT 1996

TABLE 2

	3µm suspension	1µm suspension
mean(SD)AUC	95 (62)µg/mL.h	247(85)µg/mL.h
mean(SD)C_{max}	1.2(0.7)µg/mL	5.0(1.6)µg/mL
median T_{max}	5 hours	1 hour

The mean (95% CI) increase for the AUC of the 1µm suspension relative to the 3µm suspension was 2.6-fold (1.9-3.5) and for C_{max} was 4.1-fold (2.5-6.6).

This Sheet Has Been Replaced



THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
17 OCT 1996

CLAIMS

1. Atovaquone in the form of particles wherein at least 95% of the particles have a volume diameter in the range 0.1-2μm.

2. Microfluidised particles of atovaquone.

3. Microfluidised particles of atovaquone wherein at least 95% of the particles have a volume diameter in the range 0.1-2 μm.

4. A pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1 - 2 μm.

5. A pharmaceutical composition according to claim 4 wherein the particles are microfluidised particles.

6. A pharmaceutical composition according to claim 4 or claim 5 in suspension form.

7. A pharmaceutical composition according to any of claims 4 to 6 wherein the pharmaceutically acceptable carriers include a suspending agent.

8. A pharmaceutical composition according to claim 7 wherein the suspending agent is xanthan gum.

9. A pharmaceutical composition according to any of claims 4 to 8 for use in therapy.

10. A pharmaceutical composition according to any of claims 4 to 8 for use in the treatment and/or prophylaxis of protozoal parasitic infections and infections caused by P.carinii.

11. A method for the preparation of microfluidised particles of atovaquone according to claim 2 or claim 3 which comprises mixing atovaquone with a

* C L M S *

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
17 OCT 1996

liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser.

12. A method for the preparation of a pharmaceutical composition which method comprises the steps of:-

(a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL

(b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range of 0.1 - 2 μm.

(c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

13. A method according to claim 11 or claim 12 wherein the mixture is subjected to 20 to 50 passes through the Microfluidiser.

14. A method according to claim 13 wherein the mixture is subjected to 15 - 25 passes through the Microfluidiser

15. A method according to any of the claims 11 to 14 wherein the liquid vehicle is a surfactant solution.

16. A method according to claim 15 wherein the surfactant solution is Poloxamer 188 solution.

17. A method according to claim 12 wherein the pharmaceutically acceptable carriers include a suspending agent.

18. A method according to claim 17 wherein the suspending agent is xanthan gum.

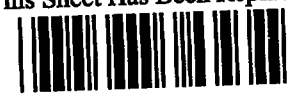
* C L M S *

THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
17 OCT 1996

19. A pharmaceutical composition produced by a process according to any of claims 12 to 18.

This Sheet Has Been Replaced





THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
17 OCT 1996

ATOVAQUONE PHARMACEUTICAL COMPOSITIONS

ABSTRACT

The invention relates to microfluidised particles of atovaquone and to a method of preparing them. More particularly, the invention is concerned with a pharmaceutical composition containing microfluidised particles of atovaquone which has improved bioavailability and its use in therapy.

This Sheet Has Been Replaced



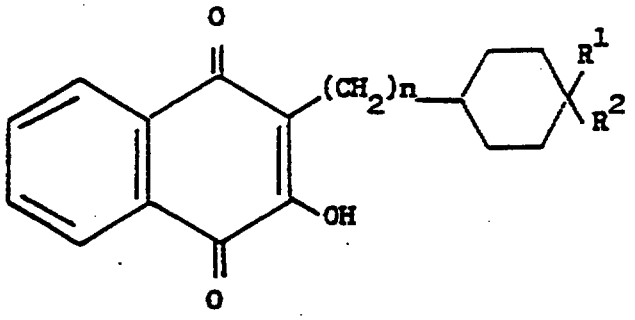
THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
20 MAR 1997

PHARMACEUTICAL PREPARATION

The present invention relates to microfluidised particles of 2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone and to a method for preparing them. More particularly the invention is concerned with a pharmaceutical composition containing microfluidised particles of 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone ("atovaquone") and its use in therapy.

