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(54) Title: DERMATOLOGICAL TREATMENT COMPOSITIONS CONTAINING DIMETHYLSULPHONE AND A SULFUR CONTAINING AMINO ACID

(57) Abstract

The present invention relates to synergistic compositions comprising methylsulphonylmethane and a sulphur containing amino acid and their use in formulations and methods of treatment for protecting and improving skin condition.

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DERMATOLOGICAL TREATMENT COMPOSITIONS CONTAINING DIMETHYLSULPHONE AND A SULFUR CONTAINING AMINO ACID.

The present invention relates to a pharmaceutical synergistic composition suitable for use in the treatment of dermatological diseases and disorders and for improving the condition of the skin.

Due to its susceptibility to environmental pollutants, irritants and noxious agents and due to its large surface area, the skin is a common site for a wide variety of diseases and disorders be they degenerative, inflammatory, endocrinal, metabolic or neoplastic. While there is a large number of dermatological agents on the market, these do little to combat the mediators of skin injury or diseases, or to enhance the repair of skin in the aftermath of injury or diseases, or to sustain the integrity of skin against recurrence of such disorders or protect against their development. Moreover, many of the therapeutic modalities currently available have limitations pertaining to patient selection or application. An object of the present invention is, thus, to introduce a synergistic pharmaceutical composition which not only overcomes these limitations but also combats the forces directly involved in mediating disease or injury processes in the skin, enhances the repair of any injury or damage sustained and upholds the integrity of the skin by increasing its resistance against the recurrence of the diseases or disorders which had been treated.

The present invention provides a synergistic pharmaceutical composition comprising methyl sulphonylmethane and a sulphur containing amino acid such as cysteine, cystine, cysteamine, methionine carboxyl esterified and S-methyl methionine sulphonium derivatives. The carboxyl group may have been esterified,

preferably by lower alkyl having 1 to 6 carbon atoms e.g. methyl. Also included are S-methyl substituted, ternary sulphonium, derivatives of methionine such as methionine-S- methylsulphonium bromide, iodide and chloride.

It will be noted that at least some of the abovementioned compounds have one or more optically active centres, in particular in the case of the amino acids at the amino- and carboxyl-substituted carbon. For the avoidance of doubt therefore it is observed that the present invention extends to both individual isomers such as D-and L-isomers and enantiomers, and, in the case where two or more optically active centres are present, diastereoisomers, as well as mixtures of isomers including racemic DL mixtures.

While it has unexpectedly been found that methyl sulphonylmethane reverses the degenerative and ageing processes occurring in the skin, affords therapy against a wide variety of diseases be they inflammatory, metabolic, endocrinal or traumatic, such as wounds and ulcers, and protects the skin against the recurrence of these diseases and disorders by sustaining its physiochemical properties thereby increasing its resistance to injury from noxious substances; it was particularly surprising to discover that the addition of a sulphur-containing amino acid augments these therapeutic advantages in a synergistic manner. This action refers to the sum total of the actions of the individual active ingredients being less than that achieved by their combination together. It was also observed that the composition has the advantageous property of adhesion to the skin thereby affording prolonged contact with the treatment area and an enhanced therapeutic delivery. In vivo and in vitro experiments demonstrated that the compositions of the present

invention exhibit the following actions

1. scavenging oxygen-derived free radicals which are cytotoxic agents implicated in tissue damage and injury besides impairing the process of healing and repair.
2. cytoprotection which refers to sustaining the physio-chemical properties of biological tissues, thus increasing their resistance to noxious stimuli.
3. biosynthesis and donation of sulphur which effects enhanced repair and healing.

While not limiting the scope of this invention, it is believed that one or more of these actions is to a greater or lesser extent responsible for the beneficial effects afforded by the compositions of the invention.

In accordance with the present invention application onto the skin improves its condition in a number of ways including:

a. ameliorating the severity of degenerative changes that had already occurred.
b. protection against the progression of such changes.
c. increasing the resistance of the skin to damage by sustaining its physiochemical properties, thus affording protection against the adverse effects of environmental irritants, pollutants and noxious agents.
d. affording a potent sunscreening effect thereby protecting against ultraviolet sunrays which precipitate skin degeneration and premature ageing besides increasing the susceptibility to malignant transformation.
e. enhancing the healing of inflamatory, endocrinal and metabolic disorders.
f. enhancing the healing of wounds, ulcers, fissures and sinuses in addition to increasing the take and healing of skin grafts.

