TRIMELAMOL-CONTAINING PHARMACEUTICAL COMPOSITIONS

This invention relates to pharmaceutical compositions containing trimelamol.

Trimelamol (N^2,N^4,N^6-trihydroxymethyl-N^2,N^4,N^6-trimethylamine) is known to possess anti-tumour properties, and is reported to have a similar activity to pentamethylmelamine (PMM), another known anti-tumour agent, against the murine PC6 plasmacytoma but enhanced activity with respect to PMM against the Walker 256 carcinosarcoma in the rat. Moreover, trimelamol causes less acute neurotoxicity in animal toxicological testing than does PMM. Trimelamol is therefore a potentially valuable addition to the armoury of cancer-treating drugs.

A problem which has hitherto hindered the development of trimelamol-containing pharmaceutical compositions is that it is chemically quite unstable. It is also poorly soluble in water. Much research effort has therefore been devoted with a view to providing a stable injectable formulation of trimelamol which could be manufactured on a commercial scale, but so far the results have not been fully satisfactory.

These problems are addressed in GB-A-2170710 which teaches that the rate of dissolution of trimelamol in aqueous media can be improved if the trimelamol is freeze-dried from a solution additionally containing mannitol, and that such freeze-dried products can be formulated in aqueous dextrose to give a more stable solution. Our own experience of this technique shows, however, that at least when it is applied on the commercial scale, there is a considerable risk of an unacceptably high degree of degradation of the active compound occurring. For example, we recently prepared a 100 litre batch of trimelamol in an aqueous mannitol-containing solution according to the procedure of

GB-A-2170710, and we found that over the 6-7 hour period which it took to dissolve the trimelamol, filter sterilize the resulting solution and fill it into vials, the trimelamol assay value of the final product was only 82.7% of the initial value.

We have now found that trimelamol is more soluble in aqueous vehicles containing certain polyethylene glycols than it is in water containing mannitol, and that the resulting solutions are considerably more stable than the aqueous mannitol solutions. This discovery permits the manufacture of freeze-dried trimelamol compositions, suitable for reconstitution to provide a formulation for parenteral administration, with a significantly reduced risk of any substantial trimelamol degradation occurring during the preparation of the freeze-dried material.

Furthermore, we have also found that when the freeze-dried trimelamol composition of this invention is reconstituted with water or other infusion liquid for intravenous administration, the resulting pharmaceutical preparation is significantly more stable than the dextrose-containing trimelamol preparations which we have been able to obtain by following the teachings of GB-A-2170710. Accordingly, a greater time can elapse between reconstituting the trimelamol in an aqueous vehicle and administering the resulting preparation to a patient before there is any significant loss of trimelamol activity, which is a considerable advantage in many clinical situations.

These findings are quite unexpected, especially since the use of other polyhydric alcohols such as mannitol, TRIS, polyvinyl alcohol and sodium gluconate, in place of the polyethylene glycol has been found to confer no benefit.

The polyethylene glycols which are useful herein are those with a molecular weight of at least

about 600, preferably in the range 600-4000. Polyethylene glycols with a molecular weight in the range of 900-1100, and more especially of about 1000, are preferred for optimum improvement in the stability of the trimelamol solutions. However, such preparations formed using such polyethylene glycols are somewhat difficult to completely freeze-dry, requiring low temperatures and/or prolonged drying times. Accordingly, on the commercial scale it may sometimes be more convenient and economical to use polyethylene glycols of somewhat higher molecular weight. We have found in our work that polyethylene glycol of molecular weight of approximately 3400 offers a good compromise between trimelamol stability, on the one hand, and commercially more acceptable freeze-drying conditions on the other. It will be understood that references herein to a polyethylene glycol of a specified molecular weight refers to the average molecular weight of the polyethylene glycol. As normally supplied commercially, polyethylene glycols consist of a mixture of polyethylene glycols of different molecular weights, and are designated by the average molecular weight of each particular sample. Hereafter, polyethylene glycol will sometimes be abbreviated to "PEG", in accordance with usage in the art.

Thus, in accordance with one aspect, the present invention provides a solid freeze-dried composition comprising trimelamol and a polyethylene glycol having a molecular weight of at least 600.

The invention also provides a process for preparing a solid freeze-dried composition comprising trimelamol, which process comprises dissolving trimelamol in a mixture of water and a polyethylene glycol having a molecular weight of at least 600, freezing the resulting solution and subjecting it to lyophilisation.