Atovaquone has previously been disclosed, for example in European Patent No. 0123238 and US Patent No. 5053432 (incorporated herein by reference) which relates to 2-substituted-3-hydroxy-1,4-naphthoquinones of formula (I):



(I)

wherein either R^1 is hydrogen and R^2 is selected from C_{1-6} alkoxy, aralkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, phenyl substituted by one or two groups selected from halogen and C_{1-6} alkyl, halogen and perhalo-C_{1-6} alkyl or R^1 and R^2 are both C_{1-6} alkyl or phenyl, and n is zero or 1, and physiologically acceptable salts thereof. The compounds are said to have antiprotozoal activity. Specifically, compounds of formula (I) wherein n is zero are said to be active against the human malaria parasite Plasmodium falciparum and also against Eimeria species such as E.tenella and E.acervulina, which are causative organisms of coccidiosis and compounds of formula (I) where n is 1 are said to be active against protozoa of the genus Theileria, in particular T.annulata or T.parva. Amongst the compounds specifically named and exemplified is the compound of formula (I) wherein n is zero, R^1 is hydrogen and R^2 is 4-chlorophenyl, i.e. atovaquone.

EP 0362996 discloses the use of atovaquone in the treatment and/or prophylaxis of Pneumocystis carinii pneumonia.

REPLACEMENT SHEET
20 MAR 1997

Further uses of atovaquone against Toxoplasmosis and Cryptosporidiosis are disclosed in European patent application nos. 0445141 and 0496729 respectively.

The efficacy of atovaquone as a therapeutic agent is limited by its bioavailability. Accordingly it is an object of the present invention to provide atovaquone in a more bioavailable form.

It has now been found that the bioavailability of atovaquone can be increased by ensuring that the particle size is within a certain defined range of small particles. However, conventional methods of reducing the particle size of atovaquone were found to be unsuccessful in producing particles of the size required to improve bioavailability.

The Microfluidiser has been marketed by the Microfluidics Corporation since 1985. The principle of its operation is based on a submerged jet technology. It was designed, primarily, as a homogenizing device for use in the food and pharmaceutical industries, for the preparation of e.g. emulsion and liposomal systems and has subsequently been used for cell-rupture purposes in biotechnology applications.

It has now surprisingly been found that microfluidised particles of atovaquone produced using a Microfluidiser have improved bioavailability of the compound. It is believed that this is due to the small size and narrow range of sizes of the microfluidised atovaquone particles.

During operation of the Microfluidiser, the feed stream is pumped into a specially designed chamber, in which fluid streams interact at very high velocities and pressures. Fixed microchannels within the interaction chamber provide an extremely focussed interaction zone of intense turbulence, causing the release of energy amid cavitation and shear forces. Without wishing to be bound by theory it is believed that since all product passes through a dimensionally fixed area of energy release, greater size uniformity and smaller sizes are achieved by using the Microfluidiser rather than conventional methods for producing fine particles.

Thus, in a first aspect, the present invention provides small particles of atovaquone. Preferably the particles are microfluidised particles. Suitably at least 90% of the

REPLACEMENT SHEET
20 MAR 1997

particles have a volume diameter in the range of 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0.1-2μm.

In a second aspect, the present invention provides a pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1-2μm. Preferably the particles are microfluidised particles.

The carriers must be acceptable in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof.

According to a third aspect, the present invention provides a method for the preparation of microfluidised particles of atovaquone which comprises mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser in order to provide the atovaquone in the form of particles wherein at least 90% of the particles have a volume diameter in the range 0.1-3μm. Preferably at least 95% of the particles have a volume diameter in the range 0-1-2μm.

In a further aspect the present invention provides a method for the preparation of a pharmaceutical composition comprising the steps of:-

a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450mg/mL.

b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range 0.1-2μm.

c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

Suitably, the mixture is subjected to 10-50 passes through the Microfluidiser, e.g. 25-30 passes. Preferably the mixture is subjected to 15-25 passes through the Microfluidiser.

REPLACEMENT SHEET
20 MAR 1997

In one embodiment, the liquid vehicle is a surfactant. Preferably, the liquid vehicle is a surfactant solution. In a particularly preferred embodiment the surfactant is Poloxamer 188 solution. In another preferred embodiment the pharmaceutically acceptable carriers include a suspending agent. Suitable suspending agents include methyl cellulose and xanthan gum. Preferably the suspending agent is xanthan gum.

Pharmaceutical formulations include those suitable for oral and parenteral (including subcutaneous, intradermal, intramuscular and intravenous) administration as well as administration by naso-gastric tube. Suitable formulations within the scope of the present invention include, for example, solid dosage forms such as tablets and liquid dosage forms, such as suspensions, which are preferred formulations. The formulation may, where appropriate, be conveniently presented in discrete dosage units and may be prepared from the microfluidised particles using methods known in the art of pharmacy.

