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(54) Title: USE OF SUCCINIC ACID OR SALTS THEREOF FOR THE TREATMENT OF DIABETES MELLITUS AND WOUND HEALING

(57) Abstract

The invention relates to the use of succinic acid or pharmaceutically acceptable salts thereof for manufacture of medicaments or nutritional supplements effective for treating disease states caused by dysfunctional signal transdunction, preferably diabetes mellitus, and for treating wounds. The invention provides methods of inhibiting protein tyrosine phosphatase activity and of treating disease states caused by dysfunctional signal transduction, which comprises administering succinic acid or a pharmaceutically acceptable salts thereof alone or in combination with activators of protein tyrosine kinases, preferably with insulin.

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USE OF SUCCINIC ACID OR SALTS THEREOF FOR THE TREATMENT OF DIABETES MELLITUS AND WOUND HEALING

A61K 031/19, 031/135, 38/28.

Technical Field

The present invention relates to the use of succinic acid or a pharmaceutically acceptable salts thereof, and methods of inhibiting protein tyrosine phosphatases and treating disease states caused by dysfunctional signal transduction.

Background art

Cellular signal transduction is a fundamental mechanism whereby external stimuli regulate diverse cellular processes. The reversible phosphorylation of tyrosine residues in proteins involved in signaling pathways is one of the key signal transduction mechanism which is governed by the opposing actions of protein tyrosine kinases (PTKases) and protein tyrosine phosphatases (PTPases).

Protein tyrosine kinases are a family of transmembrane and cytoplasmic enzymes that specifically phosphorylate tyrosine residues in proteins. More than 50 receptors involved in regulation of cell growth, differentiation, and metabolism are known to have intrinsic protein tyrosine kinase activity. For example, the receptors to insulin, insulin-like growth factors (IGF), epidermal growth factor (EGF), transforming growth factor alpha (TGF alpha), platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and vascular endothelial growth factor (VEGF) contain intrinsic tyrosine kinase activity. The antigen receptor on T and B cells, as well as receptors for growth hormone (GH), erythropoietin, cytokines (e.g. IL-2, IL-3,IL-6), and interferon, do not have intrinsic tyrosine kinase activity but stimulate tyrosine phosphorylation by association with cytoplasmic tyrosine kinases like Src kinases (e.g.,Src, Fyn), Janus kinases (e.g., JAK1, JAK2, TYK2). External stimuli (e.g. insulin, EGF, TGF alpha, and PDGF)

frequently mediate its response through tyrosine phosphorylation of the receptor itself and substrates (e.g. insulin receptor substrate-1 (IRS-1). In each case, binding of external stimuli to receptors activates the associated tyrosine kinases to transduce a signal throughout the cell and into the nucleus. Taylor, S.S. et al., Annu. Rev. Cell. Biol. 8:429-62 (1992); White, M.F. and Kahn, C.R. J. Biol. Chem. 269:1-4 (1994); Hibi, M. and Hirano, T. Int. Rev. Immunol. 17:75-102 (1998); Taniguchi, T. Science 268(5208):251-5 (1995); Heldin, C.H. Cancer Surv. 27:7-24 (1996); al-Obeidi, F.A. et al., Biopolymers 47:197-223 (1998); Ortega, N. Front. Biosci. 4:d141-152 (1999).

Signaling pathways regulated by phosphorylation of tyrosine residues on proteins are crucial for the regulation of numerous cell functions including growth, mitogenesis, motility, cell-cell interactions, metabolism, gene transcription, and the immune response. Burke, T.R.Jr and Zhang, Z.R., Biopolymers 47:225-41 (1998). For example, levels of tyrosine phosphorylation of the receptor-like PTPase CD45 is vital for the initiation of antigen-induced lymphocyte proliferation and essential for T- and B-cells signaling and immune response. Justement, L.B. et al., Science 252:1839-42 (1991); Koretzky, G.A. et al., Proc. Natl. Acad. Sci. USA 88:2037-41 (1991); Kung, C. and Thomas, M.L., Front. Biosci. 2: d207-21 (1997). The tyrosine phosphorylation was shown to be involved in gamma-interferon signaling mechanism that controls a diverse set of biological responses including antiviral protection and antiproliferation action, as well as plays a role in inflammation, tissue repair and host defense. Igarashi, K. et al., Mol. Cell. Biol. 13:1634-40 (1993); Igarashi, K. et al., Mol. Cell. Biol. 13:3984-9 (1993). The tyrosine phosphorylation was shown to be involved in propagation of alpha-interferon mediated signal response. David, M. et al., J. Biol. Chem. 268:6593-9 (1993); David, M. et al., J. Biol. Chem. 271:15862-5 (1996). A transcription factor NF-kappaB which regulates immune and inflammatory response was demonstrated to be activated by tyrosine phosphorylation. Imbert, V. et al., Cell 86:787-98 (1996). Growth factors like FGF, TGF alpha,and PDGF play important roles in wound healing by modulating signal transduction of receptor tyrosine kinase. Kim, W.J. et al., Arch. Pharm. Res. 21:487-95 (1998). Wound healing depends on tyrosine kinase activity associated with insulin and a several growth factors. For example, EGF receptor kinase activity was elevated more than 200% within 30 minutes, moreover, EGF receptor levels were increased by 36-fold

within 24 hours after gastric mucosal injury and these increases were closely correlated with mucosal regeneration. Relan, N.K. et al., Lab. Invest. 73:717-26 (1996); Majumdar, A.P. et al., J. Lab. Clin. Med. 128:173-80 (1996); Majumdar, A.P. et al., Am. J. Physiol. 274: G863-G870 (1998). EGF receptor tyrosine kinase plays an essential role in regulating gastric mucosal cell proliferation in gastric ulcer healing. Tarnawski, A.S. et al., J. Clin. Gastroenterol. 27 Suppl. 1:S12-20 (1998). It was reported that increased expression of EGF receptor in the epithelial cells of gastric ulcer margins and increased the receptor phosphorylation levels leading to a dramatic increase in MAP-ERK-1 and ERK-2 kinase phosphorylation levels more than 440% and 880% respectively during early stages of gastric ulcer healing. Moreover, tyrphostin A46, an inhibitor of EGF receptor kinase dependent cell proliferation, was reported to inhibit significantly both the signaling pathways and ulcer healing. Tarnawsky, A. et al.,Gastroenterology 102:695-698 (1992); Pai, R. et al., Gastroenterology 114:706-13 (1998). Expression of VEGF was demonstrated to be accompanied by forming new microvessels during tissue injury repair. Jones, M.K. et al., Gastroenterology 114:A163 (1998). Growth factors was found to enhance cutaneous wound epitelialization. Flour, M. and Degreef, H. Semin. Cutan. Med. Surg. 17:260-5 (1998). EGF and TGF alpha binding to a common EGF receptor tyrosine kinase initiates a series of events which ultimately regulate cell proliferation during repair of human burn wounds. Wenczak, B.A. et al., J. Clin. Invest. 90:2392-401 (1992). Insulin was reported to improve wound matrix formation in massively burned patients. Pierre, E.F. et al., J. Trauma 44:342-5 (1998). Insulin and EGF was reported to accelerate gastric eithelial wound repair by stimulating both the migration and the proliferation of gastric epithelial cells. Maehiro, K. et al., J. Gastroenterol. 32:573-8 (1997). Neurotrophins and their receptors play roles in nerve repair. Ebadi, M. et al., Neurochem. Int. 30:347-74 (1997). Insulin was reported to enhance wound healing in fetal rat partietal bones. Yano, H. et al., J. Oral. Maxillofac. Surg. 54:182-6 (1996). PDGF and IGF-1 in combination was shown to enhance periodontal regeneration. Giannobile, W.V. et al., J.Periodontal Res. 31:301-12(1996). Giannobile, W.V., Bone 19:23-27 (1996). IGF was reported to play a role in bone fracture healing. Trippel, S.B. Clin. Ortop. 355Suppl:S301-13 (1998).