The improvements in skin condition can also include maintenance of its vitality, smoothness, firmness and texture.

Advantageously, there is also included a vasodilator substance such as menthol in order to further increase the effectiveness of the composition in the skin. Moreover, enhanced therapeutic gains have been noted with the incorporation of vitamins A and E in compositions of the present invention.

In addition, the present invention provides a pharmaceutical composition comprising methyl sulphonylmethane and a sulphur containing amino acid, in intimate admixture with a physiologically acceptable carrier therefor, for use in improving the condition of the skin.

In a further aspect, this invention provides a topical formulation comprising methylsulphonylmethane and a suphur containing amino acid in intimate admixture with a pharmaceutically acceptable vehicle. This vehicle should not be deleterious to biological tissues or incompatible with any of the ingredients of the formulation. Since some individuals have more sensitive skin than others, alternative vehicles to those used normally may have to be tried.

Suitable vehicles are well known in the art and are presented in all its standard publications such as the British National Formulary and British Pharmacopoeia. They include ointment and cream bases, lotions, pastes, jellies, sprays, aerosols and bath oils. Ointments and creams may contain oleaginous absorption colloidal clays, thickening agents such as gum tragacanth or sodium alginate and other pharmaceutically acceptable accessory

ingredients such as humectants, preservatives, buffers and antioxidants which have utility in such formulations. In general cream formulations are preferred as being most acceptable to the majority of users. A particularly convenient base is one utilizing cetomacrogol, comprising for example 30% w/v cetomacrogol emulsifying ointment (30% w/v cetomacrogol emulsifying wax, 20% w/v liquid paraffin wax, 50% white soft paraffin) in freshly boiled and cooled purified water with for example 0.1% w/v chlorocresol or 0.08% w/v propyl hydroxybenzoate, 0.15% w/v methyl hydroxybenzoate and 1.5% w/v benzyl alcohol.

The topical formulations of the invention contain at least 0.5% w/w of each of its active ingredients, preferably from 1 to 20% w/w and most preferably from 1 to 10%, e.g. 5% methyl sulphonylmethane and 2% cysteine or methionine. Where menthol is included, this is generally used in an amount from 1 to 20% w/w and most preferably from 1 to 5% w/w.

In addition, the invention being presented can be administered orally or parenterally in a suitable vehicle, in particular by intravenous injection.

For oral administration the ingredients of the invention and any accompanying material may be presented as a draught in water or in a syrup, in capsules, sachets, boluses or tablets, as an aqueous or oleaginous solution or suspension or in suspension in a syrup, such suspensions optionally including suspending agents or as an oil-in-water or water-in-oil emulsion.

Included in the oral route, the compositions of the invention can be taken in an alcoholic drink be that a spirit, wine or beer. The non-alcoholic forms of these drinks may also serve as vehicles for the oral consumption

of the invention. Moreover, the compositions of the present invention may be added to fruit juices, mineral waters be they carbonated or not, and to all forms of soft drinks.

The compositions of the invention can be directly delivered to the lung via smoke and in this respect, they can be added as a powder or solution to tobacco leaves or to the tobacco of cigarettes, cigars and pipes. The compositions of the invention may also be included as a solution or powder in cigarette filters or small delivery compartments incorported in the cigarette. This compartment may also contain the composition of the invention in granules which evaporate upon contact with the smoke thereby delivering their active ingredients to be carried by the smoke.

Where desirable or necessary flavouring, sweetening, preserving, thickening or emulsifying agents may be included in the formulation.

Tablets may contain the ingredients of the invention and any accompanying material as a powder or granules optionally mixed with binders, lubricants, inert diluents or surface-active or dispersing agents.

For parenteral administration the compositions of this invention and any accompanying material may be presented in sterile solutions or suspensions in aqueous or oleaginous vehicles, which may also contain preservatives, antioxidants and material for rendering the solution or suspension isotonic with the recipient's blood. Such formulations may conveniently be presented in unit-dose or multi-dose sealed containers.

For administration orally or parenterally the compositions of this invention are preferably presented in solution, suspension, or emulsion at a concentration of from 0.5% to 20% w/v, more preferably 2 to 5% w/v in unit or multidose form. When presented in unit dose form, each unit dose preferably contains from 100 to 500 mg of each of its ingredients. This dosage may be given once or more daily preferably at intervals of from 2 to 8 hours, most preferably every 6 hours. Advantageously, the ingredients of the invention are administered in a slow release or a sustained release vehicle, various suitable vehicles of this type being known in the art.

