(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 February 2010 (11.02.2010)

PCT

(10) International Publication Number
WO 2010/015870 A1

(51) International Patent Classification:
A61K 31/502 (2006.01) *A61P 11/06* (2006.01)
A61K 45/06 (2006.01) *A61P 17/00* (2006.01)
A61P 9/08 (2006.01) *A61P 17/06* (2006.01)
A61P 9/10 (2006.01) *A61P 25/28* (2006.01)
A61P 9/12 (2006.01)

(21) International Application Number:
PCT/HU2009/000073

(22) International Filing Date:
5 August 2009 (05.08.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
P0800498 6 August 2008 (06.08.2008) HU

(71) Applicant *(for all designated States except US)*: **SEMMELWEIS EGYETEM** [HU/HU]; Üllöi út 26., H-1085 Budapest (HU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MÁTYUS, Péter** [HU/HU]; Vályog u. 12., H-1032 Budapest (HU). **NÉMETH, János** [HU/HU]; Berzenczey u. 37., H-1094 Budapest (HU). **MAGYAR, Kálmán** [HU/HU]; Csokonai u. 1., H-1028 Budapest (HU). **SOMOGYI, Anikó** [HU/HU]; Stromfeld A. u. 28., H-1124 Budapest (HU). **DUNKEL, Petra** [HU/HU]; Práter u. 59., H-1083 Budapest (HU). **SOMFAI, Gábor Márk** [HU/HU]; Szalay u. 5/b., H-1055 Budapest (HU). **BALOGH, Balázs** [HU/HU]; Zöldlomb u. 24/a., H-1025 Budapest (HU). **TÚRÓS, György** [HU/HU]; Pesti u. 46/a., H-1173 Budapest (HU).

(74) Agent: **DANUBIA PATENT AND LAW OFFICE LLC**; Bajcsy-Zsilinszky út 16., H-1051 Budapest (HU).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2010/015870 A1

(54) **Title**: USE OF DIHYDRALAZINE FOR THE TREATMENT OF DISEASES RELATED TO ELEVATED SEMICARBAZIDE SENSITIVE AMINE-OXIDASE (SSAO) ACTIVITY

(57) **Abstract**: The present invention relates to the use of dihydralazine for the treatment of diseases related to elevated level of SSAO activity and its use for the manufacture of pharmaceutical compositions for the treatment of such diseases.

Use Of Dihydralazine For The Treatment Of Diseases Related To Elevated Semicarbazide Sensitive Amine-Oxidase (Ssao) Activity

Field of the invention

The present invention relates to the use of dihydralazine for the treatment of diseases related to elevated level of SSAO activity and its use for the manufacture of pharmaceutical compositions for the treatment of such diseases.

Said diseases related to elevated SSAO activity include: acute or chronic inflammatory conditions and diseases related to elevated SSAO activity, connective tissue inflammatory conditions and diseases, rheumatoid arthritis, ankylosing spondylitis, lupus erythematosus, vasculitis, synovitis, gastrointestinal inflammatory conditions and diseases, ulcerative colitis, Crohn's disease, irritable bowel syndrome, central nervous system inflammatory conditions and diseases, Alzheimer's disease, multiple sclerosis, pulmonary inflammatory conditions and diseases, asthma, inflammatory skin conditions, psoriasis, contact dermatitis, atopic dermatitis, liver inflammatory conditions and diseases, inflammatory conditions and diseases of the eye (eyeball, accessory and protective structures of the eye, orbit), macular edema, diseases related to carbohydrate metabolism, diseases related to adipocyte differentiation or dysfunction, diseases related to smooth muscle cell dysfunction, atherosclerosis, obesity, vascular diseases, arteriosclerosis, Raynaud's disease.

In a preferred embodiment of the invention, dihydralazine is used to decrease SSAO activity in such a dose, that does not lead to appreciable drop in blood pressure in the animal or human treated.

Background of the invention

Semicarbazide sensitive amine oxidase (SSAO) catalyzes the oxidative deamination of primary aliphatic and arylalkylamines, resulting in the formation of the corresponding aldehyde, with the concomitant production of H_2O_2 and NH_3. The physiological substrates of SSAO are aminoacetone and methylamine. SSAO differ from the other main group of amine oxidases including MAO-A and MAO-B with respect to cofactor, subcellular distribution, biological function, substrates and inhibitors. In the human genome, three SSAO genes have been identified, AOC1 corresponding to diamine oxidase (DAO) (Chassande, O. et al., J. Biol. Chem., 1994, 269, 14484-14489), AOC2 coding for retina-specific SSAO (Imamura, Y. et al., Genomics, 1997, 40, 277-283; Imamura, Y. et al., Genomics, 1998, 51, 293-298; Zhang, Q. et al., Gene, 2003, 318, 45-53), and AOC3 for human placental amine oxidase/VAP-1 (Zhang, X. and McEntire, W.S., Gene, 1996, 179, 279-286; Smith, D.J. et al., J. Exp. Med., 1998, 188, 17-27).