Protein tyrosine phosphatases (PTPases, EC 3.1.3.48) are a family of transmembrane and cytoplasmic enzymes that specifically dephosphorylate phosphotyrosine residues in proteins. The substrates of PTPases may be proteins which possess phosphotyrosine residues including PTKases (e.g. receptors to insulin, EGF, VEGF, TGF alpha, FGF, and PDGF) or substrates of PTKases (e.g. IRS-1). Hunter, T., Cell 58:1013-16 (1989); Saito, H. and Streuli, M., Cell Growth Differ. 2:59-65 (1991); Pot, D.A. and Dixon, J.E., Biochim. Biophys. Acta 1136:35-43 (1992); Vincent, J.B. and Crowder, M.W. in "Phosphatases in cell metabolism and signal transduction: structure, function, and mechanism of action", R.G.Landes Co., 60-98 (1995). Some of PTPases are negative regulators of insulin and growth factors receptor signal transduction. For example, the PTP 1B was reported to be a negative regulator of insulin and IGF-1 signal transduction. Kenner, K.A. et al., J. Biol. Chem. 271:19810-6 (1996); Byon, J.C. et al., Mol. Cell. Biol. 182:101-8 (1998). The transmembrane PTPase LAR was shown to have a direct impact on insulin action. Goldstein, B.J. et al., Mol. Cell. Biochem. 182:91-9 (1998). Low molecular wight LMW PTPase was shown to be a negative regulator of insulin-mediated mitotic and metabolic signaling. Chiarugi, P. et al., Biochem. Biophys. Res. Commun. 238:676-82 (1997). As reported, PTPase LMW inhibits cell proliferation by dephosphorylation of the phosphorylated PDGF receptor. Chiarugi, P. et al., FEBS Lett. 372:49-53 (1995). The PTPases was shown to mediate decreasing both EGF and PDGF receptor tyrosine phosphorylation. Sorby, M. and Ostman, A., J. Biol. Chem. 271:10963-6 (1996).

Since tyrosine phosphorylation is reversible and dynamic in vivo, both PTKases and PTPases play key roles in various growth factor- or cytokine-mediated signal transduction pathways. The levels of tyrosine phosphorylation required for normal cell growth and differentiation at any time are achieved through the coordinated action of PTKases and PTPases. An imbalance between these enzymes results in dysfunctional signal transduction that may impairs normal cell functions leading to metabolic disorders, immune disorders, cellular transformation, and wound healing disorders.

For example, the overexpression of protein tyrosine phosphatases was demonstrated to correlate significantly with breast and ovarian cancer. Weiner, J.R. et al., J. Natl.

Cancer Inst. 86:372-8 (1994); Weiner, J.R. et al., Gynecol. Oncol. 61:233-40 (1996); Ottenhoff-Kalff, A.E. et al., Breast Cancer Res. Treat. 33:245-56 (1995).

The overexpression of the PTPase LAR was reported to cause a reduction in insulin receptor autophosphorylation and insulin-stimulated cellular responses. Li, P.M. et al., Cell. Signal. 8:467-73 (1996). The increasing PTPase activity toward insulin receptor was reported to be a pathogenic factor in a human insulin resistance, while improving sensitivity to insulin in human adipose tissue is accompanied by decreasing of PTPase activity. Ahmad, F. et al., J. Clin. Invest. 95:2806-12 (1995); Ahmad, F. et al., Metabolism 46:1140-5 (1997). The imbalance between PTPase and PTKase activity was shown to play a role in impairment of insulin signal transduction under diabetes mellitus. Ahmad, F. et al., J. Clin. Invest. 100:449-58 (1997);.

Diabetes and systemic glucocorticoid treatment caused a severe defect in wound healing accompanied by reduced expression of PDGF receptors. Beer, H.D. et al., J. Invest. Dermatol. 109:132-8(1997). The levels of IGF-1 was reported to be reduced in the wound enviroment of diabetics, whereas treatment of these wounds with IGF-1 reverses diabetes-induced wound healing impairment. Bitar, M.S., Horm. Metab. Res. 29:383-6 (1997). Wound healing is impaired under ageing due to delay in appearance of growth factors such as PDGF, EGF and their receptors in the wound enviroment. Ashcroft, G.S. et al., J. Anat. 190 (Pt.3):351-65 (1997). Patients with gastric or duodenal ulcers was found to have a significantly lower salivary content of EGF compared to healthy subjects. Konturek, S.J., Gastroenterol. Clin. North. Am. 19:41-65 (1990). *Helicobacter pylori* infection, a predominant cause of chronic gastritis and ulcer disease in human, was shown to delay of ulcer healing by producing of the vacuolating cytotoxin that interferes with the EGF-triggered signal transduction cascade. Pai, R. et al., Am. J. Pathol. 152:1617-24 (1998). Growth factors like EGF, VEGF, TGF alpha and their receptors play important roles in tissue injury repair and ulcer healing. Jones, M.K. et al., Front. Biosci. 4:d303-9 (1999).

In general, it is sometimes useful to regulate tyrosine phosphorylation in order to treat diseases caused by dysfunctional signal transduction, and to enhance wound healing. Since a regulation of PTPase activity is a direct method of modulating

dysfunctional signal transduction, a need exerts in the art for novel methods of inhibiting or modulating the PTPase activity.

All PTPases contain in the active site an essential cystein residue which exhibits extremely low pK_a values 4.7 to 5.6 (versus 8.5 of a typical cystein residue) and forms under physiological conditions a readily oxidized thiolate-anion. Lohse, D.L. et al.,Biochemistry 36:4568-75 (1997); Denu, J. et al., Biochemistry 34:3396-3403 (1995); Zhang, Z.-Y., and Dixon, J.E., Biochemistry 32:9340-45 (1993).

