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DES INFECTIONS DUES AU VIH
(54) Title: 2,4-DICHLOROBENZYL ALCOHOL AND AMYLMETACRESOL AGAINST HIV INFECTION

(57) **Abrégé/Abstract:**

The use of a composition comprising 2,4-dichlorobenzyl alcohol and amylmetacresol in the preparation of a medicament for the treatment or prevention of HIV viral infections.





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<p>(57) Abstract</p> <p>The use of a composition comprising 2,4-dichlorobenzyl alcohol and amylmetacresol in the preparation of a medicament for the treatment or prevention of HIV viral infections.</p>		

2,4-DICHLOROBENZYL ALCOHOL AND
AMYLMETACRESOL AGAINST HIV INFECTION

5 The present invention relates to the use of formulations
of 2,4-dichlorobenzyl alcohol (or 2,4 DCBA) and
amylmetacresol (or AMC) in the treatment or prevention of
Human Immunodeficiency Virus (HIV) viral infections.

10 HIV infections are now found in every country in the
world and the most important method of transmission is
sexual. The virus is present as free virus particles or
as intracellular virions in monocyte cells in semen. The
virus is thereby transmitted relatively easily from male
15 to female or male to male. Alternatively, in an infected
female, virus either free or cell bound is found in fluid
in the vagina.

Existing antivirals have been found which inhibit
important enzymes of the virus such as reverse
20 transcriptase (RT), protease or integrase. The first
clinically used drug namely AZT (or zidovudine,
RetrovirTM), inhibits the virus RT enzyme. However, AZT
does not represent a cure of infection and has not been
demonstrated to prevent person to person spread. Indeed
25 one of the clinical problems with the drug, apart from
toxicity, is the emergence of drug resistant virus and
the actual spread of the drug resistant virus in the
community. A new approach against HIV is therefore to
search for novel compounds which may destroy or
30 inactivate free virus and/or cell associated virus
directly on contact. These are so-called virucidal
compounds. Such molecules have been described in the
past (Oxford et al in *App. Microbiology* 21 606-610
(1971)), but have been little investigated recently.

Since the HIV virion is surrounded by a lipid bilayer it would be logical to test detergent like molecules for their ability to disrupt and dissolve the lipid membrane of the virus. For example the non-ionic surfactant nonoxynol-9 has been used in clinical practice as a spermicide and has direct anti-HIV activity (Malkovsky et al in *The Lancet* 645 (1988)). However, it has little selective anti-HIV effect and because it causes toxicity and cell destruction following application to the vagina its use may actually enhance infection with HIV (Kreiss et al in *J.A.M.A.* 268 477-482 (1992)).

The compound 2,4-dichlorobenzyl alcohol is known as an antiseptic agent i.e. as an antibacterial and antifungal agent, see for example Martindale "The Extra Pharmacopoeia" 28th edition, page 561, The Pharmaceutical Press (1982). Amylmetacresol or 6-pentyl-*m*-cresol is also known as a disinfectant used in mouth-washes or lozenges in combination with 2,4-dichlorobenzyl alcohol to treat mouth infections, see for example Martindale "The Extra Pharmacopoeia" 28th edition, page 549, The Pharmaceutical Press (1982). An antiseptic is defined in The Concise Oxford Dictionary (Oxford University Press, Oxford (1982)) as an agent which counters the development of sepsis, especially by preventing the growth of bacteria. Sepsis is defined in Black's Medical Dictionary (A & C Black, London (1990)) as poisoning by the products of the growth of micro-organisms in the body, and the general symptoms which accompany it are those of inflammation. Neither 2,4-dichlorobenzyl alcohol or amylmetacresol has previously been shown to demonstrate anti-viral or anti-retroviral activity and, in particular, anti-HIV activity.

Unexpectedly it has now been discovered that a formulation of 2,4-dichlorobenzyl alcohol and amylmetacresol has anti-retroviral activity and causes inactivation of HIV infectivity.

5

According to the present invention there is provided the use of a composition comprising 2,4-dichlorobenzyl alcohol and amylmetacresol in the preparation of a medicament for the treatment or prevention of HIV viral infections.

10

In a particularly preferred embodiment of the present invention there is provided the use of a StrepsilTM-like formulation of 2,4-dichlorobenzyl alcohol and amylmetacresol in the preparation of a medicament for the treatment or prevention of HIV viral infections.

15

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the vaginal, rectal or oral routes. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

25

For the preparation of solutions and syrups used in formulating the composition, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions. Other suitable excipients include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc.