For topical therapy the composition is applied onto the skin from 1 to 3 times a day whereby it is spread over the whole area to be treated and massaged in for about 3 to 5 minutes. It is advisable to leave the evening application overnight if repair of any skin damage is to be realised. It is not necessary to wash away the previous application so as to apply a fresh one, however this may be simply done using warm water alone.

Further preferred features and advantages of the invention will be realized by way of the following examples which are being presented for illustration purposes only.

Example 1 -

Preparation of Topical Formulations for Treating the Skin

A. Methylsulphonylmethane 5g
DL-cysteine hydrochloride 2g
cetomacrogol 'A' add to 100g

B. methylsulphonylmethane 5g
DL-cysteine hydrochloride 2g
menthol crystals 1g
cetomacrogol 'A' add to 100g

C. methylsulphonylmethane 5g
methylmethionine sulphonium chloride 2g
cetomacrogol 'A' add to 100g

D. methylsulphonylmethane 5g
methylmethionine sulphonium chloride 2g
menthol crystals 1g
cetomacrogol 'A' add to 100g

These formulations are prepared in a medium at 25°C temperature. Five grams of methylsulphonylmethane are mixed with 2 grams of cysteine hydrochloride or methylmethionine sulphonium chloride in a glass or stainless steel container. One gram of finely ground menthol is then added. Addition of cetomacrogol to 100g is undertaken and the whole composition mixed for 10 minutes. The product is then left to stand for at least 15 minutes before being placed in opaque airtight glass containers and stored at an optimal temperature of 26°C, and most preferably no more, away from direct sunlight. After preparation, none of these formulas should be used for at least 24 hours, should not be left exposed to the air for long periods of time and should not be directly exposed to the sun.

Example 2 - Use of Topical Cream Formulation

The creams described in Example 1 can be applied several times a day and the evening application may be left overnight and washed away the following morning using warm water with or without soap. Treatment may be for a few days or months depending on each case. Generally, for protective purposes the formulation is applied once daily onto the parts of the skin to be protected, e.g. face and limbs, prior to exposure to environmental irritants or sunlight. For therapeutic purposes, application is governed by the nature of the disorder to be treated, e.g. dermatitis 5-10 days (unless caused by varicose veins when treatment is extended to 4 weeks), wound healing 2-3 weeks, varicose ulceration 12-16 weeks. In these cases, the application is 2 to 3 times every day most preferably at 8 hourly intervals. Maintenance therapy after successful treatment or to sustain the condition of skin may require a once daily application to a particular part of the skin for months, years or even indefinitely.

Example 3 - Detailed Evaluation of the Compositions

A. In groups of twenty Sprague-Dawley rats of either sex allocated at random and weighing 220g to 290g, the influence of methylsulphonylmethane and cysteine on acute damage of the gastric mucosa was studied. Solutions of cysteine or methylsulphonylmethane were prepared with double distilled water. All drugs were gavaged into the stomach under light ether anaesthesia by orogastric instillation using a 6FG feeding tube. Animals were fasted for 24 hours then one ml of cysteine and/or methylsulphonylmethane or double distilled water was instilled into the stomach. One hour later gavage with 1 ml of 40% ethanol or double distilled water was carried out. Animals were killed two hours later by ether

overdose, their gastric acid secretion was collected then analysed for the H+ output by titration to pH7 with 0.1M NaOH and their stomachs were pinned out and examined for the extent of alcohol-induced acute gastric mucosal injury (mm^2 surface area expressed as the mean $\pm$ the standard error of the mean, SEM, for each group). The following observations were made:

