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[54] THOZALINONE AS AN ANTI-PARKINSON AGENT

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[51] **Int. Cl.** **A61k 27/00**

[58] **Field of Search** 424/272

[56] **References Cited**

UNITED STATES PATENTS

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[57] **ABSTRACT**

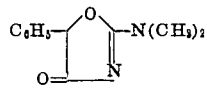
This disclosure describes a method of controlling the tremor and rigidity of Parkinson's disease or a drug-induced extrapyramidal disorder in a mammal which is afflicted with said disease or disorder which comprises administering thozalinone or anionic salts thereof to said mammal.

3 Claims, No Drawings

THOZALINONE AS AN ANTI-PARKINSON AGENT

BRIEF SUMMARY OF THE INVENTION

This invention relates to a novel method of controlling the symptoms of Parkinson's disease or a drug-induced extrapyramidal disorder in mammals, especially a human being, which comprises administering thozalinone to said mammals afflicted with said disease or disorder. Thozalinone (2-dimethylamino-5-phenyl-2-oxazolin-4-one) is a known central nervous system stimulant and is represented by the following structural formula:



Thozalinone free base forms acid-addition salts with a variety of non-toxic pharmaceutically acceptable salt forming reagents. Thus, acid-addition salts, formed by admixture of the free base with an acid in a suitable inert solvent, are formed with such acids as sulfuric, phosphoric, hydrochloric, hydrobromic, and related acids. For purposes of this invention, thozalinone free base is equivalent to its pharmaceutically acceptable acid-addition salts.

BACKGROUND OF THE INVENTION

Parkinson's disease is described as a syndrome of progressive rhythmic tremor, masklike facial expressions, slowing of movements, with increasing rigidity coming on in the fifth or sixth decade, called paralysis agitans or shaking palsy. In both Parkinson's disease and drug-induced extrapyramidal disorders, the patient exhibits one or more of the following symptoms: tremor, rigidity, increased salivation, akathisia, manifested by extreme restlessness, and dyskinesias, characterized by spastic contractions and involuntary movements.

The drugs most widely prescribed for use in the treatment of Parkinson's disease and drug-induced extrapyramidal disorders are 3-(1-pyridyl)-1-phenylcyclohexyl-1-propanol hydrochloride and 1-dopa [3-(3,4-dihydroxyphenyl)-L-alanine]. Unfortunately, these agents and the other currently available agents for treatment of Parkinson's disease possess side effects which limit their use. Thozalinone, on the other hand, is free from peripheral cardiovascular effects and is far less expensive than 1-dopa. Many of the patients afflicted with Parkinson's disease are refractory and do not respond favorably to treatment with any of the presently available agents so that, obviously, additional agents such as those of the present invention are needed.

In the last few years several reports have indicated that the brain levels of dopamine, norepinephrine, and serotonin are lowered in Parkinson's disease [Ehringer and Hornykiewicz, Klinische Wochenschrift 38(24): 1,236–1,239, (Dec. 1960); Bernheimer, Birkmayer, and Hornykiewicz, Klinische Wochenschrift 41(10): 465–469 (May 1963); Hornykiewicz, Wiener Klinische Wochenschrift 75(18): 309–312 (May 1963)]. In more than 40 patients who died with Parkinson's disease (postencephalitic and idiopathic), the greatest reduction was found in striatal and nigral dopamine [Hornykiewicz, Metabolism of brain dopamine in human parkinsonism; neurochemical and clinical aspects. In: Biochemistry and Pharmacology of the Basal Ganglia. Proceedings of the Second Symposium of the Parkinson's Disease Information Research Center, College of Physicians and Surgeons, Columbia University, New York, 1965, E. Costa, L. J. Cote and M. D. Yahr, eds. Hewlett, N. Y. Raven Press, 1966, pp 171–185].