In another aspect, the present invention provides a pharmaceutical preparation suitable for parenteral administration, which comprises a freeze-dried trimelamol-containing composition of this invention dissolved in an infusion liquid suitable for parenteral administration.

The trimelamol-containing freeze-dried composition of this invention may be prepared by dissolving trimelamol in a mixture of the selected polyethylene glycol and water, and then freezing and lyophilising the resultant solution by conventional methods. It is often preferred that the amount of water in the initial solution should be the minimum necessary to achieve complete solubilization of the polyethylene glycol, in order to optimize trimelamol solubility and stability in the solution. This amount varies with different polyethylene glycols, ranging from about 25% by weight in the case of polyethylene glycol, of molecular weight about 1000, to about 75% by weight in the case of polyethylene glycols having molecular weights of about 4000-8000. However, possible practical disadvantages of using high concentrations of PEG 1000 in the initial solution are the longer processing times required because of the increased vehicle viscosity, which in turn leads to a reduction in the rate of trimelamol dissolution, and in the filtration rate, whilst the temperatures required for freeze-drying the products can be substantially lower, sometimes to below the capabilities of some freeze-drying equipment.

The amount of the trimelamol present in the aqueous polyethylene glycol solution is not critical, but it is an important advantage of the present invention that it permits comparatively highly concentrated trimelamol solutions to be formed without encountering the trimelamol polymerization problem which is referred

to in GB-A-2170710. Accordingly, relatively large amounts of trimelamol can be present in a vial or other container of a given volume into which, in accordance with conventional procedures, the solution is normally filled prior to the freezing and lyophilisation and in which the freeze-dried material is then stored until it needs to be reconstituted for use.

The relatively high solubility of trimelamol in the polyethylene glycol-water mixtures used in accordance with this invention is illustrated by the tabulation below:

Solvent	Active Ingredient	Solubility (mg/ml)
Water[1]	Trimelamol	<1
Water	Trimelamol with reduced particle size[1] or freeze dried from water	5
PEG 1000[2] (75% in Water)	Trimelamol	58.8
PEG 1000[2] (70% in Water)	Trimelamol	52.0
PEG 1000[2] (60% in Water)	Trimelamol	40.9
PEG 1000[2] (50% in Water)	Trimelamol	29.2
PEG 1500[2] (50% in Water)	Trimelamol	37.1
PEG 3400[2] (50% in Water)	Trimelamol	34.3
Mannitol (1% in Water)	Trimelamol	7.9

[1] Rutty et al, Cancer Chemother. Pharmacol, 17, 251-8 (1986)

[2] PEG is an abbreviation for polyethylene glycol, the number following indicating the average molecular weight.

In one preferred embodiment of this invention, a PEG 1000-water mixture is formed which contains about 40-50 mg of trimelamol per ml of the mixture, more especially about 44 mg/ml of the active ingredient, and which, following freeze-drying, can be diluted ten-fold with Water for Injection or other suitable infusion liquid to provide an aqueous solution for parenteral administration to human subjects at trimelamol concentrations of about 4 mg/ml even allowing for up to 10% degradation of active ingredient. However, and as shown by the above solubility table, such high trimelamol concentrations are not obtainable with PEGs of higher molecular weight, so that this is a disadvantage of using the higher molecular weight polyethylene glycols, to be weighed against their easier freeze-drying capabilities.

The freeze-dried compositions of this invention typically will contain from 5 - 6.5% by weight of trimelamol.

Typical freezing and lyophilisation conditions used herein to prepare the freeze-dried compositions of this invention comprise freezing the product to about -40°C or below and pulling a vacuum. The product is then allowed to rise to +5° to +10°C to complete the drying stage. With PEGs of higher molecular weights, lower cooling temperatures and/or longer cooling times are required as compared with those of higher molecular weight.

As previously indicated, the freeze-dried trimelamol-containing compositions of this invention can be reconstituted with water or other aqueous infusion liquid to provide a pharmaceutical preparation suitable for parenteral administration in anti-tumour therapy. More particularly, it is preferred to use an aqueous dextrose solution, eg with a dextrose concentration of 2.5% or 5%, as the infusion liquid, for better

compatibility with human blood. However, there is some reduction in trimelamol stability in dextrose-containing solutions as compared with water alone, although it is found that this reduction in stability can be minimised by adjusting the pH to above pH 6, and preferably to pH 9.0 - 9.5, as by the addition of sodium hydroxide.