30

Alternatively the composition may be formulated as an ointment, cream, suspension, lotion, powder, solution, paste, gel, spray, aerosol or oil. When formulated in an ointment, the active ingredient may be employed with
5 either a paraffinic or a water-miscible ointment base. The active ingredient may also be formulated in a cream with an oil-in-water cream base or a water-in-oil base. Compositions may be further prepared in which the active ingredients are dissolved or suspended in a suitable
10 carrier, especially an aqueous solvent.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

15 Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas.

It also contemplated that the compositions may include
20 anti-oxidants, buffers, bacteriostats, solutes, suspending agents and thickening agents. Excipients which may be used include water, alcohols, polyols, glycerine and vegetable oils, for example.

25 The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colourants, odourants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable
30 salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the substance of the present invention can

vary between wide limits, depending upon the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used. However, without being bound by any particular dosages, it is believed that, for vaginal, rectal or oral administration, an effective composition in accordance with the present invention (usually present as part of a pharmaceutical composition as discussed above) may be suitable in treatments of the present invention as follows:

Suitable concentration ranges for the compounds present in the composition may be as follows. 2,4-dichlorobenzyl alcohol may be present in the composition in a concentration of from 10 μ g/ml to 10mg/ml, preferably of from 0.1mg/ml to 5mg/ml and particularly of from 1.0mg/ml to 5mg/ml. The concentration of amylmetacresol in the composition may be of from 10 μ g/ml to 5.0mg/ml, preferably from 50 μ g/ml to 2.0mg/ml and particularly from 0.05mg/ml to 1.0mg/ml. If side effects develop, the amount and/or frequency of application of the composition can be reduced, in accordance with normal clinical practice.

In the context of the present invention the term "HIV" extends to HIV-1, HIV-2 and other related viruses responsible for the medical condition described as Acquired Immunodeficiency Syndrome (AIDS) or the condition described as AIDS-Related Complex (ARC). The term HIV is the internationally accepted definition of the viruses previously known by the names HTLV-III, LAV and ARV.

Alternatively, the invention provides the use of 2,4-

dichlorobenzyl alcohol and/or amylmetacresol in the preparation of a medicament for the treatment or prevention of viral infections caused by HIV.

5 The use of a formulation of 2,4-dichlorobenzyl alcohol and amylmetacresol in an acidic sucrose base in the preparation of a medicament for the treatment or prevention of viral infections caused by HIV is also in accordance with the present invention.

10

It is also part of the present invention to provide the use of a composition comprising 2,4-dichlorobenzyl alcohol and/or amylmetacresol in the preparation of an agent for the treatment or prevention of HIV viral
15 infections. The agent may a barrier contraceptive device, or it may adapted for vaginal or rectal administration.

The present invention, therefore, also extends to a
20 method of treatment or prevention of HIV viral infections, the method comprising administration of an effective amount of 2,4-dichlorobenzyl alcohol and/or amylmetacresol.

25 Compositions of the present invention may be suitably used for vaginal, rectal or oral administration. Where the route of administration is vaginal, the composition may be provided in the form of a pessary, cream, gel, lotion or spray. Where the route of administration is
30 rectal, the composition may be provided in the form of an enema.

Since an identical formulation has been used for two decades or more as StrepsilTM mouth lozenges to combat

bacterial infections it is speculated that the mixture has little deleterious effect on mucosal cells in this region. It is proposed that should such an absence of cellular toxicity in a human mucosal surface be accompanied by the virucidal effects now discovered against HIV-1, that the formulation or a similar formulation could be used as vaginal or rectal application, or as a constituent in a barrier contraceptive device to inactivate HIV on contact, or used orally to destroy extra-cellular virus and hence reduce or prevent spread of the virus. Such a composition or a barrier contraceptive device containing such a composition may also contain other active agents, for example a spermicide. The barrier contraceptive device may also be provided with a lubricant, such as for example VaselineTM or KY JellyTM.

Barrier contraceptive devices include the male sheath or condom, the cap and the female condom or FemidomTM. As described above it is also contemplated that compositions according to the present invention could be applied to or contained in such devices.

Thus, in other aspects the invention provides (a) a composition of the invention for use in vaginal, rectal or oral application or to be applied to a barrier contraceptive device, such as a condom or cap; and (b) a barrier contraceptive device, such as a condom or cap, incorporating a composition of the invention.

The present invention also includes a product comprising 2,4-dichlorobenzyl alcohol and amylmetacresol as a combined preparation for simultaneous, separate or sequential use in the treatment or prevention of HIV

viral infections.