Hydrogen peroxide (H_2O_2) was demonstrated to be universal inhibitor of PTPase activity that inhibits specifically both cytoplasmic (PTP 1B) and transmembrane (LAR) PTPases under physiological conditions by reversible oxidation of the catalytic cysteine thiolate. Denu, J.M. and Tanner, K.G., Biochemistry 37:5633-42 (1998). Also, it was reported that hydrogen peroxide transiently generated during growth factor stimulation is concomitant with relevant tyrosine phosphorylation. Bae, Y.S. et al., J. Biol. Chem. 272:217-21 (1997); Sundaresan, M. et al., Science 270(5234):269-9 (1995); Finkel, T., Curr. Opin. Cell Biol. 10:248-53 (1998). It was reported that exogenous hydrogen peroxide mimics insulin action. Moreover, insulin stimulates production of endogenous hydrogen peroxide. Mukherjee, S.P. et al., Biochem. Pharmacol. 27:2589-94 (1978); May, J.M. and de Haen, C., J. Biol. Chem. 254:2214-20 (1979). May, J.M. and de Haen, C., J. Biol. Chem. 254:9017-21 (1979); Lawrence, J.C.Jr and Larner, J., J. Biol. Chem. 253:2104-13 (1978); Little, S.A. and de Haen, C., J. Biol. Chem. 255:10888-95 (1980). H_2O_2 was reported to inhibit LMW PTPase which downregulates PDGF and insulin receptor signal transduction. Caselli, A. et al., J. Biol. Chem. 273:32554-60 (1998). EGF-induced intracellular H_2O_2 was reported to inhibit PTPase 1B, and this inhibition was found to be a fully reversible. Lee, S.-R. et al., J. Biol. Chem. 273:15366-72 (1998). Thus, stimulation of endogenous hydrogen peroxide production is a physiologically occurring way to enhance tyrosine phosphorylation in mammalian tissues through inhibiting PTPase activity.

Succinic acid is the physiologically occuring substrate of succinate dehydrogenase that play a role in cellular respiration and energy metabolism.

We have found for the first time that administering an effective amount of succinic acid or salt thereof causes an increase in endogenous hydrogen peroxide production in

dose- and time-dependent manner by mammalian tissues that is accompanied by reversible inactivation of PTPase activity. This biological effect is unexpected to prior arts. Although respiratory chain of isolated mitochondria can generate hydrogen peroxide as a minor by-product with a numerous of respiratory substrates including succinate, the inactivation of PTPases by H_2O_2 generated in mitochondrial respiratory reactions is unknown from prior art. Boveris, A. et al., Biochem. J. 128:617-30 (1972); Boveris, A. and Chance, B., Biochem. J. 134:707-16 (1973); Swaroop, A. and Ramasarma, T., Biochem. J. 194:657-65 (1981); Turrens, J.F. et al., Arch. Biochem. Biophys. 273:408-14 (1985). Moreover, the evidences was presented that mitochondrial respiratory chain does not mediate the receptor-triggered hydrogen peroxide generation. Meier, B. et al., Biochem. J. 263:539-45 (1989).

The present invention shows for the first time that administering to a mammal an effective amount of succinic acid or salt thereof is effective for inhibiting PTPase activity. Since inhibiting PTPase activity is effective for modulating dysfunctional signal transduction, the administering of succinic acid or salt thereof to mammals is accordingly useful in treating disease states caused by dysfunctional signal transduction.

It is an object of the present invention to provide a methods for inhibiting protein tyrosine phosphatase activity and treating deisease states caused by dysfunctional signal transduction, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof.

Vanadates and peroxovanadates are known to be non-specific PTPases inhibitors that mimic a variety effects of insulin. Bevan, A.P. et al., Mol. Cell. Biochem. 153:49-58 (1995). However, this class of compounds is toxic because each of compound contains a heavy metal atom. Moreover, evidences was presented that vanadate as well as peroxovanadate attenuates optimal mammary epithelial cell growth stimulated by combination of insulin and EGF. McIntyre, B.S. et al., Proc. Soc. Exp. Biol. Med. 217:180-7 (1998).

Disclosure of Invention

The present invention relates to the use of succinic acid or salt thereof for inhibiting the activity of protein tyrosine phosphatases (PTPases). The invention is further relates to the regulation of cellular processes governed by signal transduction through the

inhibition of the activity of PTPases by the succinic acid or salt thereof. The invention further provides for the use of succinic acid or salt thereof in the treatment of a mammal having a disorder caused by dysfunctional signal transduction, preferably diabetes mellitus. The invention further provides for the use of succinic acid or salt thereof in the treatment of a wound in a mammal.

The present invention provides a method of inhibiting protein tyrosine phosphatase activity, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof. The succinic acid or a pharmaceutically acceptable salt thereof inhibits PTPases not directly by binding to molecule of a PTPase, but through stimulating production of endogenous hydrogen peroxide which is a non-specific inhibitor both cytoplasmic and transmembrane PTPases. Various procedures known in the art may be used for identifying, evaluating or assaying the inhibition of PTPase activity by the succinic acid or a pharmaceutically acceptable salt thereof in the method of the invention, for example, exposing tested cells to the succinic acid or a pharmaceutically acceptable salt thereof and detection of PTPase activity levels in comparison with a control.

PTPases of the invention play a role in cellular signal transduction. The inhibiting PTPase activity by succinic acid or a pharmaceutically acceptable salt thereof is useful for modulating signal transduction and regulation cellular processes which are governed by signal transduction. The term "signal transduction", as used herein, includes the multiple pathways that are regulated by reversible phosphorylation of specific tyrosine residues on proteins involved in signaling. The term "modulating", as used herein, means upregulation or downregulation of a signaling pathway. Cellular processes under the control of signal transduction may include, but are not limited to, normal processes such as proliferation, differentiation, transcription of specific genes, adhesion, apoptosis, and survival; and abnormal processes such as transformation, and blocking differentiation.

A signal may be triggered by the binding of a ligand to its receptor which transduces the signal by the phosphorylating or dephosphorylating specific tyrosine residues on proteins inside the cell. Such proteins may include, but are not limited to, the receptor or its subunits, the receptor substrates, cytoplasmic kinases, cytoplasmic phosphatases,

adapter molecules, and transcription factors. The term "receptor", as used herein, include, but are not limited to, insulin receptor, members of insulin-like growth factor receptor family, epidermal growth factor receptor family, transforming growth factor receptor family, fibroblast growth factor receptor family, hepatocyte growth factor receptor family, vascular endothelial growth factor receptor family, platelet-derived growth factor receptor, the T-cell receptor, the B-cell receptor, members of the type 2 to 4 cytokine receptor families, and erythropoietin receptor. The adapter molecules may include the Grb proteins, IRS-1, and Zap-70. The transcription factors may include nuclear factor kappa-B, and STAT proteins. The ligand, as used herein, includes, but are not limited to, insulin, insulin-like growth factors, epidermal growth factor, platelet-derived growth factor, vascular endothelial growth factor, fibroblast growth factors, transforming growth factor, and neurotrophins, and cytokines such as growth hormone, erythropoietin, tumor necrosis factor, interleukins, and interferons.

The inhibiting PTPase activity by succinic acid or a pharmaceutically acceptable salt thereof can be used to upregulate signal transduction in mammals so that the effect of ligand binding to a receptor is enhanced, or mimicked if the ligand is not present. The succinic acid or a pharmaceutically acceptable salt thereof exerts this effect in the signaling pathway which is normally downregulated by tyrosine dephosphorylation. The means by which PTPases normally downregulate signal transduction may involve the dephosphorylation of specific phosphotyrosine residues on receptors which needed in phosphorylation of its own tyrosine residues to achieve optimal activity in the signaling pathway. The inhibiting PTPase activity can be used to prevent dephosphorylation of phosphotyrosine residues on receptors which normally becomes phosphorylated upon ligand binding, thereby prolong receptor mediated signal transduction. The inhibiting PTPase activity can also be used to prevent the dephosphorylation of the receptor in which the tyrosine residues become autophosphorylated due to basal activity of the receptor. In these receptors, a signal may be triggered even in the absence of ligand binding.