The freeze-dried trimelamol-containing compositions of this invention can be reconstituted simply by dissolving the freeze dried material directly in the aqueous infusion liquid. It is, however, usually more convenient to effect the reconstitution in two stages, first adding a small volume of water for injection to the freeze-dried material in the vial, and then subsequently adding the reconstituted solution to a larger volume of dextrose solution or other infusion liquid in an infusion bag in order to achieve the required dilution. Dissolution is substantially instantaneous when the infusion liquid is added in the second stage.

In order to enhance the rate of rehydration of the lyophile, mannitol may if desired be added to the solution of trimelamol in the mixture of polyethylene glycol and water prior to the freezing and lyophilising of the solution, suitably in an amount of 0.1 to 2% by weight of the solution, and preferably at a concentration of about 1% w/w. However, the addition of mannitol may increase the time and/or reduce the temperature required for freeze-drying.

Whilst it is generally preferred that the freeze-dried product should be reconstituted just before use, to avoid any risk of undue loss of trimelamol activity, the improved stability of the present pharmaceutical preparations means that it is less critical should there be some delay between reconstitution and administration.

The pharmaceutical preparations of the present invention ie the reconstituted freeze-dried compositions, are suitable for administration by intravenous infusion. We prefer that the concentration of the trimelamol in the i.v. infusion preparations should be about 4 mg/ml. With higher concentrations of trimelamol there is a greater risk of degradation of the active compound, which may lead to precipitation of a solid, believed to be a polymer of trimelamol, out of solution.

As for the preparations taught in GB-A-2170710, the present trimelamol-containing pharmaceutical preparations may be administered as an anti-tumour agent in the treatment of cancer such as ovarian cancer and malignant lymphoma, by an injection regimen providing up to about 3 grams of trimelamol per square metre of body surface area of the patient.

The invention is illustrated by the Examples which follow:

Example 1

Trimelamol was dissolved in a number of liquid vehicles at a concentration of 0.5 mg/ml of liquid vehicle unless otherwise indicated, and the stability of the resulting solutions was tested by a HPLC procedure using a 150 mm x 4.6 mm i.d. Spherisorb S5 C-8 column, a mobile phase of 15% acetonitrile in water with 0.005M tetrabutyl-ammonium hydroxide, and U.V. detection at 225 nm. At a flow rate of 1.5 ml/minute the trimelamol retention time was 3.2 minutes.

The results are presented in Table 1 below:

TABLE 1

Solvent	Temperature	$t_{50\%}$ (Hours)	$t_{5\%}$ (Hours)
Water	RT	18.99	1.41
Mannitol 0.1%	RT	18.88	1.39
Mannitol (8 mg/ml)[1]	RT	16.1	1.19
PEG 200	RT	23.7	1.76
PEG 300	RT	30.3	2.25
PEG 400	RT	37.6	2.78
PEG 600 (50%) H_2O	RT	17.3	1.28
PEG 600	RT	41.6	3.08
PEG 1000 (75%) H_2O	RT	306.0	22.70
PEG 1500 (50%) H_2O	RT	130.0	9.60
PEG 3400 (50%) H_2O	RT	46.29	3.43
PEG 4000 (25%) H_2O	RT	35.2	2.60
PEG 6000 (25%) H_2O	RT	58.7	4.35
PEG 1000 (75%) H_2O[2]	RT	547.8	40.60
PEG 1000 (70%) H_2O[2]	RT	285.2	21.16
PEG 1000 (60%) H_2O[2]	RT	166.6	12.34
PEG 1000 (50%) H_2O[2]	RT	127.2	9.43
PEG 1000 (75%) H_2O[2]	3-4°C	689.5	51.07
PEG 1000 (75%) H_2O	3-4°C	824.4	61.00

[1] Trimelamol concentration was 4 mg/ml

[2] Trimelamol concentration was 44 mg/ml

PEG = polyethylene glycol

RT = 20-25°C

$t_{5\%}$ = time taken for 5% of the trimelamol in the solution to decompose

$t_{50\%}$ = the time taken for 50% of the trimelamol in the solution to decompose

From Table 1, it will be noted that all the polyethylene glycol solutions improved the stability of the trimelamol at 0.5 mg/ml as compared with either water by itself or water containing 0.1% by weight of mannitol, with the exception of the 50:50 PEG 600/water solution. However, in the case of the lower molecular weight polyethylene glycols (ie PEG 200, PEG 300 and PEG 400), the increase in the half-life of the trimelamol was comparatively small, and since these polyethylene glycols are liquid at room temperature they cannot be freeze-dried. Their use, therefore, is not contemplated by the present invention. PEG 600 represents about the minimum molecular weight polyethylene glycol that is useful, but as the table shows an improvement in stability with this material depends on the use of less than 50% by weight of water.