Treatment (n=20)	% Incidence of animals showing injury	Injury area in mm^2 (mean ± SEM)
distilled water + distilled water	0%	0
distilled water + ethanol	100%	40 ± 1.2
0.5% MSM + ethanol	80%	31.7 ± 1.4
1% MSM + ethanol	70%	25.1 ± 0.9
5% MSM + ethanol	60%	18.3 ± 1.1
10% MSM + ethanol	60%	17.9 ± 1.5
20% MSM + ethanol	60%	19.1 ± 1.2
0.5% cysteine + ethanol	90%	32.3 ± 1.6
1% cysteine + ethanol	70%	25.1 ± 1.1
5% cysteine + ethanol	50%	16.2 ± 0.8
10% cysteine + ethanol	50%	16.1 ± 0.9
20% cysteine + ethanol	50%	15.9 ± 1.2
0.5% MSM & 0.5% cysteine + ethanol	50%	12.3 ± 1.4
1% MSM & 1% cysteine + ethanol	25%	3.2 ± 0.4
5% MSM & 5% cysteine + ethanol	0%	0
10% MSM & 10% cysteine + ethanol	0%	0
20% MSM & 20% cysteine + ethanol	0%	0

MSM : methylsulphonylmethane

Alcohol disrupts the gastric mucosal barrier causing hydrogen ion back diffusion and coagulative necrosis. The alcohol-induced acute gastric mucosal injury has been

shown to be mediated by oxygen-derived free radicals. Dose dependent protection against this injury was afforded by each of cysteine and methylsulphonylmethane. Moreover, administration of these agents together exhibited a synergistic influence in protection against tissue damage. No influences on the gastric acid secretion were associated with these actions.

It is, thus, construed that each of cysteine and methylsulphonylmethane exhibit cytoprotective activities against tissue injury and interact with each other in this respect synergistically. The mechanism of this action is believed to be scavenging the oxygen-derived free radicals which mediate tissue damage.

B. The ability of methylsulphonylmethane and/or cysteine or methylmethionine sulphonium chloride to influence the healing rate of the alcohol-induced acute gastric mucosal injury was then examined. Groups of twenty Sprague-Dawley rats of either sex allocated at random and weighing 190g to 270 g were fasted for 24 hours then 1ml of 40% ethanol or double distilled water was gavaged into the stomach by orogastric instillation under ether anaesthesia using a 6FG feeding tube. One hour, 24 hours and 48 hours later animals were similarly gavaged with 1ml of double distilled water or solutions of cysteine, methylmethionine sulphonium chloride (MMSC) and/or methylsulphonylmethane (MSM) prepared in double distilled water. Ten animals from each group were killed by ether overdose six hours after each of the second and third instillations, their gastric acid secretion collected and analysed for the H+ output as stated above and their stomachs pinned out and examined to assess the integrity of the mucosa and to determine the presence or absence of injury. The following observations were made:

Treatment n = 20	% Incidence of animals showing injury after the second dose	after the third dose
distilled water + distilled water	0%	0%
ethanol + distilled water	100%	80%
ethanol and 0.5% MSM	80%	60%
ethanol + 1% MSM	60%	50%
ethanol + 5% MSM	50%	50%
ethanol + 10% MSM	50%	50%
ethanol + 20% MSM	50%	50%
ethanol + 0.5% cysteine	90%	80%
ethanol + 1% cysteine	80%	70%
ethanol + 5% cysteine	60%	60%
ethanol + 10% cysteine	60%	60%
ethanol + 20% cysteine	60%	60%
ethanol + 0.5% MMSC	90%	80%
ethanol + 1% MMSC	80%	70%
ethanol + 5% MMSC	70%	60%
ethanol + 10% MMSC	60%	60%
ethanol + 20% MMSC	60%	60%
ethanol + 0.5% MSM + 0.5% cysteine	60%	30%
ethanol + 1% MSM + 1% cysteine	30%	10%
ethanol + 5% MSM + 5% cysteine	0%	0%
ethanol + 10% MSM + 10% cysteine	0%	0%
ethanol + 20% MSM + 20% cysteine	0%	0%
ethanol + 0.5% MSM + 0.5% MMSC	50%	30%
ethanol + 1% MSM + 1% MMSC	20%	10%
ethanol + 5% MSM + 5% MMSC	0%	0%
ethanol + 10% MSM + 10% MMSC	0%	0%
ethanol + 20% MSM + 20% MMSC	0%	0%

MSM : methylsulphonylmethane

MMSC : methylmethionine sulphonium chloride

Administration of methylsulphonylmethane and/or cysteine or methylmethionine sulphonium chloride did not influence gastric acid secretion in a significant manner, thus, the actions of these agents is independent of and not mediated via acid output. The results show that each of cysteine, methylmethionine sulphonium chloride, and methylsulphonylmethane stimulates the healing of acute mucosal damage and that they interact synergistically with each other towards this objective. Since no influences on the state of acid secretion were noted, it is construed that the enhancement of healing was achieved via mechanisms operating at cellular levels such as biosynthesis and sulphur donation in addition to scavenging oxygen-derived free radicals which impair healing by a direct deleterious effect upon tissues.