SSAO is involved in the metabolism of biogenic and xenobiotic amines.

The human SSAO enzyme exists as a membrane-bound and as a soluble form in the plasma, its activity displaying a wide tissue distribution. The major sources of the enzyme are the endothelial cells, smooth muscle cells and adipocytes. Sequence identity of SSAO was reported with vascular adhesion protein-1 (VAP-1), which has a role in the adhesion of lymphocytes, therefore in inflammation (Smith, D.J. et al., J. Exp. Med., 1998, 188, 17-27).

Elevated SSAO levels or overexpression of the enzyme have been reported in various pathological conditions (e.g. congestive heart failure (Boomsma, F. et al., Cardiovasc. Res., 1997, 33, 387-391), end-stage renal disease (Kurkijarvi, R. et al., Eur. J. Immunol., 2001, 31, 2876-2884), inflammatory liver diseases (Kurkijarvi, R. et al., J. Immunol., 1998, 161, 1549-1557), multiple sclerosis (Airas, L. et al., J. Neuroimmunol., 2006, 177, 132-135), psoriasis (Madej, A. et al., J. Eur. Acad. Dermatol. Venerol., 2007, 21, 72-78), Alzheimer's disease (del Mar Hernandez, M. et al., Neurosci. Lett., 2005, 384, 183-187; Ferrer, I. et al., 2002, 321, 21-24) and myopathies (Olive, M. et al., Muscle Nerve, 2004, 29, 261-266)).

Elevated levels of SSAO activity have been observed in type I and type II diabetes, markedly in the presence of diabetic complications (Boomsma, F. et al., Biochim. Biophys. Acta, 2003, 1647, 48-54; Boomsma, F. et al., Clin. Sci., 1995, 88, 675-679; Garpenstrand, H. et al., Diabetic. Med., 1999, 16, 514-521; Mészáros, Z. et al., Metab. Clin. Exp., 1999, 48, 113-117; Boomsma, F. et al., Diabetologia, 1999, 42, 233-237; Salmi, M. et al., Am. J. Pathol., 2002, 161, 2255-2262). Elevated SSAO activity has been reported in diabetic retinopathy, supporting the hypothesis that SSAO may be involved in the pathogenesis of this diabetic complication.

Thus, pharmacological inhibition of the SSAO enzyme may exert beneficial effects in various diseases.

As a consequence of the elevated activity of the enzyme in diabetes, there is an increased formation of the cito- and/or angiotoxic enzyme products, that may contribute to the pathogenesis of diabetic complications. Since there is a high expression of SSAO in the endothel and the plasma, the SSAO-related citotoxic effects might be more significant in the highly vascularised tissues, such as for example in the eyes (Ekblom, J., Pharmacol. Res., 1998, 37, 87-92). The citotoxic enzyme products in case of the physiological substrates are formaldehyde, methylglioxal, NH_3 and H_2O_2, that may increase the deleterious effects of oxidative stress, moreover may lead to the formation of protein cross-links and advanced-glycation end-products (AGEs). The said processes have an important role also in the pathogenesis of diabetic complications. Thus, inhibitors of SSAO activity may be useful for the treatment of diabetic complications, particularly diabetic retinopathy and diabetic macular edema.

Diabetic retinopathy and diabetic macular edema are severe complications of diabetes even in an epidemiological aspect. Diabetic retinopathy is the second leading cause of acquired blindness in adults. Currently, the primary treatment of diabetic retinopathy and macular edema is retinal laser photocoagulation therapy. However, this therapy is relatively time consuming besides leading to a beneficial outcome only in a moderate number of cases, mostly helping to reduce the progression of the disease. A routine treatment is pars plana vitrectomy, i.e. surgical clearing of the vitreous body. The surgical technique however is rather elaborate, costly and has certain risks. On the other hand, the currently available pharmacological therapies are not effective enough, they can lead only to a transient amelioration of the condition. Therefore, there is a great demand for novel pharmacological agents, that are more effective than the currently available therapies (Ciulla, A.T. et al., Diabetes Care, 2003, 26, 2653-2664).

Of the potential pharmacological therapies of retinopathy, clinical trials have been conducted with AGE inhibitors and antioxidants (Soro-Paavonen, A., Curr. Med. Chem., 2006, 13, 1777-1788). Retinal leukostasis, capillary occlusion and on the other hand, chronic, low-grade subclinical inflammation were suggested to have central and causal role in the formation of the signature vascular lesions of diabetic retinopathy (Joussen, A.M., FASEB J., 2001, 18, 1450).