The inhibiting PTPase activity by succinic acid or a pharmaceutically acceptable salt thereof can be used to downregulate signal transduction in cells so that the effect of ligand binding to a receptor is abolished or attenuated. The succinic acid or a

pharmaceutically acceptable salt thereof exert this effect in the signaling pathway which normally downregulates by tyrosine dephosphorylation. Such signaling pathways include, but are not limited to, Src family tyrosine kinases which are activated by dephosphorylation of their inhibitory site.

Preferably, the inhibiting PTPase activity by succinic acid or a pharmaceutically acceptable salt thereof is useful in insulin-sensitive tissues for triggering or enhancing insulin receptor signal transduction by inhibiting the constitutive dephosphorylation of the phosphotyrosine sites on the activated insulin receptor. This would allow to prolong or enhance phosphorylation of the insulin receptor, thus prolong or enhance insulin receptor signal transduction. The succinic acid or a pharmaceutically acceptable salt thereof can be used to trigger a signal even in absence of insulin, since insulin receptor was demonstrated to be phosphorylated at a low level even in absence of insulin. Goldstein, B.J., J. Cell. Biochem. 48:33-42(1992).

The present invention provides a method of treating disease states caused by dysfunctional signal transduction, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof.

Dysfunctional signal transduction can result from imbalance between activity of PTPases and proteine tyrosine kinases which play roles in a signaling pathway. The disease states may be caused by dysfunctional signal transduction of insulin receptor, members of insulin-like growth factor receptor family, epidermal growth factor receptor family, transforming growth factor receptor family, fibroblast growth factor receptor family, hepatocyte growth factor receptor family, vascular endothelial growth factor receptor family, transforming growth factor, platelet-derived growth factor receptor, the T-cell receptor, the B-cell receptor, members of the type 2 to 4 cytokine receptor families, and erythropoietin receptor. The disease states may include, but are not limited to, diabetes mellitus and its chronic complications such as nephropathy, encephalopathy, microangiopathy, retinopathy, diabetic foot ulcers caused by deficient insulin signal transduction; immune disorders caused by deficient cytokine signal transduction such as anemia and immunodeficiency; neurodegenerative disorders caused by deficient neurotrophin signal transduction; cancer; and wound healing disorders caused by

deficient insulin and growth factor signal transduction under ageing, diabetes, or infections. Preferably, the disease state is diabetes mellitus.

The present invention provides a method of treating wounds, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof. Such wounds may include, but are not limited to, incised wounds, burn wounds, gastrointenstinal mucosal erosions and ulcers, periodontal wounds, and bone fractures.

Further, the invention provides a method of treating disease state caused by dysfunctional signal transduction in a mammal, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof stepwise or in physical combination with an effective amount of a protein tyrosine kinase activator.

The term "protein tyrosine kinase activator" includes ligands to receptors which contain intrinsic tyrosine kinase activity, or ligands to receptors which do not have intrinsic tyrosine kinase activity, but can stimulate tyrosine phosphorylation through association and activating cytoplasmic PTKases. The term "ligand", as used herein, include, but are not limited to, insulin, insulin-like growth factors, epidermal growth factor, platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, fibroblast growth factor, and neurotrophins, and cytokines such as growth hormone, erythropoietin, tumor necrosis factor, interleukins, and interferons. The term "receptor", as used herein include but are not limited to, insulin receptor, members of insulin-like growth factor receptor family, epidermal growth factor receptor family, fibroblast growth factor receptor family, hepatocyte growth factor receptor family, vascular endothelial growth factor receptor family, nerve growth factor, platelet-derived growth factor, the T-cell receptor, the B-cell receptor, members of the type 2 to 4 cytokine receptor families, and erythropoietin receptor.

The new combination of succinic acid or a pharmaceutically acceptable salt thereof with the protein tyrosine kinase activator is synergistically effective for modulating signal transduction. The combination of the invention is synergistically effective in upregulating signal transduction that normally upregulated by phosphorylation of tyrosine residues in proteins involved in the signaling pathways. The combination of the

invention is synergistically effective in downregulating signal transduction that normally downregulated by dephosphorylation of phosphotyrosine residues in proteins involved in signaling pathways.

Preferably, the protein tyrosine kinase activator of the composition is insulin. Insulin of the invention is any standard insulin prepared by methods well-known in the art. The new combination of succinic acid or a pharmaceutically acceptable salt thereof with insulin is synergistically effective in triggering or enhancing insulin receptor signal transduction that is accordingly effective for treating disease states caused by dysfunctional signal transduction of insulin receptor. Preferably, the disease states caused by dysfunctional insulin receptor signal transduction is diabetes mellitus. Insulin is administered parenterally in the method of the invention. The effective amount of insulin is selected from 2 units to 100 units per day per mammalian subject.

Preferably, the mammal of the invention is a human.

The succinic acid has the chemical structure given below:

$HOOCCH_2CH_2COOH$

The pharmaceutically acceptable salt of the succinic acid is prepared by known methods from organic and inorganic bases. Such bases include, but are not limited to, nontoxic alkali metal and akaline earth bases, for example, calcium, magnesium, lithium, sodium, and potassium hydroxide; ammonium hydroxide and nontoxic organic bases, such as triethylamine, butylamine, diethanolamine, triethanolamine, and 2-ethyl-6-methyl-3-hydroxypyridine. In addition, the salt of succinic acid can be in form of solvates with water or suitable organic solvents.

The succinic acid or a pharmaceutically acceptable salt thereof is preferably administered orally and topically in the methods of the invention. The succinic acid or a pharmaceutically acceptable salt thereof may also be administered by a variety of other routes such as parenterally, e.g. intravenously, or subcutaneously, or intramuscularly; transdermally; or rectally. The effective amount of succinic acid or a pharmaceutically acceptable salt thereof for use in the methods of the invention is selected from 0.1 milligram to 50 milligrams, more preferably from 1 mg to 20 mg per day per kilogram

of body weight of the mammalian subject. Preferably, the succinic acid or a pharmaceutically acceptable salt thereof is administered for period of 1 day or longer, more preferably, 3 to 7 days.

The invention provides for the use of succinic acid or a pharmaceutically acceptable salt thereof for the manufacture of a medicament or a nutritional supplement effective for treating disease state caused by dysfunctional signal transduction in a mammal, preferably in a human. Succinic acid or a pharmaceutically acceptable salt thereof is useful in nutritional and medicinal formulations according to the present invention for treating disease state caused by dysfunctional signal transduction in a mammal, preferably in a human. The medicament or nutritional supplement of the invention is prepared by known procedures using well-known ingredients. In making the medicament or nutritional supplement, the active ingredients will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, and may be in the form of a capsule, tablet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid, or liquid material which acts as a vehicle, excipient, or medium for the active ingredient. The nutritional supplements can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules. The medicament can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, aerosoles, suppositories, sterile injectable solutions, and sterile packaged powders. Some examples of suitable carriers, diluents, and excipients include lactose, dextrose, sorbitol, mannitol, calcium phosphate, alginates, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propyl hydroxybenzoates, talc, magnesium stearate, stearic acid, and mineral oil. The medicaments or nutritional supplements can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents, or flavoring agents.