On the basis of these two sets of experiments it is realized that sulphur containing aminoacids and methylsulphonylmethane protect tissues, including the skin, against injury (cytoprotection) and enhance the healing of the damage they have incurred and that these beneficial actions are synergistically enhanced by their administration together. Moreover, it appears that the concentrations of each of these two agents as used in the formulations of Example 1 is generally optimal.

Example 4 - Clinical Trials

Prospective randomized double blind controlled trials were carried out in patients who were randomized to the control (cetomacrogol A) or active therapy groups by drawing sealed envelopes. The treatment code was only broken at the end of the trial period. All the formulations used were prepared as detailed in Example 1.

After exclusion of the patients who were not fully evaluable, the following observations were made:

A. The sunscreening effect of the formulation described in Example 1.A and its ability to protect patients against skin burns, erythema, itching and scaling following a few hours' exposure to the sun were examined. There were 12 controls (10 women and 2 men, age range 18 to 31 years, mean 25) and 14 treatment cases (8 women and 6 men, age range 18 to 39 years, mean 28), who were treated for 7 days (the period of direct exposure to sunlight) by the daily application of the cream prior to the exposure, in a liberal amount over the area to be protected. Complete protection (100%) was afforded by active therapy against all the adverse effects produced by exposure to the sunlight. This protection extended to prevention of skin burn or irritation. Controls had no protection at all.

B. The therapeutic effect of the formulation described in Example 1.C. in the treatment of contact dermatitis was examined. Treatment was applied twice every day for 5 days. There were 10 controls (5 men and 5 women, age range 18 to 34 years, mean 22) and 12 treatment cases (8 men and 4 women, age range 20 to 39 years, mean 26). While complete healing of the dermatitis was noted at the end of the study in all the active treatment cases (100%), only one control (10%) demonstrated this favourable response.

C. Hyperkeratosis is a proliferative skin disorder which represents hyperplasia of the epidermis and may have a malignant potential. The condition affects the exposed parts of the skin of middle aged people, particularly the face and upper limbs. Treatment of these lesions by the twice daily application of the formulation described in Example 1.D. for four weeks (21 cases, 12 women and 11 men

with an age range of 55 to 69 years, mean 63) caused complete shedding of the lesions and their replacement by normal skin in all cases. No response was noted in any of the control cases (20 patients, 13 women and 7 men with an age range of 54 to 67 years, mean 61). It is, thus, construed that the formulation used stimulates repair of skin lesions.

D. The therapeutic efficacy of the formulation described in Example 1.B. in controlling the symptoms caused by skin burns resulting from exposure to the sun and in treating this condition was examined by its application onto the affected parts of skin every 8 hours for 10 days. There were 11 treatment cases (6 men and 5 women with an age range of 21 to 33 years, means 26) and 13 controls (8 women and 5 men with an age range of 19 to 35 years, mean 25). Within 24 hours of treatment all the symptoms of the burns (hyperaesthesia, itching, pain) were completely controlled in all of the members of the active therapy group but in none of the controls. By the 5th to the 7th day of active treatment all the burnt skin had been shed off and a return to normal skin was achieved by the 10th day of treatment in all cases. Only 2 controls (15%) had symptomatic relief within 24 hours and 4 controls only (31%) had shedding of their burnt skin after 5 to 7 days and appearance of normal skin by the 10th day of treatment.

E. The efficacy of the formulation described in Example 1.D in maintaining the skin smoothness and avoiding its roughening and/or fissuring was examined in a group of women who were already using some form of beauty cream to this end and who had no previous history of any skin diseases. All the cases entered into the study abstained from using their original cream and were then randomized to the control or active therapy group and instructed to

use their cream whether for the hands and/or face once daily before retiring to bed and to leave it overnight for 2 months. There were 20 cases in the active therapy group (age range 18 to 26 years, mean 23) and 21 cases in the control group (age range 18 to 25 years, mean 21). At the end of the study, 3 controls (14%) and 18 active therapy cases (90%) stated that the cream they had used was superior to their original one in terms of acting as a beauty cream which keeps the skin smooth and avoids its roughening. Thus, the ability of the formulation used in maintaining the vitality of the skin and in increasing its resistance to environmental irritants and damage is established.