Inhibition of the SSAO activity leads to a decrease in the formation of AGEs and in oxidative stress. Moreover, a specific VAP-1 inhibitor was reported to have beneficial effects in an uveitis model (endotoxin-induced uveitis) (Noda, K., FASEB J., 2008, 22, 1094-1103). Thiazole VAP-1 inhibitors were disclosed as useful for the treatment of macular edema (WO 2006/028269, WO 2006/011631, WO 2004/067521). In an animal model of diabetes, the treatment with the inhibitor exhibited a beneficial effect on the increased vascular permeability in the eyes (WO 2004/067521). The strong SSAO inhibitor aminoguanidine has beneficial effect on diabetic retinopathy in animal model (Ellis, E.N., et al., Metabolism, 1991, 40, 1016), its effect might be related to its SSAO inhibition.

Considering the above detailed aspects, it is reasonable to suggest that application of pharmaceutical compositions of SSAO/VAP-1 inhibitors could be a novel, effective approach for the treatment of inflammatory eye diseases. Currently there is not any drug available in therapy exploiting this approach.

In SSAO enzyme catalysis the carbonyl group containing topaquinone cofactor has a central role, analysis of its structure may serve as a sound basis for the design of inhibitor compounds. The name of SSAO derives from its sensitivity to inhibition by semicarbazide. Besides semicarbazide, the well-known inhibitor of SSAO, also other classes of compounds were described as inhibitors, such as several hydrazine derivatives. Among the drugs already used in therapy containing hydrazino group, several were reported to have SSAO inhibitory effect, such as hydralazine, carbidopa and benserazide (Mátyus, P. et al., Curr. Med. Chem., 2004, 11, 1285-1298). None of these compounds has been used however for the treatment of diseases related to elevated SSAO activity, said diseases including diabetic complications, such an application of these drugs was not even suggested previously.

An underlying reason of that could be, that the primary effect of these drugs that serve as the basis of their recent use in therapy (e.g. antihypertensive effect), may be a serious adverse effect in course of their use for the treatment of diseases related to elevated SSAO activity, such as retinopathy.

The growing interest in hydrazino derivatives however is well illustrated by the recent disclosure of several SSAO inhibitor hydrazino derivatives, e.g. some hydrazinoalcohol derivatives (WO 2005/080319, WO 2002/002090); hydrazinoindane derivatives (WO 2003/006003); and phenylallylhydrazine derivatives (Wang, E.Y. et al., J. Med. Chem., 2006, 49, 2166-2173, WO 2006/094201). According to the patent publications, these compounds could be useful for the treatment of acute and chronic inflammations, diseases related to carbohydrate metabolism, diseases related to adipocyte differentiation or dysfunction, diseases related to smooth muscle dysfunctions and vascular diseases.

Disclosure of the invention

Dihydralazine has long been used in human therapy as an antihypertensive agent. Despite its structural analogy with hydralazine (dihydralazine may be regarded as a substituted hydralazine), no data have been published previously on the effect of dihydralazine on SSAO. In spite of the structural resemblance of dihydralazine to hydralazine, the SSAO inhibitory activity of dihydralazine is far from being evident, due to the observations that even slight structural modifications in hydrazine derivatives may significantly influence the type and magnitude of SSAO inhibitory activity (Lizcano, J. M. et al., Biochem. Pharmacol., 1996, 52, 187-195). The SSAO inhibition by dihydralazine was confirmed by our experiments described in the examples. Therapeutic application of dihydralazine as an SSAO inhibitor may be more advantageous than that of

hydralazine, as a consequence of the presence of one more hydrazino group in dihydralazine. It is well known that the hydrazino group of hydralazine and one of the hydrazino groups of dihydralazine undergo metabolic inactivation (Schneider, T. et al., Pharmazie, 1988, 43, 33-36, ibidem. 704-706), the other, metabolically intact hydrazino group of dihydralazine may therefore be available for SSAO inhibition that may result in particularly preferable pharmacokinetic and phamacodynamic properties of dihydralazine as SSAO inhibitor.

The present invention relates to the use of dihydralazine for the treatment of diseases related to elevated levels of SSAO activity.

In another embodiment, the present invention relates to the use of dihydralazine for manufacture of a pharmaceutical composition for the treatment of diseases related to elevated levels of SSAO activity.

The diseases related to elevated SSAO activity of the above embodiments include: acute or chronic inflammatory conditions and diseases related to elevated SSAO activity, connective tissue inflammatory conditions and diseases, rheumatoid arthritis, ankylosing spondylitis, lupus erythematosus, vasculitis, synovitis, gastrointestinal inflammatory conditions and diseases, ulcerative colitis, Crohn's disease, irritable bowel syndrome, central nervous system inflammatory conditions and diseases, Alzheimer's disease, multiple sclerosis, pulmonary inflammatory conditions and diseases, asthma, inflammatory skin conditions, psoriasis, contact dermatitis, atopic dermatitis, liver inflammatory conditions and diseases, inflammatory conditions and diseases of the eye (eyeball, accessory and protective structures of the eye, orbit), macular edema, diseases related to carbohydrate metabolism, diseases related to adipocyte differentiation or dysfunction, diseases related to smooth muscle cell dysfunction, atherosclerosis, obesity, vascular diseases, arteriosclerosis, Raynaud's disease.