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that the administration of thozalinone or salts thereof to mammals has a marked effect on the synthesis of dopamine in the brain. Thozalinone, as opposed to dextro-amphetamine, causes a marked increase in the synthesis of dopamine in the brain. Thozalinone is thus superior to dextro-amphetamine which has been used for the treatment of Parkinson's disease. Thozalinone may be administered orally or parenterally in amounts ranging from about 0.1 mg. to about 20 mg. per kg. of body weight per day in the form of the free base or as non-toxic acid addition salts thereof for the treatment of Parkinson's disease. A preferred dosage regimen for optimum results would be from about 0.5 mg. to about 5 mg. per kg. of body weight per day, and such dosage units are employed that a total of from about 25 mg. to about 250 mg. for a subject of about 50 kg. body weight are administered in a 24 hour period. The dosage regimen can be adjusted to provide the optimum therapeutic response. For example, several doses of 50–100 mg. may be administered per day.

Thozalinone and its non-toxic acid-addition salts may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft gelatin capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds of this invention may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gum, and the like. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 5 percent to about 75 percent or more of the weight of the unit. The amount of active compound in such therapeutically useful compositions or preparations is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 5 and 250 milligrams of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit, for instance, tablets, pills or capsules may be coated with shellac, sugar, or both. A syrup or elixir may contain the active compounds, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed.

Compositions having the desired clarity, stability, and adaptability for parenteral use are obtained by dissolving from 0.10 percent to 10.0 percent by weight of thozalinone or its salts in a vehicle consisting of a mixture of non-volatile, normally liquid polyethylene glycols which are soluble in both water and organic liquids and which have molecular weights of from about 200 to about 1,500. Such mixtures of polyethylene glycols are commercially available and are usually obtained by condensing glycol with ethylene oxide. Although the amount of thozalinone or salt thereof dissolved in the above vehicle may vary from 0.10 to 10.0 percent by weight, it is preferred that the amount employed be from about 3.0 percent to about 9.0 percent by weight. Although various mixtures of the aforementioned non-volatile polyethylene glycols may be employed, it is preferred to use a mixture of non-volatile polyethylene glycols having an average molecular weight of about 400. Such a mixture is usually referred to as polyethylene glycol 400. A preferred embodiment comprises a clear solution of from about 3.0 percent to about 9.0 percent by weight of thozalinone dissolved in an aqueous solution of polyethylene glycol 400. In addition to

thozalinone or salts thereof, the parenteral solutions may also contain various preservatives which may be used to prevent bacterial and fungal contamination or chemical degradation.

Thozalinone and dextro-amphetamine were compared at equi-stimulant doses, 30 mg./kg. and 3 mg./kg. intraperitoneally being about the median excitant doses, respectively. Doses of 100 mg./kg. of thozalinone and 10 mg./kg. of dextro-amphetamine are considered to be excessive. This comparison was carried out in a twofold manner. Firstly, to establish whether steady-state conditions prevailed following drug treatment as measured by pool size (concentration) of amines in brain, and secondly, to measure the effect of these two drugs under steady-state conditions on the synthesis of norepinephrine and dopamine in brain, measured by effect on turnover rate and turnover time.

Thozalinone has no effect on the concentration of the catecholamines in the brain of warm-blooded mammals. Dextro-amphetamine caused a significant depletion of norepinephrine at 10 mg./kg. and significantly increases the concentrations of dopamine at 3 mg./kg. Steady-state conditions for norepinephrine and dopamine prevailed at the doses of thozalinone used and for norepinephrine and dopamine at 3 and 10 mg./kg., respectively of dextro-amphetamine.