The orally ingestible nutritional supplement of the invention can comprise deionized water, succinic acid or salt thereof, maltodextrin, sodium caseinate, corn syrup solids, medium chain triglycerides, canola oil, calcium caseinate, soy protein, potassium citrate, magnesium chloride, sodium citrate, tricalcium phosphate, soy lecithine, sodium

ascorbate, choline chloride, potassium chloride, coenzyme Q, vitamin E, molibdenium yeast, selenium yeast, carrageenan, chromium yeast, biotin, niacinamide, zink sulfate, ferrous sulfate, calcium pantothenate, vitamin A, ascorbic acid, cyancobalamine, manganese sulfate, copper gluconate, vitamine K, thiamin, pyridoxine hydrochloride, vitamine D, riboflavin, folic acid, potassium iodide, methionine, antifoam agents and natural or artificial flavors and sweeteners. Ingredients which may also be added to nutritional supplement of the invention include fructose, soybean oil, sunflower oil, canola oil, carnitine, taurine, and other natural components. The various compositions of the invention can also contain other conventional ingredients such as suspending or wetting agents, preservatives, antioxidants, thickening agents and the like.

The medicament of the invention in form of oral formulations (e.g. paste, toothpaste, gel, or liquid formulations) can comprise a water; tetrasodium pyrophosphate or other pyrophosphate source; sodium fluoride, stannous fluoride, indium fluoride, sodium monofluorophosphate, or other fluoride source; alkali metal bicarbonate salt; xylitol; thickening agents such as carboxyvinyl polymers, carrageenan, hydroxyethyl cellulose, sodium carboxymethylcellulose and other water soluble salt of cellulose ethers, natural gum like gum karaya, xanthan gum, gum arabic, and gum fragacanth; colloidal magnesium aluminium silicate, lithium aluminium silicate; humectants such as glycerin, sorbitol, polyethylene glycol, propylene glycol, and other alcohols; abrasive polishing materials such as silicas, calcium carbonate, dicalcium orthophosphate dihydrate, calcium pyrophosphate, tricalcium phosphate, calcium polymetaphosphate, insoluble polymetaphosphate, hydrated alumina, and resinous abrasive materials such as condensation product of urea and formaldehide; buffering agents which adjust the pH of 9.0 to 10.5 such as alkali metal hydroxides, carbonates, borates, and silicates; titanium dioxide; coloring agents; flavoring agents like menthol; surfactants including nonionic, amphoteric, zwitterionic, cationic, anionic such as water soluble long-chain alkylsulfates and their salts, sodium lauryl sulfate, and sodium coconut monoglycerides sulfonates; and sweeting agents such as sodium saccharin, dextrose,sucrose,lactose,maltose,levulose, aspartame,sodium cyclamate, D-tryptophan, and dihydrochalcones.

The medicament of the invention in form of topical formulations may include, but are not limited to, solutions, suspensions, salves, ointments, creams, gels, aerosoles, jellics, emulsions, powders, liniments, and the like. If desired, these may be sterilized or mixed with auxiliary agents, e.g. preservatives; stabilizers such as albumine, a dissacharide, a cyclic oligosaccaride like hydroxypropyl-beta-cyclodextrin; surfactants; wetting agents; perfumes; solvents such as water; or buffers.

The medicament of the invention may be in form suitable for parenteral, e.g. intravenous, subcutaneous, or intramuscular administration. For intravenous use, the succinic acid or a pharmaceutically acceptable salt thereof is administered in a pharmaceutically acceptable carrier such as a commonly used intravenous fluid and administered by infusion. Such fluids, for example, physiological buffer saline or Ringer's solution can be used. For intramuscular preparations, a sterile the active components of composition can be dissolved and administered in a pharmaceutically acceptable carrier such as pyrogen-free water (distilled) or physiological saline.

The medicament or nutritional supplement of the invention can be used in combination with other biologically active compounds known to the art that exhibit antidiabetic action such as peroxovanadates, and oral hypoglicemic drug like sulfonylureas and biguanides. The medicament or nutritional supplement of the invention can be used in combination with other biologically active compounds known to the art that exhibit activities directed to enhancing of wound healing such as antimicrobial agents, peroxocompounds like peroxovanadates, and

Treating, as used herein, describes the managment and care of a mammal for the purpose of combating the disease, condition, or disorder and includes the administration of a compound of present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder.

The following examples are presented to demonstrate the invention. The examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLE 1

This example shows that disodium succinate hexahydrate is effective in inhibiting PTPase activity.

Animals. Male albino Wistar rats 8-10 weeks of age 200-250 grams of body weight were used. The rats were housed at the temperature of 18 ± 21^{0}C on a 12 hour light-dark cycle. Rats were fed on a stock laboratory diet (59 % carbohydrates; 17 % protein; 3 % fat; 21 % minerals, water, cellulose) and allowed water *ad libitum*.

Adipocytes preparation. Isolated rat adipocytes were prepared by the method of Rodbell. Rodbell, M., J. Biol. Chem. 239:375-80 (1964). Epididymal adipose tissues were digested with collagenase (*Clostridium histolyticum*) at 37°C for 1 h in Krebs-Ringer phosphate buffer, pH 7.4, containing 3% bovine serum albumin (BSA). The composition of the Krebs-Ringer buffer was 126 mM NaCl, 1.4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5.2 mM KCl, 10 mM $NaHPO_4$, pH 7.4. At the end of digestion, the cells were filtered through double cheesecloth, washed three times with 5 volume of 1% BSA buffer, and resuspended in this medium. The homogenate was centrifuged at 3000 g for 15 min, washed with 1% BSA buffer, and resuspended in this medium again. Then the homogenate was centrifuged at 17000 g for 30 min and washed with 1% BSA buffer. Further, prepared homogenate was centrifuged again at 30000 g for 30 min, washed with 1% BSA buffer, and rat adipocytes (4×10^6 cells/ml) were used in the experiments.

Liver supernatant preparation. Rat livers were washed with cold phosphate-buffered saline and homogenized in buffer containing 100 mM Tris-HCl, 1.15% KCl, and pH 7.4. The homogenate was centrifuged at 200 g for 10 min to remove debris, and a postnuclear supernatant (35.6 mg of protein per ml) was used in the experiments.

Hydrogen peroxide assay. To determine hydrogen peroxide production in the rat tissue fractions, the assay based on the horseradish peroxidase-mediated oxidation of phenol red by H_2O_2 was used. Pick, E. and Keisari, Y., J. Immunol. Methods 38: 161-70(1980).

The rat adipocytes or the liver supernatant were incubated at 37°C in a buffer containing 50 mM Tris-HCl, 5 mM EDTA, 0.02% digitonin, pH 7.6, and with or without

(control) 2 mM disodium succinate hexahydrate. An aliquot of the cellular fraction was used to estimate H_2O_2 release at 0, 5, 10 and 15 min of the incubation. Results are presented in Tables 1 to 2 as mean±S.E.M., and as H_2O_2 concentration in % of control.