In the above embodiments, diseases related to carbohydrate metabolism include diabetes (type 1 or type 2), diabetes related vascular complications and/or neuropathy and/or retinopathy and/or nephropathy, particularly diabetic retinopathy and macular edema.

It should be noted, that according to our animal experiments, dihydralazine is able to exhibit significant SSAO inhibition in such a low dose, that does not cause appreciable (i.e. appreciable to the animal or human treated) blood pressure lowering effect. Thus, in a preferred embodiment of the invention dihydralazine is used for the inhibition of SSAO activity in such a dose, which does not cause appreciable lowering in blood pressure in the animal or human treated under the normal levels. However, in patients with hypertension, lowering of blood pressure, i.e. antihypertensive effect could be beneficial, therefore, in case of such patients, even higher doses (depending on the degree of hypertension) than the ones described below as preferred embodiments of the invention can be used.

In one embodiment, the invention relates to the use of dihydralazine in therapy per se or with one or more additional compounds in combination. In another embodiment, dihydralazine can be used *per se* or in combination with one or more additional compounds for the manufacture of a pharmaceutical composition.

Detailed description of the invention

The pharmaceutical compositions of the present invention can be administered by any means that achieve their intended purpose. Routes of administration of the present invention can be, for example, oral, intravenous, intramuscular, subconjunctival, parabulbar, retrobulbar, subtenon, intracameral, intravitreal and other injections, sublingual, transdermal, ocular, eye drops, eye gels or ointments. Compositions of the present invention can be solid, semisolid or liquid.

Potential liquid compositions of the present invention include but are not limited to solutions, tinctures, syrups, emulsions and suspensions.

The pharmaceutical compositions of the present invention can contain one or more non-toxic, pharmaceutically acceptable excipients (e.g. agents used for formulation, carriers, surface acting agents, colours, sweeteners, solvents, suspending agents, coatings etc.). Preferred embodiments are controlled-release compositions and compositions providing an organ specific targeted delivery of the active ingredient.

Pharmaceutical compositions of the present invention can be prepared by admixing the active ingredient dihydralazine or its salt (preferred are for example sulphate, hydrochloride salts) with one or more excipients and by formulation to a pharmaceutical composition. Pharmaceutical compositions can be prepared by methods and contain excipients which are well known in the art. A generally recognized compendium of such methods and ingredients is Remington's Pharmaceutical Sciences.

In one embodiment, dihydralazine is administered in a dose of 0.005-1.5 mg/bodyweight kg. In a preferred embodiment, dihydralazine is administered in a dose of 0.2-1 mg/bodyweight kg, in a more preferred embodiment, in a dose of 0.4-0.8 mg/bodyweight kg, if the preferred embodiment of administering the active ingredient without causing appreciable lowering of blood pressure is intended to be carried out. These dosage intervals are related to administration to humans and can be used for the administration to animals just with the appropriate interpolation.

Pharmaceutical compositions of the invention in a preferred embodiment contain single doses. The exact dosage administered will be dependent upon a variety of factors and can be chosen by the individual physician in view of the appropriate parameters (weight of the recipient, age, stage and degree of the disease etc).

As demonstrated by Example 3, in adults of average weight (approx. 70 kg), administration of 14.5 mg dihydralazine sulphate two times daily was found to be preferable (no appreciable lowering of blood pressure was observed).

The effect of dihydralazine was studied in Micromedex/Drugdex evaluations. In the treatment of hypertension, dihydralazine is indicated as supplemental therapy as a second or third drug. Dihydralazine therapy is initiated with lower doses (eg, 12.5 to 25 milligrams twice daily), with gradual dose increases based on clinical response and tolerability to total daily doses of 25 to 100 milligrams in 2 or 3 divided doses (Strocchi E. et al., Int. J. Clin. Pharmacol. Ther. Toxicol., 1983, 21, 519-523; Komajda M. et al., Am. J. Nephrol., 1986, 6(suppl 2), 106-112; Thibonnier M. et al., Br. J. Clin. Pharmacol., 1980, 9, 561-567; Salmela P.I. et al., Ann. Clin. Res., 1981, 13, 433-438; Sassano P. et al., Am. J. Med., 1987, 83, 227-235). Higher doses (150 milligrams/day) have been used in some studies (Kirch W. & Axthelm T., J. Cardiovasc. Pharmacol., 1982, 4, 562-566).