Thozalinone at the doses used and dextro-amphetamine at 3 mg./kg. have no significant effect on the pool size, turnover rate or turnover time of norepinephrine. Because of the absence of steady-state conditions, measurements of the effect of 10 mg./kg. of dextro-amphetamine on turnover time and rate of norepinephrine could not be made. For the same reason, the effect of 3 mg./kg. on dopamine turnover time and rate could not be measured. At 10 mg./kg. of dextro-amphetamine, there appeared to be a decrease in turnover time and an increase in the turnover rate of dopamine, but the changes were not statistically significant. In contrast thozalinone very markedly decreases the turnover time and increases the turnover rate of dopamine in the brain of warm-blooded mammals at 30 mg./kg. and 100 mg./kg. Turnover rate refers to the renewal of a substance in the body or in tissue during steady-state conditions. Renewal may be accomplished by synthesis in the tissue or exchange between the circulation and the tissue. Because both circulating dopamine and norepinephrine are excluded from the brain by the blood-brain barrier, turnover rate in this study refers to synthesis. Because dopamine is concentrated in the corpus striatum, effects on pool size and turnover apply almost exclusively to this region.

The following examples are presented in order to more fully disclose the invention. It should be understood, however, that the examples are not intended to limit the invention in any way.

EXAMPLE 1

Effect of *d*-Amphetamine and Thozalinone on Brain Amine Concentration and Motor Activity of Grouped Mice

Motor Activity:

A circular actophotometer (Metro Industries, Long Island City, New York), 12 inches in diameter and containing six photocell units, was employed. The measure of activity was the number of times that five mice crossed the crossed beams of light during a five minute session in the activity chamber. Counts were recorded on a digital counter connected to the photocell unit. The top of the chamber was covered so that external visual stimuli would be kept homogeneous. A total of five groups of five mice each were used on each treatment to provide sufficient data for statistical comparison. Each group of five mice was housed in individual 14 × 20 cm. rectangular wire cages for at least 4 hours prior to their session in the actophotometer. In all experiments motor activity was recorded 30 minutes after intraperitoneal administration of d-amphetamine, thozalinone or control (water). Control and drug treated animals were run in parallel. The results appear in Table I below.

Measurement of Brain Amines

Those groups of five mice used for recording motor activity were immediately removed from the activity counter and sacrificed by cervical fracture. The brains were removed, weighed, quick frozen and stored at −40° C. until assayed. In the determination of norepinephrine and dopamine, tissues were extracted by a modification of the method of Shore and Olin, J. Pharmac. Exp. Ther. 122: 295–300 (1958), which modification was described by Gray and Rauh, J. Pharmac. Exp. Ther. 155: 127–134 (1967). Brain amine concentrations were determined spectrophotofluorometrically. The method of Maynert and Klingman, J. Pharmac. Exp. Ther. 135: 285–295 (1962) was used for the measurement of norepinephrine and the method of Carlsson and Waldeck, Acta physiol. scand. 44: 293–298 (1958) was used to measure dopamine. Three pooled mouse brains were assayed, using the same aliquot of homogenate for the determination of both monoamines. The results appear in Table I below.

At essentially equi-stimulant doses, thozalinone differed from dextro-amphetamine in having no action on the concentrations of norepinephrine and dopamine in brain, i.e. steady-state conditions prevailed at both doses of thozalinone for norepinephrine and dopamine; for dextro-amphetamine steady-state conditions existed for norepinephrine at 3 mg./kg. and at 10 mg./kg. for dopamine. At the present time no functional significance can be attached to the increase in the pool size (concentration) of dopamine in brain after 3 mg./kg. of dextro-amphetamine or to the depletion of norepinephrine in brain after the administration of 10 mg./kg. of this agent.