Tests to measure the bioavailability of atovaquone in vivo indicate that formulations of microfluidised atovaquone have improved bioavailability compared to prior art formulations. The invention therefore provides, in a further aspect, formulations comprising microfluidised atovaquone for use in therapy, in particular in the treatment and prophylaxis of protozoal parasitic infections, e.g. malaria and toxoplasmosis, and infections caused by P.carinii.

The invention will now be further illustrated by the following non-limiting examples:-

Example 1

Preparation of Microfluidised particles of atovaquone

Atovaquone was prepared by methods according to the prior art, e.g. US patent no. 5053432 (incorporated herein by reference). 600mL of a mixture consisting of 2.5% w/v atovaquone in 0.25% w/v aqueous Celacol M2500 was prepared and 100mL were retained in a glass jar as a control. A laboratory scale model 120B Microfluidiser was connected to a 90 psi pneumatic supply and adjusted to produce a fluid pressure of 15000 psi. The machine base, interaction chamber and pipework of the Microfluidiser

REPLACEMENT SHEET
20 MAR 1997

were immersed in a bath of cold water. 500mL of the mixture were loaded into the Microfluidiser's bulk vessel and passed through the Microfluidiser interaction chamber before being returned to the top, and side, of the bulk chamber. The mixture was recirculated continuously through the interaction chamber, and samples were taken at 10, 20, 30, 45 and 60 minutes. The number of passes to which each of these samples had been subjected was calculated and is shown in Table 1 below.

TABLE 1

Sample	Microfluidisation time (minutes)	Sample Volume (ml)	Number of passes
Control	0	100	0
1	10	105	8
2	20	105	9-19
3	30	110	31-35
4	45	105	65-77
5	60	35	142-244

Microscopic observations of the control and samples at 40x magnification were made and the results were as follows:-

Control - Varied shapes, plates, rods and spheroids, around 5x5μm generally and up to 7.5x10μm, loosely aggregated.

Sample 1 - More rounded smaller shapes, some "large" crystals, lots of small fragments 2.5x2.5μm, more dispersed.

Sample 2 - More rounded, smaller shapes, more fragments.

Sample 3 - Still more rounded, smaller shapes, more fragments.

Sample 4 - Yet more rounded, smaller shapes, more fragments.

REPLACEMENT SHEET
20 MAR 1997

Sample 5 - Very small particles, all under 2.5μm, all rounded, monodisperse.

Example 2

Pharmaceutical Formulation

An oral suspension formulation was prepared by mixing the following ingredients:-

Microfluidised particles of atovaquone		150.0mg
Poloxamer 188		5.0mg
Benzyl alcohol		10.0mg
Xanthan gum		7.5mg
Purified water	to make	1.0mL

Example 3

Biological Test

Nine healthy fasted male volunteers received single doses of 5mg/mL suspensions containing 250mg atovaquone as a 3μm mean particle size suspension and 1μm Microfluidised suspension in a randomised crossover study. Plasma samples were taken at intervals up to two weeks after the last dose and assayed by HPLC. The results are given in table 2 below:

REPLACEMENT SHEET
20 MAR 1997

TABLE 2

	3μm suspension	1μm suspension
mean(SD)AUC	95 (62)μg/mL.h	247(85)μg/mL.h
mean(SD)C_{max}	1.2(0.7)μg/mL	5.0(1.6)μg/mL
median T_{max}	5 hours	1 hour

The mean (95% CI) increase for the AUC of the 1μm suspension relative to the 3μm suspension was 2.6-fold (1.9-3.5) and for C_{max} was 4.1-fold (2.5-6.6).

This Sheet Has Been Replaced

REPLACEMENT SHEET
20 MAR 1997

CLAIMS

1. Atovaquone in the form of particles wherein at least 95% of the particles have a volume diameter in the range 0.1-2μm.

2. Microfluidised particles of atovaquone.

3. Microfluidised particles of atovaquone wherein at least 95% of the particles have a volume diameter in the range 0.1-2 μm.

4. A pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 95% of the particles have a volume diameter in the range of 0.1 - 2 μm.

5. A pharmaceutical composition according to claim 4 wherein the particles are microfluidised particles.

6. A pharmaceutical composition according to claim 4 or claim 5 in suspension form.

7. A pharmaceutical composition according to any of claims 4 to 6 wherein the pharmaceutically acceptable carriers include a suspending agent.

8. A pharmaceutical composition according to claim 7 wherein the suspending agent is xanthan gum.

9. A pharmaceutical composition according to any of claims 4 to 8 for use in therapy.

10. A pharmaceutical composition according to any of claims 4 to 8 for use in the treatment and/or prophylaxis of protozoal parasitic infections and infections caused by P.carinii.