In one embodiment, the invention relates to the use of dihydralazine in therapy per se or with one or more additional active ingredients in combination. In another embodiment, dihydralazine can be used *per se* or in combination with one or more additional active ingredients for the manufacture of a pharmaceutical composition. Preferred additional active ingredients of the invention are agents inhibiting SSAO activity and/or anti-inflammatory agents and/or VEGF inhibitors and/or VEGF small interfering RNA agents and/or tyrosine kinase inhibitors and/or protein kinase C inhibitors and/or angiotensin converting enzyme (ACE) inhibitors

and/or PPAR-γ inhibitors and/or acetylsalicylic acid and/or statins (statin type compounds, abbreviated: statins), and/or compounds used for the treatment of diabetes and/or its pathological consequences.

Preferred additional active ingredients can be: VEGF inhibitors, as for example pegaptanib (Macugen), ranibizumab (Lucentis), bevacizumab (Avastin), vatalanib; VEGF Trap; VEGF small interfering RNA agents, as for example bevasiranib, Sirna-027; PPAR-γ inhibitors, as for example glitazones; tyrosine kinase inhibitors, as for example ALN-VEG01, AG-013958; steroid or non-steroid anti-inflammatory agents.

The invention is illustrated by the following non-restrictive experimental examples.

Example 1

Study of the effect of dihydralazine on human serum SSAO activity

In the experiment human serum was used in the following way: blood samples were collected without anticoagulant, then stored at 4°C for 15 minutes and centrifuged (4°C, 2500 g/10 min). The supernatant was stored at -80°C until the measurement of the enzyme activity.

A radiochemical procedure (Yu P.H., Zuo D.M., Biochem. Pharmacol., 1992, 43, 307-312) was adapted for determining SSAO activities in the human serum. It is based on the determination of the quantity of ^{14}C-benzaldehyde formed by the enzyme reaction of ^{14}C-benzylamine.

The preparations containing the serum and the dihydralazine enzyme inhibitor were pre-incubated at room temperature for 20 min. The enzyme reaction was started with ^{14}C-benzylamine substrate (~ 200000 dpm/tube). During the experiments 200 µl was the final volume of the incubation media, the composition of which is shown in Table 1. The samples obtained accordingly were incubated at 37°C for 40 min, then the reaction was stopped by adding 200 µl 2M citric acid solution. In case of the 'blank' sample, citric acid solution was added before adding the substrate, therefore, in this case no enzyme reaction took place. The samples were extracted with 1000 µl 1:1 toluene – ethyl acetate mixture, by vortex method, then centrifuged (2500 g / 5 min). The enzyme activity was determined by liquid scintillation counting (600 µl supernatant + 5ml Aquasafe), using the formula below:

$$\frac{[\text{dpm (sample)} - \text{dpm } 0'] \times 1.6 \times 10 \times 1.5}{\text{measured substrate activity dpm} \times \text{mg protein}}$$

standing for: 1.6: extraction volume; 10: molar activity; 1.5: correction factor for the incubation time; dpm: disintegrations per minute (unit of radioactivity)

The inhibition was calculated in the % inhibition compared to the control ('control' sample not containing the inhibitor compound) after correction with the background ('blank' sample).

The method used for the determination of the protein quantity of the samples is based on comparison of the 1000× dilution of the plasma to the standard calibration curve of serum albumin (Bradford M.M., Anal. Biochem. *72*, 248, 1976). The % inhibition can be determined also of the dpm values of the control and the sample.

Table 1.

Composition of the incubation media

Solutions	'blank' (0')	'control'	sample
0.1 M phosphate buffer, pH=7.4	100 µl	100 µl	100 µl
1 mM clorgyline.HCl	20 µl	20 µl	20 µl
inhibitor (dihydralazine)	-	-	20 µl
serum	40 µl	40 µl	40 µl
0.5 mM ^{14}C-benzylamine (BZA)	20 µl	20 µl	20 µl
distilled water	20 µl	20 µl	-
[2 M citric acid (to stop the enzyme reaction)	200 µl	200 µl	200 µl]

According to the assay, the IC_{20} value of dihydralazine on human serum SSAO is 0.5 µM.

Example 2

Effect of dihydralazine on the SSAO activity *in vivo* in rats

Rats were treated sc with 0.1 or 0.01 M dihydralazine solution (0.1 ml/100 g) once daily for 1-5 days. On the appropriate day, 2 hours after the treatment the rats were decapitated and their blood was collected without anticoagulant in plastic centrifuge tubes. Blood samples were centrifuged at a low frequency (800 rpm/min) and the supernatant was used for the determination of SSAO activity applying ^{14}C-benzylamine substrate.

SSAO inhibition expressed in pmol/mg protein/hour units and in % values (mean ± S.D.) in the two treated groups is shown in table 2 and 3.