TABLE I

Treatment	Norepinephrine		Dopamine		Motor activity	
	Concentration, mcg./gm.	Change, percent	Concentration, mcg./gm.	Change, percent	Count (5 min.)	Change, percent
Water	0.362±0.04		0.652±0.03		578±38	
d-Amphetamine, 3 mg./kg.	0.370±0.04	+2	[a] 0.770±0.05	+18	[a] 861±160	+49
d-Amphetamine, 10 mg./kg.	[a] 0.302±0.02	−17	0.655±0.04	0	[a] 739±170	+28
Thozalinone, 30 mg./kg.	0.359±0.05	−1	0.681±0.12	+4	[a] 852±62	+47
Thozalinone, 100 mg./kg.	0.365±0.03	+1	0.705±0.04	+8	[b] 998±109	+73

[a] = Different from control at $p \lesssim 0.05$.
[b] = Different from control at $p \lesssim 0.01$.
Brain amine concentrations are means ± standard deviations of 5 samples of 3 brains each (15 mice). Motor activity was measured in 5 groups of 5 mice each per dose level. Positive (+) values mean an increase and negative (−) values mean a decrease from control.

EXAMPLE 2

Effect of Dextro-amphetamine and Thozalinone on Norepinephrine and Dopamine Pool Size and Turnover

The turnover rate of norepinephrine and dopamine was calculated according to the steady-state kinetics [Brodie et al., J. Pharmac. Exp. Ther. 154: 493–498 (1966)] from the endogenous norepinephrine or dopamine level and from the decline in the level of these amines 3 hours after their synthesis was blocked by α-methyltyrosine (AMT). d-Amphetamine (3 and 10 mg./kg.), thozalinone (30 and 100 mg./kg.) or control vehicle were administered to groups of 15 mice each, 30 minutes prior to a single dose (200 mg./kg.) of AMT. Three hours after AMT administration the norepinephrine and dopamine content of whole brain was determined. Initial amine pool sizes (control levels) were obtained from additional groups of mice which received only the stimulants or

vehicle and were sacrificed 30 minutes later. In each instance, brains of three mice were pooled and norepinephrine and dopamine were extracted and assayed by the methods described in Example 1. The results appear in Table II below. Assessed by turnover rates and times, thozalinone at the doses used had no effect on the synthesis of norepinephrine but significantly increased the synthesis of dopamine. Dextroamphetamine, on the other hand, under steady-state conditions had no statistically significant effect on the synthesis of the two catecholamines. Possibly at 10 mg./kg., dextroamphetamine increased the synthesis of dopamine to some degree.

TABLE II

Treatment	Norepinephrine			Dopamine			Motor activity counts/5 min.
	Pool size, mcg./g.	Turnover time, hours	Turnover rate, mcg./g./hr.	Pool size, mcg./g.	Turnover time, hours	Turnover rate, mcg./g./hr.	
Control (vehicle)	.360 (.339–.381)	5.2 (4.3–6.5)	.070 (.055–.085)	.635 (.592–.681)	5.5 (3.8–10.2)	.115 (.067–.163)	578±38
Amphetamine, 3 mg./kg. I.P.	.369 (.333–.409)	6.3 (5.5–7.4)	.059 (.040–.078)	.813[a] (.659–1.002)			861[c]±60
Amphetamine, 10 mg./kg. I.P.	.309[b] (.279–.342)			.712 (.577–.878)	3.5 (3.0–4.3)	.201 (.117–.285)	739[c]±170
Thozalinone, 30 mg./kg. I.P.	.393 (.355–.435)	5.0 (4.5–5.8)	.078 (.057–.099)	.746 (.605–.919)	3.2[c] (2.8–3.8)	.231[a] (.139–.323)	852[c]±162
Thozalinone, 100 mg./kg. I.P.	.392 (.354–.434)	5.1 (4.6–5.8)	.076 (.055–.097)	.709 (.575–.874)	2.6[d] (2.3–2.9)	.277[c] (.181–.373)	998[c]±109

Level of significance when compared with vehicle control: [a]P=.04; [b]P=.01; [c]P=.05; [d]P=.002; [e]P=.01.

The amine parameters are the means and 95% confidence limits of 5 samples of 3 mice each (15 mice). Motor activity is the mean 5 minute count (±S.D.) for 5 group of 5 mice each Amphetamine, thozalinone or vehicle was administered ½ hr. prior to AMT (200 mg./kg. I.P.) or sacrifice; mice which received AMT were sacrificed 3 hrs. later.