F. Women who presented with obvious dermatological signs of skin ageing mainly manifested on the face and hands (loss of firmness, roughening, thickening, keratosis, fissuring and wrinkles), were randomized to be treated with the formulation described in Example 1.A or to the control group and were treated twice daily for six months then a single overnight application for another six months. There were 43 active treatment (age range 53 to 67 years, mean 56) and 41 control (age range 52 to 65 years, mean 57) cases who were fully evaluable. After 3 months, all cases in the active therapy group were observed to have acquired smoother non-fissured skin, which had shed off all the keratotic lesions. By six months, these advantages had extended to making the skin firmer with much less conspicuous wrinkles. None of the cases in the control group realized any benefits in terms of ameliorating the severity of degenerative changes.

After one year of treatment, all the active therapy cases had smooth, firm, non-fissured skin without any keratosis and the wrinkles were much less conspicuous and almost invisible in at least 36 cases (84%). These gains were

not realized in any member of the control group.

It was, thus, construed that the formulation used is extremely effective in arresting the degenerative skin changes incurred by ageing and in ameliorating the severity of those changes which have already occurred.

G. Women with obvious facial wrinkles were randomized to receive the formulation described in Example 1.D or to the control group and were treated twice daily for 6 months then once daily (overnight application) for 18 months. The wrinkles were mapped on special charts and any improvements in their appearance, in terms of making them less visible, were calculated as percentage improvements. While the control group (n = 45, age range 50 to 59 years, mean 54) had after six months a 5% improvement, the active therapy group (n = 48, age range 48 to 59 years, mean 56) achieved a 71% improvement over this time period. At the end of the study (2 years) the percentage improvement in the control group has extended to 10% but this improvement had reached 89% in the active therapy group. The significant improvement in the wrinkles' appearance was associated with a similar improvement in the smoothness and firmness of the facial skin. The latter is probably a major factor behind the wrinkles becoming less conspicuous and apparent. Moreover, at the end of the study, no new wrinkles were observed to have developed.

These advantages reflect the benefits of using the formulation in combating the severity of skin degenerative changes and in impairing their progression.

H. Following mass closure and subcuticular approximation of the skin for upper midline laparotomy incisions, 50 patients were randomized to the active treatment group (22

men and 28 women with an age range of 18 to 53 years, mean 31) where the formulation described in Example 1.D was applied once every day with a conventional dressing onto the wound for 10 days and another 52 patients (23 men and 29 women with an age range of 18 to 57 years, mean 34) were randomized to the control group and similarly treated. None of the active treatment cases were observed throughout one year and six months of follow-up to develop wound dehiscence, incisional herniation, wound pigmentation or hypertrophic scarring. In the control group one patient developed wound dehiscence, 2 patients (4%) developed incisional herniation, 3 patients (6%) developed wound hyperpigmentation, and another 3 patients (6%) developed keloids.

It is, consequently, concluded that the formulation used not only stimulated wound healing but also sustains the integrity of the repair.

I. Twelve quadriplegic patients (8 men and 4 women, age range 23 to 59 years, mean 31) were randomized to twice daily application of the formulation listed under Example 1.C with physiotherapy for six months, and another 10 patients (6 men and 4 women, age range 19 to 53 years, mean 29) were randomized to the control group and similarly treated. No pressure ulceration (bed sores) occurred in any member of the active therapy group but developed in 3 members of the control group (30%).

Further evidence is, therefore, provided that the formula used increases the resistance of the skin to mechanical trauma.

J. In patients who had sustained a third degree burn of a surface area between 9 and 18%, treatment was applied onto the burnt area twice daily for 3 weeks. Twenty-five

patients (15 men and 10 women, age range 18 to 41 years, mean 29) were randomized to receive the formulation described in Example 1.B and another 26 patients were randomized to the control group (18 men and 8 women, age range 18 to 44 years, mean 30). Active treatment was significantly more effective than the control cream in reducing the degree of pain, discomfort, and itching produced by the burn and resulted in a cosmetically superior healing with much less pigmentation or discolouration. Therefore, the formulation used enhances the healing process of the skin.