The blood pressure of the animals was determined in an invasive way. In treated animals, compared to the control group, the treatment did not cause lowering of the blood pressure.

Table 2

Effect of dihydralazine on SSAO activity *in vivo* (1.)

Serum SSAO activity in case of 10 µmol/kg dose (1.9 mg/kg)							
Group	Specific activity (pmol/mg/h)	Mean	SD	p (t-test)	Inhibition (%)	Mean	SD
Control	3.52 3.08 3.52	3.373	0.252		-4 9 -4	0	7

10 µmol/kg 24 h	2.11 1.19 1.44	1.580	0.477	0.010	37 65 57	53	14
10 µmol/kg 48 h	1.52 1.66 1.61	1.595	0.067	0.004	55 51 52	53	2
10 µmol/kg 72 h	1.25 1.06 1.43	1.244	0.187	0.000	63 69 58	63	6
10 µmol/kg 96 h	0.97 1.94 1.12	1.344	0.523	0.010	71 42 67	60	16
10 µmol/kg 120 h	0.78 2.90 0.59	1.425	1.284	0.114	77 14 82	58	38

Table 3

Effect of dihydralazine on SSAO activity *in vivo* (2.)

Serum SSAO activity in case of 100 µmol/kg dose (19 mg/kg)							
Group	Specific activity (pmol/mg/h)	Mean	SD	p (t-test)	Inhibition (%)	Mean	SD
Control	3.52 3.08 3.52	3.373	0.252		-4 9 -4	0	7
100 µmol/kg 24 h	1.13 1.05 1.11	1.094	0.041	0.003	67 69 67	68	1
100 µmol/kg 48 h	1.31 1.33 1.80	1.481	0.279	0.001	61 60 47	56	8
100 µmol/kg 72 h	1.11 1.26 1.15	1.177	0.079	0.002	67 63 66	65	2
100 µmol/kg 96 h	0.55 1.12 0.91	0.859	0.288	0.000	84 67 73	75	9
100 µmol/kg 120 h	1.29 0.34 0.56	0.730	0.495	0.004	62 90 83	78	15

Example 3

Study of the effect of dihydralazine on the macular edema of type 2 diabetic patients and on the SSAO activity

The effect of orally administered low dose dihydralazine on macular edema and serum SSAO activity was studied in type 2 diabetic patients in an early stage of macular edema formation.

After giving their informed consent, patients participating in the study were treated for 56 days taking ½ tablet twice daily of a pharmaceutical composition containing 28.9 mg dihydralazine sulphate (i.e. the ½ tablet contained 14.5 mg dihydralazine sulphate).

To measure the efficacy and monitor the safety of the treatment, the following examinations were carried out before starting the treatment and at the 1., 7., 14., 28. and 56. day of the treatment:

1. ocular examinations: visual acuity (ETDRS chart), slit-lamp examination, measurement of intraocular pressure with applanation tonometry, fundus biomicroscopy with pupil dilation, digital fundus photography. The grade of macular edema was determined via measuring retinal thickness with optical coherency tomography (OCT).

2. internal medicine examinations: anamnesis, physical examinations, blood pressure, pulse, urine examination.

3. laboratory examinations: blood glucose, HbA1c, liver function, kidney function, microalbuminuria, metabolic panel of the blood , lipids, CRP.

Serum SSAO activity was determined from blood samples collected before starting the treatment, and at the 14., 28. and 56. day of the treatment. The blood samples were centrifuged and the supernatant was stored at -80°C until the measurement of the enzyme activity. SSAO activity was determined applying the method described in the example above.

The table below (Table 4) summarizes the data of 2 treated patients. In the presented cases, significant decrease of the macular edema was observed in the fovea, the most important anatomical location of the retina responsible for central sharp vision, with a parallel decrease of serum SSAO activity. As illustrated by the data, the applied dose did not cause significant change of blood pressure.

In the table the following abbreviations were used:

BMI: body mass index – weight (kg) / height^2 (m^2)

RRsyst: blood pressure (systolic) (Hgmm)

RRdiast: blood pressure (diastolic) (Hgmm)

OCT CST (central subfield thickness): the average thickness of the central 1-mm area of the macula

OCT CPT (center point thickness): the thickness of the center point of the retina; normally that is the thinnest point of the macula

Table 4.