The concentrations of amines are probably only significant to two decimal places.

Because steady-state conditions do not prevail, turnover times and rates could not be calculated for dopamine and norepinephrine after 3 and 10 mg./kg., respectively, of dextro-amphetamine.

EXAMPLE 3

[Preparation of tablet formulation]

Ingredient	Per tablet (g.)	For 10,000 tablets (g.)
Active ingredient: 2-dimethylamino-5-phenyl-2-oxazolin-4-one	0.0500	500
Lactose	0.0800	800
Corn starch (for mix)	0.0150	150
Corn starch (for paste)	0.0100	100
Totl	0.1550	1,550
Magnesium stearate (1%)	0.0013	12.5
Total	0.1563	1,562.5

The active ingredient, lactose and corn starch (for mix) are blended together. The corn starch (for paste) is suspended in 800 milliliters of water and heated with stirring, to form a paste. This paste is then used to granulate the mixed powders. Additional water is used, if necessary. The wet granules are passed through a No. 8 hand screen and dried at 120° F. The dry granules are then passed through a No. 16 screen. The mixture is lubricated with 1 percent magnesium stearate and compressed into tablets in a suitable tableting machine.

EXAMPLE 4

[Preparation of oral syrup formulation]

Ingredient	Amount
Active ingredient: 2-dimethylamino-5-phenyl-2-oxazolin-4-one, mg.	1,000
Sorbitol solution (70% N.F.), ml.	40
Sodium benzoate, mg.	150
Sucaryl, mg.	90
Saccharin, mg.	10
Red dye (F.D. & C. No. 2), mg.	10
Cherry flavor, mg.	50
Distilled water, q.s. ad, ml.	100

The sorbitol solution is added to 40 ml. of distilled water and the active ingredient is suspended therein. The sucaryl, saccharin, sodium benzoate, flavor and dye are added and dissolved in the above solution. The volume is adjusted to 100 ml. with distilled water.

Other ingredients may replace those listed in the above formulation. For example, a suspending agent such as bentonite magma, tragacanth, carboxymethylcellulose or methylcellulose may be used. Phosphates, citrates or tartrates may be added as buffers. Preservatives may include the parabens, sorbic acid and the like and other flavors and dyes may be used in place of those listed above.

EXAMPLE 5

Preparation of Parenteral Formulation

In a solution of 119 ml. of propylene glycol and 30 ml. of distilled water was dissolved 8.5 g. of 2-dimethylamino-5-phenyl-2-oxazolin-4-one hydrochloride, with stirring. After dissolution was complete, a solution of 850 mg. of sodium formaldehyde sulfoxylate in 3.0 ml. of distilled water was then added to the formulation. The pH of this solution was then adjusted to 7.0 with ethanolamine and the volume was made up to 170 ml. with distilled water. The formulation was filtered through a fine sintered glass filter, filled into 5.0 ml. ampoules each containing 2.0 ml., and sealed under nitrogen.

We claim:

1. The method of controlling the tremor and rigidity of Parkinson's disease or a drug-induced extrapyramidal disorder in a mammal which is afflicted with said disease or disorder which comprises administering orally to said mammal an effective amount of a compound selected from the group consisting of 2-dimethylamino-5-phenyl-2-oxazolin-4-one and a pharmaceutically acceptable acid-addition salt thereof in association with a pharmaceutical carrier to provide a daily dosage of from about 0.1 to about 20 milligrams per kilogram of body weight of the mammal.

2. The method as defined in claim 1 wherein the compound is 2-dimethyl-amino-5-phenyl-2-oxazolin-4-one free base.

3. The method as defined in claim 1 wherein the compound is 2-dimethyl-amino-5-phenyl-2-oxazolin-4-one hydrochloride.

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