Table 1. Stimulation of H_2O_2 production in rat adipocytes exposed to 2 mM disodium succinate (16 separate experiments).

Incubation time, min	H_2O_2 concentration, nmol/ml of medium	H_2O_2 concentration, in % of control
0	10.25 ± 0.07	100
5	17.23 ± 0.11	168
10	18.63 ± 0.24	182
15	27.66 ± 0.46**	270

**Denotes statistically significant differences from the results obtained at 0 min ($P < 0.001$).

Table 2. Stimulation of H_2O_2 production in rat liver supernatant exposed to 2 mM disodium succinate (16 separate experiments).

Incubation time, min	H_2O_2 concentration, nmol/ml of medium	H_2O_2 concentration, in % of control
0	19.8 ± 0.6	100
5	79.7 ± 0.8	403
10	209.2 ± 1.5	1056
15	192.4 ± 2.2**	971

**Denotes statistically significant differences from the results obtained at 0 min ($P < 0.001$).

Thus, disodium succinate stimulates production of endogenous hydrogen peroxide which is a well-known inhibitor of PTPase activity.

Phosphatase assay. To survey the tissue fractions for PTPase activity, the method of Kremerskothen and Barnekow with using phosphotyrosine as an artificial

substrate was used. Kremerskothen, J. and Barnekow, A., in "Tyrosine phosphorylation/dephosphorylation and downstream signaling", ed. by L.M.G.Heilmeyer Jr., Springer-Verlag Berlin Heidelberg, pp. 123-126 (1993).

Disodium succinate hexahydrate was assayed for its activity in inhibiting PTPase activity in the cellular fractions (rat adipocytes and liver supernatant). For the PTPase assay 60 µl of the cell fraction was incubated in 40 µl of a reaction buffer containing 50 mM Tris/HCl, pH 7.5 and 10 mM phosphotyrosine as the substrate, and with or without (control) 2 mM disodium succinate hexahydrate. After a 5, 10, or 20 min incubation period at 37°C, the reaction was terminated by the addition of 40 µl 10% BSA and 120 µl 25% tricloroacetic acid. The probes were centrifuged at 6000 g for 10 min and 20 µl of the supernatant were mixed with 380 µl of 50 mM Tris-HCl, pH 7.5 and 100 µl of the malachite green colour reagent (Baykov, A.A. et al. Anal. Biochem. 171:266-70 (1988). After a 10 min incubation period at a room temperature, the extinction was measured at 655 nm in microtiter plates. The specific PTPase activity was calculated by using a standard graph ranging inorganic phosphate (P_i). Results are presented in Tables 3 to 4 as mean±S.E.M., and as PTPase activity in % of control.

Table 3. Inhibiting PTPase activity in rat adipocytes exposed to 2 mM disodium succinate (14 separate experiments).

Incubation time, min	P_i concentration, nmol/ml	PTPase activity, % of control
0	0.067 ± 0.004	100
5	0.071 ± 0.001	106
10	0.060 ± 0.001	90
20	0.041 ± 0.001**	61

**Denotes statistically significant differences from the results obtained at 0 min ($P < 0.001$).

Table 4. Inhibiting PTPase activity in rat liver supernatant exposed to 2 mM disodium succinate (17 separate experiments).

Incubation time, min	P_i concentration, nmol/ml	PTPase activity, % of control
0	0.203 ± 0.003	100
5	0.158 ± 0.002	78
10	0.141 ± 0.002	69
20	0.122 ± 0.002**	60

**Denotes statistically significant differences from the results obtained at 0 min ($P < 0.001$).

Thus, the administering of disodium salt of succinic acid is effective in inhibiting PTPase activity.

EXAMPLE 2

This example shows that administering disodium succinate mimics insulin in totally pancreatectomized rats.

Assays. Plasma free fatty acids levels were determined by enzymatic method with a commercially available kit (Waho Chemicals Gmbh, Neuss, Germany) with Multistat 3 centrifugal analyzer (Instrumentation Laboratories, Lexington, USA).

Serum glucose concentrations were determined by the glucose oxidase method with a kit (Lachema, Slov.) with glucose analyzer (Beckman, Fullerton, Calif., USA).

Plasma insulin concentrations were determined by a double-antibody radioimmunoassay kit (Kabi Pharmacia Diagnostics, Uppsala, Sweden) using a rat insulin standard (Novo Research Institute, Bagsvard, Denmark).

Plasma C-peptide concentrations were determined with a kit (Dako, Denmark) using a rat C-peptide standard.

Plasma triglycerides and cholesterol concentrations in High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were determined by enzymatic methods

with kits (Boeringher Mannheim, Mannheim, Germany) with Multistat 3 F/LS apparatus (Instrumentation Laboratories, Lexington, USA).

Procedure. The male Wistar rats maintained as described in example 1 of the invention were used in these experiments. The rats were anaesthetized with 1% solution of nembutal (0.2 ml/100 g of body weight, intramuscularly), and the total pancreatectomy (TP) was made. The vessels were ligated, the abdominal cavities were purified and then sewed. In addition to the laboratory diet and water, the totally pancreatectomized rats (n=10) were allowed 5% water solution of disodium succinate hexahydrate *ad libitum* or none (n=3, control). All control rats were perished during the first day after TP. The values of plasma insulin and C-peptide levels in totally pancreatectomized rats treated by the succinate were below the detection limits of the used assays during the 60 days of the experiment. The detection limit was 3 pmol/l for plasma insulin assay, and 0.0165 nmol/l for plasma C-peptide assay. Fasting plasma insulin levels, plasma C-peptide levels, serum glucose levels, plasma free fatty acid levels (FFA), plasma cholesterol levels, plasma triglycerides levels, plasma high density lipoprotein cholesterol levels (HDL), and plasma low density lipoprotein cholesterol levels (HDL) were measured in rats by tail clipping method before total pancreatectomy (TP), and after TP. The data are presented in Tables 5 through 7 as mean±S.E.M.

Table 5. Mean fasting plasma Insulin, C-peptide, and serum Glucose levels in totally pancreatectomized rats (n=10) treated by disodium succinate.

Before total pancreatectomy (TP)				
	Days	Insulin, pmol/l	C-peptide, nmol/l	Glucose, mmol/l
		117 ± 11	0.93 ± 0.05	4.26 ± 0.33
After total pancreatectomy (TP)				
Before treatment	0	< 3	< 0.0165	21.24 ± 1.28
Treatment period	10	< 3	< 0.0165	7.20±0.85**
	20	< 3	< 0.0165	5.94±0.81**
	40	< 3	< 0.0165	5.37±1.64**
	60	< 3	< 0.0165	4.98±0.67**

**Denotes statistically significant differences from the results obtained at zero day (P< 0.001).

Table 6. Mean fasting plasma FFA, Triglycerides, and Cholesterol levels in totally pancreatectomized rats (n=10) treated by disodium succinate.