K. Following excision and partial thickness skin grafting for deep burns extending over an area of 9 to 12% of the lower limbs, a liberal amount of the formulation described in Example 1A or the control cream was applied over the grafted area before dressing it up. Twelve patients were randomized to the active therapy group (8 women and 4 men, age range 18 to 38 years, mean 25) and 14 patients were randomized to the control group (7 women and 7 men, age range 18 to 43 years, mean 29). When the dressing was removed five days after grafting, a completely successful take was observed in all members of the active therapy group. Two controls (14%), however, showed failure of graft taking. This study reflects the ability of the active treatment used to enhance skin grafting.

L. Patients who presented with dermatitis of the medial side of the lower third of the leg caused by incompetent perforating veins and manifested by itching, oozing, erythema and scaling, were randomized to receive the formulation described in Example 1.C or to the control group and were treated for four weeks by elevation of the foot overnight and when sitting or resting, once daily application of the cream and below-knee graduated compression bandages applied over open-weave terylene and

cotton gauze dressings. The compression bandages exerted an ankle pressure of around 40 mmHg falling to around 16 mmHg below the knee and comprised a layer of crepe bandages followed by a layer of Elset bandages then a layer of Coban cohesive bandages. Thirty two patients (20 women and 12 men, age range 30 to 71 years, mean 55) were randomized to the active treatment group and 30 patients (19 women and 11 men, age range 32 to 69 years, mean 53) were randomized to the control group. At the end of the study, all the active treatment cases (100%) had complete healing of the dermatitis and a return to a healthy skin state. Only 21 patients (70%) in the control group reached this satisfactory outcome.

M. Patients presenting with varicose ulceration on the medial side of the lower part of the leg, less than 10 cm^2 surface area, not previously treated and not infected or associated with gross leg oedema were randomized to treatment with the formulation described in Example 1.D or to the control group. Forty patients (25 women and 15 men, age range 28 to 71 years, mean 57) were randomized to the active treatment group and 42 patients (23 women and 19 men, age range 30 to 74 years, mean 54) were randomized to the control group. Any devitalized skin surrounding the ulceration was removed by warm olive oil and the ulcer was then washed with physiological saline and any loose tissue removed. After application of the cream, the ulcer was dressed by open-weave terylene and cotton gauze. The skin surrounding the ulcer was treated with propylene glycol monostearate then a below knee graduated compression bandage as described above was applied over the dressing. This procedure was repeated every day for 7 days then weekly until the ulceration had healed or until the end point of the study at 3 months. Patients were advised to sleep with the foot of the bed raised, to avoid long periods of standing without exercising the calf pump,

to walk whenever possible and to elevate the leg when sitting.

Active treatment produced complete healing of the ulceration in 37 patients (93%) and in 28 controls (67%) thus demonstrating the ability of the formulation used to stimulate the healing of varicose ulceration.

Example 5 - Toxicity Studies

Solutions of methylsulphonylmethane (MSM) with DL-cysteine hydrochloride (CYS) or methylmethionine sulphonium chloride (MMSC) were prepared in double distilled water to provide the following compositions:

1. 1% MSM + 1% CYS
2. 5% MSM + 5% CYS
3. 10% MSM + 10% CYS
4. 15% MSM + 15% CYS
5. 1% MSM + 1% MMSC
6. 5% MSM + 5% MMSC
7. 10% MSM + 10% MMSC
8 15% MSM + 15% MMSC

Groups of ten Sprague-Dawley rats of either sex weighing 200 to 300 grams were fasted for 24 hours then given one ml of one of the above mentioned formulations by intraperitoneal injection into the left iliac fossa, intramuscular injection or orogastric instillation under light ether anaesthesia. Animals were observed for 24 hours then allowed access to food and drink and observed for another six days. They were then killed by ether overdose and subjected to a full necropsy.

The same study was repeated in the Syrian golden hamster (weighing 150 to 200 grams) and in nude mice (40 to 50 grams of weight). In the latter species the fast before

and after drug administration was reduced to 12 hours and only 0.25 ml of each formulation was given.

There were no deaths among the groups and discomfort or obvious distress, excitation, drowsiness, withdrawal, depression, vomiting or diarrhoea was not encountered in any case. Necropsy showed no changes caused by the medication.

It is, thus, construed that over a wide dosage range,the formulations employed do not exhibit any adverse effects or noticeable acute toxicity making them safe to use within the elected therapeutic range.