5 Compositions according to the present invention may also be used as part of a kit comprising a barrier contraceptive device, such as a condom or cap, and optionally a spermicide and/or a lubricant, for example Vaseline™ or KY Jelly™. The spermicide may also be formulated in any convenient form such as a gel, cream or spray for subsequent application as part of the kit.

10

Preferred features of the invention for the second and subsequent features of the invention are as for the first aspect *mutatis mutandis*.

15

The invention will now be described by way of example with reference to the accompanying Examples which are provided for the purposes of illustration and are not to be construed as being limiting on the present invention.

20

Example 1: Preparation of virucidal mixture

25

A virucidal mixture for use in the *in vitro* activity assays was prepared as follows. A commercial preparation of a tablet containing 1.6mg 2,4-dichlorobenzyl alcohol (2,4-DCB alcohol) and 0.6mg amylmetacresol (AMC) B.P. was mixed into 5ml of buffered saliva. This preparation containing the active factors is known as the virucidal mixture).

30

Example 1: In vitro activity

Virus: Laboratory Isolate of HIV-1, MN

Cells: C8166-cells

A sample of HIV-1 was added to a preparation of 2,4-

dichlorobenzyl alcohol (2,4-DCB alcohol) and
 amylmetacresol (AMC) in 5ml of buffered saliva (the
 virucidal mixture). A corresponding sample of HIV-1 was
 separately added to 5ml of untreated buffered saliva (the
 5 control mixture). The two mixtures containing virus were
 incubated for periods of 2, 4, 20 and 60 minutes at 37°C.
 At these particular time periods a sample of virus was
 removed from each of the two mixtures and titrated in
 susceptible C8166-cells for residual virus infectivity.
 10 The compound was not toxic to C8166-cells. The
 experiment was repeated four times using different
 quantities of HIV-1 in the mixture.

Results

15

The results for the experiments are shown below in Table
 1 and Table 2. The quantity of infective virus remaining
 in the mixture is expressed as syncytium inducing units
 of virus per ml.

20

Table 1

Initial virus concentration = 10^6 ID₅₀/ml

25

Incubation time (mins)	Quantity of infective virus remaining / 2,4- DCB alcohol and AMC mixture	Quantity of infective virus remaining / Control mixture
2	≤ 100	1,000,000
4	≤ 100	1,000,000
20	≤ 100	1,000,000
60	≤ 100	1,000,000

30

Table 2

5 Initial virus concentration = 10^5 ID₅₀/ml

Incubation time (mins)	Quantity of infective virus remaining / 2,4-DCB alcohol and AMC mixture	Quantity of infective virus remaining / Control mixture
2	≤ 100	100,000
4	≤ 100	100,000
20	≤ 100	100,000
60	≤ 100	100,000

10
15
20
The experiments, whose results are shown in Table 1 and Table 2, commenced by incubation of either 10^6 or 10^5 infective syncytium inducing units of HIV-1 with the two mixtures. The solution containing the 2,4-DCB and AMC preparation caused a 10^3 - 10^4 fold reduction of infectivity (syncytium inducing activity) of the virus in both experiments. Note that HIV induces cell fusion (or syncytia) in susceptible cells and that this is used as a useful and accurate estimate of the infectious ability of the virus.

25 Example 2: Effect of pH on *in vitro* activity

30 In further experiments we investigated the effect of pH on the virucidal effect of the mixture. A low pH was necessary for the anti-HIV activity of the preparation but low pH by itself did not abolish virus infectivity. Incubation of virus with 30% sucrose had no virucidal effect.

The *in vitro* data describes the surprising ability of a mixture of two organic molecules in an acid solution containing sucrose and additional ion constituents to very significantly reduce the infectivity of live infectious HIV-1. The compounds contained in the preparation may act together with possible synergistic results or they may have separate activities. It may be that they exert their activity by binding to the outside protein spikes of the virus to thereby abolish absorption of the virus to an indicator cell, by clumping virus, by partial denaturation of the spike protein, by an action on the lipid bilayer or by stabilising virus structure (Oxford and Thomas in *Principles of Bacteriology Virology and Immunology* 4 (1990) Published by Edward Arnold).

The anti-HIV efficacy of the mixture may be improved by reformulation or addition of similar molecules, but the current formulation has the advantage of extensive clinical database following the application of the 2,4-dichlorobenzyl alcohol and amylmetacresol mixture on mucosal surfaces of man to prevent or treat sore throats caused by bacterial infection.

Examples 3: Virucidal activity of 2,4 DCBA and AMC formulations

The anti-HIV effects of varying concentration of AMC and DCBA were also separately studied. The results of these experiments are shown in Tables 3 to 5.