11. A method for the preparation of microfluidised particles of atovaquone according to claim 2 or claim 3 which comprises mixing atovaquone with a

CLMS

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
20 MAR 1997

liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser.

12. A method for the preparation of a pharmaceutical composition which method comprises the steps of:-

(a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL

(b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 95% of those particles have a volume diameter in the range of 0.1 - 2 μm.

(c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

13. A method according to claim 11 or claim 12 wherein the mixture is subjected to 20 to 50 passes through the Microfluidiser.

14. A method according to claim 13 wherein the mixture is subjected to 15 - 25 passes through the Microfluidiser

15. A method according to any of the claims 11 to 14 wherein the liquid vehicle is a surfactant solution.

16. A method according to claim 15 wherein the surfactant solution is Poloxamer 188 solution.

17. A method according to claim 12 wherein the pharmaceutically acceptable carriers include a suspending agent.

18. A method according to claim 17 wherein the suspending agent is xanthan gum.

* C L M S *

THIS SHEET HAS BEEN REPLACED

This Sheet Has Been Replaced

REPLACEMENT SHEET
20 MAR 1997

19. A pharmaceutical composition produced by a process according to any of claims 12 to 18.

* C L M S *

THIS SHEET HAS BEEN REPLACED

REPLACEMENT SHEET
20 MAR 1997

PHARMACEUTICAL PREPARATION

ABSTRACT

The invention relates to microfluidised particles of atovaquone and to a method of preparing them. More particularly, the invention is concerned with a pharmaceutical composition containing microfluidised particles of atovaquone which has improved bioavailability and its use in therapy.

REPLACEMENT SHEET
21 APR 1997

CLAIMS

1. Atovaquone in the form of particles wherein at least 90% of the particles have a volume diameter in the range 0.1-3µm.

2. Microfluidised particles of atovaquone.

3. Microfluidised particles of atovaquone wherein at least 90% of the particles have a volume diameter in the range 0.1-3µm.

4. A pharmaceutical composition comprising particles of atovaquone and one or more pharmaceutically acceptable carriers therefor wherein at least 90% of the particles have a volume diameter in the range 0.1-3µm.

5. A pharmaceutical composition according to claim 4, wherein the particles are microfluidised particles.

6. A pharmaceutical composition according to claim 4 or claim 5 in suspension form.

7. A pharmaceutical composition according to any of claims 4 to 6, wherein the pharmaceutically acceptable carriers include a suspending agent.

8. A pharmaceutical composition according to claim 7, wherein the suspending agent is xanthan gum.

9. A pharmaceutical composition according to any of claims 4 to 8 for use in therapy.

10. A pharmaceutical composition according to any of claims 4 to 8 for use in the treatment and/or prophylaxis of protozoal parasitic infections caused by P.carinii.

11. A method for the preparation of microfluidised particles of atovaquone according to claim 2 or claim 3 which comprises mixing atovaquone with a

21/4/97

* C L M S *

REPLACEMENT SHEET
21 APR 1997

liquid vehicle to provide a mixture wherein the concentration of atovaquone is less that 450 mg/mL and subjecting said mixture to at least 3 passes through a Microfluidiser.

12. A method for the preparation of a pharmaceutical composition which method comprises the steps of:-

    (a) mixing atovaquone with a liquid vehicle to provide a mixture wherein the concentration of atovaquone is less than 450 mg/mL

    (b) subjecting the mixture to at least 3 passes through a Microfluidiser to provide a microfluidised preparation wherein the atovaquone is in the form of particles and at least 90% of those particles have a volume diameter in the range of 0.1-3µm.

    (c) mixing the microfluidised preparation with one or more pharmaceutically acceptable carriers therefor.

13. A method according to claim 11 or claim 12, wherein the mixture is subjected to 10 to 50 passes through the Microfluidiser.

14. A method according to claim 13, wherein the mixture is subjected to 15 - 25 passes through the Microfluidiser.

15. A method according to any of the claims 11 to 14, wherein the liquid vehicle is a surfactant solution.

16. A method according to claim 15, wherein the surfactant is Poloxamer 188 solution.

17. A method according to claim 12, wherein the pharmaceutically acceptable carriers include a suspending agent.

REPLACEMENT SHEET
21 APR 1997

18. A method according to claim 17, wherein the suspending agent is xanthan gum.

19. A pharmaceutical composition produced by a process according to any of claims 12 to 18.

* C L M S *