Table 1 further demonstrates that the optimum improvement in stability is achieved with polyethylene glycol having a molecular weight of 1000.

Example 2

Trimelamol was dissolved in a solution of PEG 1000 (75% w/w) in Water for Injection at a concentration of 44 mg/ml. For this test, the steps of freeze-drying the resulting solution and then reconstituting the solid material were omitted. Thus, aliquots (25 ml) of the resulting solution, each containing 1.1 g of trimelamol, were directly added to 250 ml of each infusion fluid listed in Table 2 to give final trimelamol concentrations of 4 mg/ml. Any pH adjustment was by adding 0.1N sodium hydroxide. Samples were taken at regular intervals for analysis of trimelamol by high performance liquid chromatography.

For comparison, a solution of trimelamol (1000 mg) and mannitol (2000 mg) was prepared in water so as to give a final trimelamol concentration of 4 mg/ml.

The results are shown in Table 2 below. All measurements were made at room temperature (20°-25°C).

TABLE 2

Formulation	Infusion Liquid	$t_{50\%}$ (Hours)	$t_{5\%}$ (Hours)
Trimelamol 44 mg/ml in PEG 1000 (75%)/H_2O (25 ml)	Water 250 ml	23.1	1.73
Trimelamol 44 mg/ml in PEG 1000 (75%)/H_2O (25 ml)	250 ml 5% Dextrose pH 9.1 (adjusted with 0.1N NaOH)	16.94	1.26
Trimelamol 44 mg/ml in PEG 1000 (75%)/H_2O (25 ml)	250 ml 5% Dextrose pH 4.1 (unadjusted)	14.38	1.07
Trimelamol 44 mg/ml in PEG 1000 (75%)/H_2O (25 ml)	2.5% Dextrose pH 9.3	21.50	1.59
Trimelamol 44 mg/ml in PEG 1000 (75%)/H_2O (25 ml)	2.5% Dextrose pH 4.3	19.35	1.43
Trimelamol mannitol	250 ml 5% Dextrose pH 9.1	11.78	0.872

The results show that there is a significant improvement in stability for the compositions prepared, as taught in this invention, from PEG-containing aqueous solutions, as compared to that obtained from a mannitol-containing solution of the kind suggested in GB-A-2170710.

The Table also demonstrates that still further improvements in stability are achieved when the pH of the

dextrose-containing infusion liquid is adjusted to within the range pH 9-9.5.

Example 3

Polyethylene glycol 1000 (3 kg) was heated at 40°C until molten and then mixed with Water for Injection (1 kg) to produce a clear to pale-amber coloured solution vehicle. Trimelamol (132.0 g) was dissolved in three litres of the above PEG 1000 (75% w/w)/H_2O vehicle by rapid stirring at room temperature, and the resulting solution filtered through a 0.2 micron filter. Aliquots (25 ml) of the resulting sterile solution were next filled into 50 ml volume vials.

Freeze-drying plugs were partially inserted into each vial, and the vials loaded into an Edwards Minifast 1700 freeze-dryer at room temperature. Product temperature was monitored using resistance thermometers. The product was cooled to -45°C and a vacuum established. Drying was completed over 48 hours, and when the temperature of the product equalled room temperature the vials were stoppered pneumatically under vacuum and the vacuum then broken. The freeze-dried product was a white waxy solid .

Reconstitution with 6.25 ml Water for Injection gave a clear particle-free solution.

Analysis by high performance liquid chromatography of the reconstituted product showed that no significant degradation had occurred during the processing steps.

Example 4

Polyethylene glycol 1000 (4.9 kg) was heated at 40°C until molten and then mixed with a solution of mannitol (0.07 kg) in Water for Injection (2.03 kg) to produce a clear to pale-amber coloured solution vehicle. Trimelamol (264 g) was dissolved in six litres of the above aqueous PEG 1000 vehicle (70% w/w/mannitol 1% w/w) by rapid stirring at room temperature, and the resulting solution filtered through a 0.2 micron filter. Aliquots of 37.5 ml of the resulting sterile solution were filled into 70 ml volume vials.