Materials and Methods

Test Reagent

AMC was purchased as a dark-yellow solution and was

dissolved first in DMSO and then further dilutions were made in artificial saliva (2.4 mg/ml, 1.2 mg/ml, 0.12 mg/ml, 0.06 mg/ml, 0.03 mg/ml, 0.015 mg/ml and 0.0075 mg/ml). DCBA was purchased as white powder and dissolved in DMSO (100 mg/ml stock) and further dilutions were made in artificial saliva (4.8 mg/ml, 2.4 mg/ml, 0.24 mg/ml, 0.12 mg/ml, 0.06 mg/ml, 0.03 mg/ml and 0.015 mg/ml).

Cell Cultures

Human T-cell line C8166 was used for virucidal tests and cells were cultivated in RPMI 1640 medium (from Sigma Chemical Company, UK) supplemented with 10% heat-inactivated (56°C, 30 mins) foetal calf serum (FCS), 2mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 5×10^{-5} m 2-Mercaptoethanol (Sigma Chemical Company, UK).

Virus Stock

A stock of laboratory strain HIV-1_{IIIB} containing cell free tissue culture supernatant, stored in liquid nitrogen, was used in the assays. Before and after freezing the virus stock was titrated to ensure the correct TCID₅₀ required for the particular assay.

Example 3: Virucidal Assay

Lymphoblastoid C8166 cells were plated out into 96 well plates (180 µl/well) at a concentration of 5×10^5 per ml in six-replicate in growth medium. Different concentrations of the compounds were mixed with a high titre of HIV-1_{IIIB} (10^6 TCID₅₀) at a ratio of 1:10 (1 ml total) and were incubated at 37°C for one or fifteen minutes. After incubation, the mixture of virus/compound (20µl) was added to the top row of C8166 cells. After

mixing, then 20 μ l was transferred into the next series of wells etc., to give a ten-fold dilution series from 10^{-1} to 10^{-10} and the plates were incubated at 37°C for 10 days.

Table 3

Initial virus concentration 10^5 ID₅₀/ml MN

Quantity of infective virus remaining after 1 minute at 37°C	Quantity of infective virus remaining after 1 minute at 37°C			Quantity of infective virus remaining in control mixture
	0.24 mg/ml 2,4 DCBA and 0.12 mg/ml AMC mixture	0.24 mg/ml 2,4 DCBA	0.12 mg/ml AMC low pH alone	
<10	<10	<10	<10	100,000

Table 4

Initial virus concentration 10^5 TCID₅₀/ml MN

Quantity of infective virus remaining after 1 min at 37°C with AMC at varying concentrations (mg/ml)				Quantity of infective virus remaining in control mixture
0.06	0.03	0.015	0.0075	
<10	10	1000	100,000	100,000

Table 5

Initial virus concentration 10^5 TCID₅₀/ml MN

Quantity of infective virus remaining after 1 min at 37°C with AMC at varying concentrations (mg/ml)				Quantity of infective virus remaining in control mixture
0.24	0.12	0.06	0.03	
<10	<10	10	1000	100,000

CLAIMS

1. The use of 2,4-dichlorobenzyl alcohol and amylmetacresol in combination, in an acidic sucrose base, in the preparation of a medicament for the treatment or prevention of HIV viral infections.
2. The use as claimed in claim 1, in which the medicament is suitable for vaginal, rectal, or oral administration.
3. A composition adapted for vaginal or rectal administration, comprising 2,4-dichlorobenzyl alcohol and amylmetacresol in an acidic sucrose base.
4. A composition as claimed in claim 3, which is in the form of a pessary, enema, cream, gel, spray, or lotion.
5. A composition as claimed in claim 4, wherein the composition is suitable for application to a barrier contraceptive device.
6. A composition as claimed in claim 5, in which the barrier contraceptive device is a condom or a cap.
7. A barrier contraceptive device incorporating a composition comprising 2,4-dichlorobenzyl alcohol and amylmetacresol in an acidic sucrose base.
8. A barrier contraceptive device as claimed in claim 7, which is a condom or a cap.
9. A kit of parts comprising 2,4-dichlorobenzyl alcohol, amylmetacresol, and an acidic sucrose base as a combined preparation for simultaneous, separate, or sequential use in the treatment or prevention of HIV viral infections.

10. A kit comprising a composition as claimed in any one of claims 3 to 6, a barrier contraceptive device, and, optionally, at least one of a spermicide and a lubricant.

11. A kit as claimed in claim 10, in which the barrier contraceptive device is a condom or a cap.