Before total pancreatectomy (TP)				
	Days	FFA, mmol/l	Triglycerides, mmol/l	Cholesterol, mmol/l
		0.42 ± 0.02	0.93 ± 0.05	1.46 ± 0.14
After total pancreatectomy (TP)				
Before treatment	0	0.58 ± 0.05	1.50 ± 0.30	1.67 ± 0.20
Treatment period	10	0.51 ± 0.08	0.92 ± 0.14	1.54 ± 0.34
	20	0.51 ± 0.04	1.46 ± 0.22	1.52 ± 0.41
	40	0.52 ± 0.06	1.27 ± 0.34	1.66 ± 0.19
	60	0.59 ± 0.04	1.30 ± 0.38	1.51 ± 0.27

Table 7. Mean fasting plasma LDL, and HDL levels in totally pancreatectomized rats (n=10) treated by disodium succinate.

Before total pancreatectomy (TP)			
	Days	LDL, mmol/l	HDL, mmol/l
		1.01 ± 0.06	0.29 ± 0.07
After total pancreatectomy (TP)			
Before treatment	0	1.60 ± 0.08	0.20 ± 0.04
Treatment period	10	1.83 ± 0.27	0.23 ± 0.05
	20	1.77 ± 0.16	0.26 ± 0.05
	40	1.38 ± 0.26	0.27 ± 0.08
	60	1.68 ± 0.20	0.27 ± 0.04

The administering disodium succinate to the totally pancreatectomized rats results in 4-fold decreasing in the pathologically elevated glucose levels even in the absence (non-detectable quantities) of insulin in the rat plasma.

Thus, the succinic acid salt exhibits insulinomimetic action even in the absence of insulin. Since pancreatectomy in rats is animal model of diabetes, the administering the succinic acid salt is effective therapy for treating diabetes mellitus.

EXAMPLE 3

This example shows that administering succinic acid and insulin is synergistically effective in treating diabetes mellitus.

Patients. Eight non-insulin dependent diabetic humans were studied. Non-insulin Dependent Diabetes Mellitus (NIDDM) was diagnosed in the humans according to the World Health Organisation criteria and had been presented for minimum 5 years. The NIDDM humans were metabolically stable and had fasting glucose levels more than 13.3 mmol/l. All patients were under an intensified insulin therapy.

The following table summarizes the characteristics of the humans:

	Mean ± SD
Number of humans	8
Age, years	64 ± 4
Gender, men/women	2/6
BMI	24.3 ± 1.1 kg/m^2
Fasting plasma glucose	14.36 ± 1.04 mmol/l
Daily dose of insulin	48.3 ± 4.40 units/day

Assays. Assays were used as described in the example 2 of the invention using human insulin and C-peptide as a standard.

Procedure. All patients were under insulin therapy before the experiment. All patients received daily injections of insulin in optimal doses to hold glucose levels < 6.7 mmol/l during 60 days of the experiments. Pharmaceutical grade succinic acid in a unit dosage form of 100 mg per gelatin capsule was used. All patients received succinic acid orally in daily dose of 200 mg for period of 4 to 7 days beginning from the first day of

the experiment. Fasting serum glucose and plasma C-peptide levels were monitored in humans during 60 days. The results are presented in Table 8 as mean ± S.E.M.:

Table 8. Mean daily dose of insulin, fasting serum glucose levels, and plasma C-peptide levels in NIDDM human patients treated by succinic acid .

	Days	Doses of insulin, units/day	C-peptide, nmol/l	Glucose, mmol/l
Before treatment by succinic acid	0	48.3 ± 4.4	2.84 ± 0.07	14.36 ± 1.04
After treatment by succinic acid	15	42.0 ± 3.1	2.72 ± 0.04	13.12± 0.10
	30	25.4 ± 3.7**	2.03 ± 0.05**	8.24± 0.08**
	45	24.1 ± 2.1**	1.72 ± 0.07**	9.35± 0.22*
	60	23.9 ± 3.2*	1.78 ± 0.07**	11.20± 0.06

*Denotes statistically significant differences from the results obtained at zero day ($P < 0.002$). **Denotes statistically significant differences from the results obtained at zero day ($P < 0.001$).

The administering of succinic acid during 4 to 7 days to NIDDM human patients which are under insulin therapy results in 1.7-fold decreasing in elevated fasting glucose levels to 30th day of the experiment in comparison with glucose levels that were observed under treatment by insulin only. Thus, administering of succinic acid to NIDDM human patients is effective for treating diabetes mellitus.

Moreover, the short-term treatment NIDDM humans by succinic acid (4 to 7 days) results in long-term multiple decreasing in the optimal daily doses of insulin (2-fold to 60th day of the experiment). Thus, administering of succinic acid to NIDDM human patients with insulin is the synergistically effective therapy for treating diabetes mellitus.

EXAMPLE 4

This example shows that administering an effective amount of disodium salt of succinic acid is effective for treating wounds.

A suppurative wounds were prepared by the method of Eliseev. Eliseev, V.G. "Connective tissue", Moscow (1961). The 30 male Wistar rats maintained as described in example 1 of the invention were used in this experiment. The rats were anaesthetized with 1% solution of nembutal (0.2 ml/100 g of body weight, intramuscularly). Incisions of 15 to 20 mm length in inguinal region of rats were made. A four celluloid balls of 2 mm diameter were entered into the each wound. The wounds were colonized by 0.01 ml (10000 cells/ml) of combination *Staphylococcus aureus* and *Proteus* in ratio 1:1, and then the wound was sewed by a silk thread. Two day after, the silk thread and the balls were removed from the wound. Then the purulent wounds were treated topically one time per day by 0.3 ml 5% water solution of disodium succinate hexahydrate (n=20) or none (control, n=10). Data are presented in Table 9 as day of the term (mean±S.E.M.) of the healing phase.

Table 9. Wound healing in rats treated by disodium succinate in comparison with control rats.

Wound healing phase	Term of the healing phase, days	
	succinate	control
wound depuration	6.3±0.14**	15.4±0.18
granuloma formation	4.5±0.06**	11.6±0.13
margin re-epithelialization	5.4±0.05**	12.3±0.04
complete re-epithelialization	8.6±0.04**	20.7±0.06

**Denotes statistically significant differences from the results obtained in control ($P < 0.001$).

The topical treating of suppurative wounds by disodium salt of succinic acid significantly enhances wound healing. The rate of healing was found to be more than 2-fold higher in the group of rats treated by the succinate in comparison with the control untreated rats for all consecutive phases of wound healing, including the complete re-epitalization, an ultimate goal in wound healing. Thus, administering the succinic acid salts is effective in treating wounds.