Data of the patients' treated

	1. patient	2. patient
Gender	male	male

Age	74	64
BMI (kg/m^2)	25.2	29.6
Blood pressure (Hgmm)		
before treatment		
RRsyst	153	153
RRdiast	63	76
pulse	60	79
14. day		
RRsyst	154	150
RRdiast	70	65
pulse	69	89
28. day		
RRsyst	152	158
RRdiast	68	74
pulse	69	93
56. day		
RRsyst	161	126
RRdiast	67	64
pulse	60	77
OCT CST		
before treatment	298	302
14. day	266	323
28. day	273	286
56. day	265	252
OCT CPT		
before treatment	272	257
14. day	215	319
28. day	219	239
56. day	209	201
SSAO activity (pmol/mg protein/hour)		
before treatment	93.02	167.56
14. day	62.42	127.96
28. day	85.73	186.36
56. day	82.41	105.1

CLAIMS

1. Dihydralazine for use in the treatment of diseases related to elevated levels of SSAO activity.

2. Use of dihydralazine for manufacture of a pharmaceutical composition for the treatment of diseases related to elevated levels of SSAO activity.

3. Method for the treatment of diseases related to elevated levels of SSAO activity in a mammal in need thereof, which comprises administering to said mammal an effective amount of dihydralazine.

4. The product or use or method according to any of of claims 1 to 3, wherein said SSAO related disease is selected from the group consisting of: acute or chronic inflammatory conditions and diseases related to elevated SSAO activity, connective tissue inflammatory conditions and diseases, rheumatoid arthritis, ankylosing spondylitis, lupus erythematosus, vasculitis, synovitis, gastrointestinal inflammatory conditions and diseases, ulcerative colitis, Crohn's disease, irritable bowel syndrome, central nervous system inflammatory conditions and diseases, Alzheimer's disease, multiple sclerosis, pulmonary inflammatory conditions and diseases, asthma, inflammatory skin conditions, psoriasis, contact dermatitis, atopic dermatitis, liver inflammatory conditions and diseases, inflammatory conditions and diseases of the eye (eyeball, accessory and protective structures of the eye, orbit), macular edema, diseases related to carbohydrate metabolism, diseases related to adipocyte differentiation or dysfunction, diseases related to smooth muscle cell dysfunction, atherosclerosis, obesity, vascular diseases, arteriosclerosis, Raynaud's disease.

5. The use of claim 4, wherein said diseas related to carbohydrate metabolism is type 1 or type 2 diabetes, diabetes related vascular complications and/or neuropathy and/or retinopathy and/or nephropathy, particularly diabetic retinopathy and macular edema.

6. The use of claim 1-5, wherein dihydralazine is administered to decrease SSAO activity in such a dose, that does not lead to appreciable lowering of blood pressure in the animal or human treated.

7. The use of claim 6, wherein dihydralazine is administered in a 0.005-1.5 mg/weight kg, preferably in a 0.2-1 mg/weight kg, more preferably in a 0.4-0.8 mg/weight kg dose.

8. The use of claim 1-7, wherein dihydralazine is administered in combination with one or more additional active ingredients, preferably in combination with other agents inhibiting SSAO activity and/or anti-inflammatory agents and/or VEGF inhibitors and/or VEGF small interfering RNA agents and/or tyrosine kinase inhibitors and/or protein kinase C inhibitors and/or angiotensin converting enzyme (ACE) inhibitors and/or PPAR-γ inhibitors and/or acetylsalicylic acid and/or statin type compounds and/or compounds used for the treatment of diabetes and/or its pathological consequences.

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2009/000073

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/502 A61K45/06 A61P9/08 A61P9/10 A61P9/12
A61P11/06 A61P17/00 A61P17/06 A61P25/28

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)
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)
EPO-Internal, WPI Data, CHEM ABS Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/054975 A1 (PANACEA BIOTEC LTD [IN]; JAIN RAJESH [IN]; JINDAL KOUR CHAND [IN]) 18 May 2007 (2007-05-18) claims 4,24 page 7, lines 16-29 page 9, lines 16-24	1-5,8
X	US 2004/005306 A1 (LOSCALZO JOSEPH [US] ET AL LOSCALZO JOSEPH [US] ET AL) 8 January 2004 (2004-01-08) claims 123,125,128,137,139,141 paragraphs [0006] - [0008], [0112] - [0116]	1-5,8

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family 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	Date of mailing of the international search report
27 October 2009	26/11/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Haider, Ursula