In groups of ten healthy male volunteers of ages ranging between 20 and 48 years, 5 grams of each of the formulations described in Example 1 was applied onto the face, neck and shoulders once in the morning, once again in the morning and then in the evening or once every eight hours. Treatment lasted for ten days and the applications were spread over the skin of the face, neck and shoulders, gently rubbed in for a few minutes then left for at least 3 hours before being washed away with warm water.

Physical examination was carried out once every day during the ten days of study. Similarly, standard haematological and biochemical tests (including liver and renal function tests, blood glucose, serum amylase and blood gases) with urine examination, were made every day. An electrocardiogram with cardiac enzymes' level estimation, were performed every other day.

All the therapeutic regimens were comfortably tolerated without any apparent allergic or adverse reactions. In addition, no toxicity was produced by the treatment used. It is, therefore, concluded that the formulations

described in Example 1 are safe for use in main within the recommended doses.

It will be appreciated that although the methylsulphonylmethane and sulphur containing amino acid are advantageously used in generally equal amounts, by weight, in the synergistic compositions of the invention, other ratios may also be used. Generally there is used a ratio of from 10:1 to 1:10, preferably from 5:1 to 1:5, most preferably about 1:1, by weight. It will be understood though that preferred proportions may differ from one amino acid to another and as noted hereinbefore preferred proportions of methylsulphonylmethane to cysteine or methionine are approximately 5:2 or 5:1.

CLAIMS

1. A synergistic composition, which composition comprises methylsulphonylmethane and a sulphur containing amino acid.

2. A composition as claimed in claim 1 wherein said amino acid is selected from cysteine, cysteamine, cystine, methionine wherein the carboxyl group has been esterified, and S-methyl substituted, ternary sulphonium, derivatives of methionine.

3. A composition as claimed in claim 2 wherein said carboxyl group has been esterified by lower alkyl having from 1 to 6 carbon atoms.

4. A composition as claimed in claim 2 wherein said methionine derivative comprises methionine-S-methyl sulphonium bromide, iodide or chloride.

5. A composition according to any one of claims 1 to 4 wherein said methylsulphonyl methane and the amino acid are present in a ratio of from 1:5 to 5:1 by weight.

6. A composition comprising methylsulphonylmethane and a sulphur containing amino acid for use in the preparation of a formulation for the treatment of dermatological conditions, diseases and injuries.

7. A formulation comprising a composition according to any one of claims 1 to 5 in intimate admixture with a physiologically acceptable carrier therefor, for use in the treatment of dermatological conditions, diseases and injuries.

8. A topical formulation according to claim 7 which

contains at least 0.5% w/w of each of methylsulphonyl methane and the amino acid.

9. A formulation according to claim 8 which contains from 1 to 20% w/w of each of methylsulphonyl methane and the amino acid.

10. An oral formulation according to claim 7 which is in unit dosage form, each unit does containing from 100 to 500 mg of each of methylsulphonylmethane and the amino acid.

11. A method of treatment or prophylaxis of dermatological conditions, diseases or injuries which comprises administering an effective dosage of a formulation according to claim 7.

12. A method according to claim 11 wherein is applied to the skin a topical formulation according to claim 8.

13. A method according to claim 12 wherein said topical formulation is applied to the skin at least 2 times per day.

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/GB 93/01875

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/215 A61K31/195 A61K31/13 //(A61K31/215,31:195, 31:13,31:10)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB,A,2 057 263 (HERSCHLER R. J.) 1 April 1981 see claims ---	1-13
A	GB,A,2 177 917 (SALIM A. S. M.) 4 February 1987 see claims -----	1-13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search
22 December 1993

Date of mailing of the international search report

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. .ional application No.
PCT/GB93/01875

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 11-13 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati Application No
PCT/GB 93/01875

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2057263	01-04-81	US-A-	4296130	20-10-81
		AU-B-	544254	23-05-85
		AU-A-	6183380	05-03-81
		CA-A-	1157380	22-11-83
		DE-A,C	3032462	19-03-81
		FR-A,B	2464069	06-03-81
		JP-C-	1619652	30-09-91
		JP-B-	2027321	15-06-90
		JP-A-	56036412	09-04-81
		US-A-	4477469	16-10-84
		US-A-	4914135	03-04-90
		US-A-	4973605	27-11-90
		US-A-	4514421	30-04-85
		US-A-	4568547	04-02-86
		US-A-	5071878	10-12-91
		US-A-	4616039	07-10-86
		US-A-	4863748	05-09-89
GB-A-2177917	04-02-87	NONE		