WE CLAIM:

1. The use of succinic acid or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for treating disease state caused by dysfunctional signal transduction.
2. The use of succinic acid or a pharmaceutically acceptable salt thereof for the manufacture of a nutritional supplement useful for treating disease state caused by dysfunctional signal transduction.
3. The use as claimed in Claim 1 or 2 wherein the disease state is diabetes mellitus.
4. The use of succinic acid or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for treating wound.
5. A method of inhibiting protein tyrosine phosphatase, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof.
6. A method of treating disease state caused by dysfunctional signal transduction, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof.
7. The method as claimed in Claim 6 wherein said disease state is diabetes mellitus.
8. A method of treating disease states caused by dysfunctional signal transduction, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof stepwise or in physical combination with an effective amount of a protein tyrosine kinase activator.
9. The method as claimed in Claim 8 wherein said protein tyrosine kinase activator is insulin.
10. The method as claimed in Claim 8 or 9 wherein said disease state is diabetes mellitus.
11. The method as claimed in any one of Claims 8 to 10 wherein the effective amount of insulin is 2 to 100 units per day per mammalian subject.
12. The method as claimed in any one of Claims 8 to 11 wherein the insulin is administered parenterally.
13. A method of treating wound, which comprises administering to a mammal in need thereof an effective amount of succinic acid or a pharmaceutically acceptable salt thereof.

14. The method as claimed in any one of Claims 5 to 13 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is 0.1 milligram to 50 milligrams per day per kilogram of body weight of the mammal.

15. The method as claimed in Claim 14 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is 1 milligrams to 20 milligrams per day per kilogram of body weight of the mammal.

16. The method as claimed in any one of Claims 5 to 15 wherein said succinic acid or a pharmaceutically acceptable salt thereof is administered for period of 1 day or longer.

17. The method as claimed in Claim 16 wherein said succinic acid or a pharmaceutically acceptable salt thereof is administered for period of 3 to 7 days.

18. The method as claimed in any one of Claims 5 to 17 wherein the succinic acid or a pharmaceutically acceptable salt thereof is administered orally.

19. The method as claimed in any one of Claims 5 to 17 wherein the succinic acid or a pharmaceutically acceptable salt thereof is administered topically.

20. The method as claimed in any one of Claims 5 to 19 wherein the mammal is a human.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/RU 99/00100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/19 A61K31/38

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 7 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.
X	EBIHARA, KIYOSHI ET AL: "Comparative effects of various organic acids on glucose-flattening activity in rats fed a glucose solution" NUTR. REP. INT. (1989), 40(5), 1041-7 , XP002123534	1-3,5-7, 14-20
Y	abstract	8-12, 14-20

X	DATABASE WPI Section Ch, Week 199414 Derwent Publications Ltd., London, GB; Class B05, AN 1994-114217 XP002123541 & JP 06 062798 A (NAKANO SUMISE KK), 8 March 1994 (1994-03-08) abstract	1-3,5-7, 14-20

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

[X] Patent family members are listed in annex.

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"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)

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Date of the actual completion of the international search	Date of mailing of the international search report
21 December 1999	27 December 1999 (27.12.99)

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 198146 Derwent Publications Ltd., London, GB; Class B05, AN 1981-84297D XP002123542 & JP 56 125312 A (LION DENTIFRICE CO LTD), 1 October 1981 (1981-10-01) abstract ---	1,2,4, 13-20
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 09, 30 September 1997 (1997-09-30) & JP 09 124472 A (NOEVIR CO LTD), 13 May 1997 (1997-05-13) abstract ---	1,2,4, 13-20
X	DATABASE WPI Section Ch, Week 199148 Derwent Publications Ltd., London, GB; Class B03, AN 1991-350854 XP002123543 & JP 03 236320 A (KOBAYASHI KOSE KK), 22 October 1991 (1991-10-22) abstract ---	1,2,4, 13-20
X	MIYAMOTO ET AL.: "Effects of cosmetics containing Bioactive substances on skin" JOURNAL OF THE SOCIETY OF COSMETIC CHEMISTS OF JAPAN, vol. 22, no. 4, 1989, pages 254-262, XP000856450 abstract ---	1,2,4, 13-20
X	DATABASE WPI Section Ch, Week 198637 Derwent Publications Ltd., London, GB; Class B05, AN 1986-242355 XP002123544 & JP 61 171417 A (WAKUNAGA SEIYAKU KK), 2 August 1986 (1986-08-02) abstract ---	1,2,4, 13-20
X	MACDONALD, MICHAEL J. ET AL: "Glyceraldehyde phosphate and methyl esters of succinic acid. Two "new" potent insulin secretagogues" DIABETES (1988), 37(7), 997-9 , XP002123535 abstract --- -/--	1-3,5-7, 14-20

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAMBERT, ANDRE E. ET AL: "Organ culture of fetal rat pancreas. II. Insulin release induced by amino and organic acids, by hormonal peptides, by cationic alterations of the medium, and by other agents" BIOCHIM. BIOPHYS. ACTA (1969), 184(3), 540-53 , XP002123536 abstract	1-3,5-7, 14-20
X	MALAISSE WILLY: "Insulinotropic Nutritient Esters" DRUGS FUTURE, vol. 23, no. 11, 1998, pages 1205-1216, XP002123537 page 1206, column 1, paragraph 3 -page 1207, column 1, paragraph 2	1-3,5-7, 14-20
X	DZVONKEVYCH, GULY: "Effects of Carboxylin, Acetate and Acids of the Tricarboxylic Cycle on the Content of Glucose in Blood and Citric acid in Urine of Rabbits with Alloxan Diabetes. " UKR. BIOKHIM. ZH., vol. 46, no. 5, 1974, pages 547-552, XP002123538 abstract	1-3,5-7, 14-20
X	KOREC, R.: "Succinic acid alkyl esters-new genericoral anti-diabetic agents in alloxan and STZ diabetic rats" DIABETOLOGIA, vol. 40, no. suppl. 1, 1997, page A375 XP002123539 abstract	1-3,5-7, 14-20
X	LECLERCQ-MEYER, V.; MALAISSE, W.J.: "Potentiation of GLP-1 Insulinotropic Action by a Nonglucidic Nutrient in the pancreas of Diabetic GK Rats." BIOCHEMICAL AND MOLECULAR MEDICINE , vol. 59, no. 1, 1996, pages 87-90, XP002123540 abstract	1-3,5-7, 14-20
Y	WO 97 48414 A (NOVONORDISK AS) 24 December 1997 (1997-12-24) abstract	8-12, 14-20

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3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/RU 99/00100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LANGTRY JA ET AL: "Topical lithium succinate ointment (Efalith) in the treatment of AIDS-related seborrhoeic dermatitis" CLINICAL AND EXPERIMENTAL DERMATOLOGY,GB,BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, vol. 22, no. 5, page 216-219 XP002105249 ISSN: 0307-6938 -----	1-20

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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/RU 99/00100

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.: 5-20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 5-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] 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:
Present claims

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:

See additional sheet

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

2. [X] As all searchable claims could be searched 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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-20

Use of Succinic acid and pharmaceutically acceptable salts for the treatment of diabetes mellitus and for wound healing.

1.1. Claims: 1-3,5-7,14-20
Use of Succinic acid and pharmaceutically acceptable salts for the treatment of diabetes mellitus.

1.2. Claims: 1,2,4,14-20
Use of Succinic acid and pharmaceutically acceptable salts for wound healing.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/RU 99/00100

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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JP 56125312 A	01-10-1981	NONE	
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JP 3236320 A	22-10-1991	JP 2844103 B	06-01-1999
JP 61171417 A	02-08-1986	NONE	
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		US 5866538 A	02-02-1999

Form PCT/ISA/210 (patent family annex) (July 1992)