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2009/000073

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/087508 A2 (NITROMED INC [US]; GARVEY DAVID S [US]) 7 November 2002 (2002-11-07) claims 3,33,37,39-43,45 page 3, lines 23-33 page 8, line 3 - page 9, line 25	1-5,8
X	US 2004/081642 A1 (LOSCALZO JOSEPH [US] ET AL) 29 April 2004 (2004-04-29) claims 44,45,47-49,53 paragraphs [0006] - [0008], [0112] - [0116]	1-5,8
X	DE 10 2006 027794 A1 (LOHMANN THERAPIE SYST LTS [DE]) 20 December 2007 (2007-12-20) claims 1,7,8,20	1-5,8
X	DATABASE WPI Section Ch, Week 200838 Thomson Scientific, London, GB; Class B02, AN 2008-F88706 XP002552311 ZHAI Y.: "Hypotensor compounds for use as medicine for hypertension contains pargyline, reserpine, sulfuric acid dihydralazine, neoflumen and/or chlorobenzene pteridine, dibazol, diazepam, promethazine, rutin and vitamins" & CN 101 091 711 A (NO 2 RETIRET CADRE S LEISURE S [CN]) 26 December 2007 (2007-12-26) abstract	1-5,8
X	PASSA P: "The treatment of hypertension in a diabetic patient, when blood pressure is higher or equal to 140/90 mmHg" REVUE DU PRATICIEN - MEDECINE GENERALE 1993 FR, vol. 7, no. 211, 1993, pages 17-20, XP008113996 ISSN: 0989-2737 page 20, left-hand column, last paragraph	1-5,8
X	WO 03/055487 A1 (UNIV ADELAIDE [AU]; BURCHAM PHILIP C [AU]; PYKE SIMON M [AU]; FONTAINE) 10 July 2003 (2003-07-10) claims 41,45,52 page 18, lines 4-20	1-7

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2009/000073

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/002473 A1 (ADELAIDE RES & INNOVATION PTY [AU]; BURCHAM PHILIP C [AU]; PYKE SIMON) 12 January 2006 (2006-01-12) page 6, lines 12-25 page 20, lines 19-24 page 21, line 1 - page 22, line 17 page 23, lines 6-11	1-7
X	US 2008/161296 A1 (DAVIS ERIC [US] ET AL) 3 July 2008 (2008-07-03) page 1, paragraph 10 page 10, paragraph 82 claims 28,29,31,46-48	1-5,8
X	WO 2006/083779 A2 (MYLAN LAB INC [US]; O'DONNELL JOHN P [US]; OWENS WALTER [US]; DUNCAN J) 10 August 2006 (2006-08-10) claims 38,39,41,56-58 page 7, paragraph 35	1-5,8
X	SARTORI G: "The treatment of arteriosclerotic macular degeneration in ophthalmology" MEDIZINISCHE MONATSSCHRIFT 1959, vol. 13, no. 8, 1959, pages 496-499, XP008113998 ISSN: 0025-8474 page 498, left-hand column, paragraph 2 - right-hand column, last paragraph	1-8
A	MATYUS P ET AL: "Semicarnazide-sensitive amine oxidase: Current status and perspectives" CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS BV, BE, vol. 11, no. 10, 1 January 2004 (2004-01-01), pages 1285-1298, XP009091626 ISSN: 0929-8673 cited in the application page 1286, right-hand column, last paragraph - page 1289 page 1296, right-hand column, paragraph 2 - page 1297, left-hand column, paragraph 1	1-5,8

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2009/000073

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SATTLER J ET AL: "Inhibition of human and canine diamine oxidase by drugs used in an intensive care unit: relevance for clinical side effects?" AGENTS AND ACTIONS 1985 CH, vol. 16, no. 3-4, 1985, pages 91-94, XP008114000 ISSN: 0065-4299 page 92, left-hand column, paragraph 9 page 93; table 2 page 92; table 1	1-5
P,A	DUNKEL P ET AL: "Semicarbazide-sensitive amine oxidase/vascular adhesion protein 1: Recent developments concerning substrates and inhibitors of a promising therapeutic target" CURRENT MEDICINAL CHEMISTRY, vol. 15, no. 18, August 2008 (2008-08), pages 1827-1839, XP002552310 ISSN: 0929-8673 page 1827, left-hand column, paragraph 1 page 1828, left-hand column, paragraph 4 page 1829, left-hand column, paragraph 2 - right-hand column, paragraph 3	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/HU2009/000073

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007054975 A1	18-05-2007	NONE	
US 2004005306 A1	08-01-2004	NONE	
WO 02087508 A2	07-11-2002	AT 437637 T	15-08-2009
		CA 2446064 A1	07-11-2002
		EP 1406608 A2	14-04-2004
		JP 2004528337 T	16-09-2004
US 2004081642 A1	29-04-2004	NONE	
DE 102006027794 A1	20-12-2007	CA 2654211 A1	21-12-2007
		CN 101472557 A	01-07-2009
		EP 2029099 A2	04-03-2009
		WO 2007144082 A2	21-12-2007
CN 101091711 A	26-12-2007	NONE	
WO 03055487 A1	10-07-2003	US 2006160848 A1	20-07-2006
WO 2006002473 A1	12-01-2006	NONE	
US 2008161296 A1	03-07-2008	NONE	
WO 2006083779 A2	10-08-2006	AU 2006210952 A1	10-08-2006
		CA 2596426 A1	10-08-2006
		EP 1848424 A2	31-10-2007
		JP 2008528626 T	31-07-2008
		KR 20080016527 A	21-02-2008