Freeze-drying plugs were partially inserted into each vial, and the vials loaded into an Edwards Minifast 1700 freeze-dryer at room temperature for freeze-drying under vacuum at -45°C. It was found that these conditions had to be maintained for 10 days to complete the drying.

The freeze-dried product was a white waxy but porous solid.

Reconstitution with 11 ml of Water for Injection was much more rapid than that in Example 3 due to the inclusion of mannitol in the formulation. A clear particle-free solution resulted. Analysis by HPLC of the reconstituted product showed that no significant degradation had occurred during processing stages.

Example 5

Polyethylene glycol 3400 (0.6 kg) was dissolved in Water for Injection (0.6 kg) to produce a clear to pale-amber coloured solution vehicle. Trimelamol 30 g was dissolved in 1000 mls of the above aqueous-PEG 3400 (50:50) vehicle by rapid stirring at room temperature to give a solution of trimelamol (30 mg/ml). The resulting

solution was filtered through a 0.22 micron filter and aliquots (18.33 ml) of the resulting sterile solution were filled into 70 ml volume vials. Freeze-drying plugs were partially inserted into each vial, and the vials loaded into the freeze-dryer for freeze-drying according to the conditions described for Example 4. However, in this experiment, freeze-drying was completed in five days.

The freeze-dried product was a white waxy, but porous solid, the bulk of which reconstituted almost instantaneously upon the addition of 9.7 ml Water for Injection.

CLAIMS:

1. A solid freeze-dried composition comprising trimelamol and a polyethylene glycol having a molecular weight of at least 600.

2. A composition according to Claim 1, wherein said polyethylene glycol has a molecular weight of from 600 to 4000.

3. A composition according to Claim 2, wherein said polyethylene glycol has a molecular weight of from 900 to 1100.

4. A composition according to Claim 3, wherein said polyethylene glycol has molecular weight of approximately 1000.

5. A composition according to Claim 2, wherein said polyethylene glycol has a molecular weight of approximately 3400.

6. A composition according to any preceding claim, containing from 5% to 6.5% by weight of trimelamol.

7. A composition according to any preceding claim and comprising also mannitol.

8. A process for preparing a solid freeze-dried composition comprising trimelamol, which process comprises dissolving trimelamol in a mixture of water and a polyethylene glycol having a molecular weight of at least 600, freezing the resulting solution and subjecting it to lyophilisation.

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9. A process according to Claim 8, wherein the proportion of water in said mixture is not substantially in excess of that required to achieve complete solubilization of said polyethylene glycol.

10. A process according to Claim 8 or Claim 9, wherein said polyethylene glycol has a molecular weight of from 600 to 4000.

11. A process according to Claim 10, wherein said polyethylene glycol has a molecular weight of from 900 to 1100.

12. A process according to Claim 11, wherein said polyethylene glycol has a molecular weight of approximately 1000.

13. A process according to Claim 12, wherein said trimelamol is dissolved in said mixture of water and polyethylene glycol at a concentration of from 40-50 mg of trimelamol per ml of said mixture of water and polyethylene glycol.

14. A process according to Claim 13, wherein said concentration is approximately 44 mg/ml.

15. A process according to Claim 10, wherein said polyethylene glycol has a molecular weight of approximately 3400.

16. A process according to any one of Claims 8-15, wherein mannitol is also dissolved in said mixture of water and polyethylene glycol in amount such that the resulting solution contains from 0.1 to 2% by weight of mannitol.

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17. A pharmaceutical preparation suitable for parenteral administration, which comprises a composition as defined in any one of Claims 1-7, or as prepared by a process according to any one of Claims 8-16, dissolved in an infusion liquid suitable for parenteral administration.

18. A preparation according to Claim 17, and having a pH adjusted to from 6 to 9.5.

19. A preparation according to Claim 18, and having a pH adjusted to from 9.0 to 9.5.

20. A preparation according to any one of Claims 17-19, wherein said infusion liquid is an aqueous 2.5% dextrose solution.

21. A preparation according to any one of Claims 17-20, and having a concentration of trimelamol of approximately 4 mg/ml of infusion liquid.

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