Journal of Genes and Proteins

Research Article

A SCITECHNOL JOURNAL

Comparison of the Hypoproteinemic Capacities of Erythropoietin and U-74389G

Tsompos C[1]*, Panoulis C[2], Toutouzas K[3], Triantafyllou A[4], Zografos CG[3] and Papalois A[5]

Abstract

Aim: This study compared the hypoproteinemic effects of erythropoietin (Epo) and antioxidant drug U-74389G based on 2 preliminary studies. The provided results at serum total protein (TP) levels decline were co-evaluated in a hypoxia reoxygenation protocol of an animal model.

Materials and methods: TP levels were evaluated at the 60th reoxygenation min (for groups A, C and E) and at the 120th reoxygenation min (for groups B, D and F) in 60 rats. Groups A and B received no drugs, rats from groups C and D were administered with Epo; whereas rats from groups E and F were administered with U-74389G.

Results: The first preliminary study recommended a hypoproteinemic effect of Epo (p-value=0.4430). The second preliminary study proved the hypoproteinemic effect of U-74389G (p-value=0.0005). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that U-74389G has at least 4-fold hypoproteinemic action than Epo (p-value=0.0000).

Conclusions: The neurologist must take into consideration the powerful hypoproteinemic effect of the 2 drugs; mainly that of U-74389G whether implicated in neurological diseases.

Keywords

Hypoxia; Erythropoietin; U-74389G; Total protein; Reoxygenation

Introduction

The short-term hypoproteinemic [1] action of U-74389G is significant (p-value=0.0005). U-74389G is a novel antioxidant factor. It implicates just only 255 known biomedical studies at present. 4.31% of these studies concern tissue hypoxia and reoxygenation (HR) experiments. The promising effect of U-74389G in tissue protection has been noted in these HR studies. U-74389G or also known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation. It protects against HR injury in animal heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain micro vascular endothelial cells monolayers. The biochemical capacities of U-74389G were summarized as Na^+, K^+, Mg^{2+}-ATPase inhibition in the perihematomal basal ganglia territory; cholinergic response enhancement; membrane-bound enzymes stabilization; 3,4-dihydroxyphenylacetic acid (DOPAC) activity increase in the ipsilateral striatum of oligemic nigrostriatal system; antiapoptotic properties prove; caspase-3 immunoreactivity down-regulation; local cerebral extracellular superoxide anion concentrations reduce; 2,3-DHBA levels and the excitatory amino acid attenuation in hippocampus; brain total sulfhydryl groups levels restoration to normal values and the oxidant peroxynitrite consumption attenuation produced by brain microglia. However, the hypoproteinemic capacity of U-74389G gets more comprehensible whether is compared with the same capacity of a standard known drug. Such one of the more well studied drug; whereas without significant hypoproteinemic [2] action (p-value=0.4430) is erythropoietin (Epo). Actually, Epo implicates over 30,162 known biomedical studies at present. 10.41% at least of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Certainly, the concept has been moved away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, not covering completely the specific matter with total protein.

The special aim of this experimental work was to compare the hypoproteinemic effects of U-74389G and Epo on a rat model and mainly in an HR protocol. Their effects were tested by measuring the serum mean total protein (TP) levels.

Materials and Methods

Animal preparation

The Vet licenses of the research were provided under 3693/12-11-2010 & 14/10-1-2012 decisions. The granting company and the place of the experiment are mentioned in related references [1,2]. Accepted standards of humane animal care were adopted for Albino female WISTAR rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Continuous intra-experimental anesthesiologic techniques, oxygen supply, electrocardiogram and acidometry were provided. Post-experimental awakening and preservation of animals was not permitted, even if euthanasia was needed. Rats 16-18 weeks old were randomly delivered to four (6) groups (n=10), using the following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A); hypoxia for 45 min followed by reoxygenation for 120 min (group B); hypoxia for 45 min followed by immediate Epo intravenous (IV) administration and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate Epo IV administration and reoxygenation for 120 min (group D); hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group E); hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group F). The dose height selection criteria of Epo and U-74389G were assessed at preliminary studies as 10 mg/Kg body mass of animals for both drugs.

Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. Reoxygenation was induced

***Corresponding author:** Tsompos Constantinos, Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios", 2 Elenis Zografou street, Thessaloniki 54634, Greece, Tel: +00302313322223; Fax: +00302106811215; E-mail: tsomposconstantinos@gmail.com

Received: December 15, 2017 **Accepted:** December 20, 2017 **Published:** December 27, 2017

by removing the clamp and restoration the inferior aorta patency. After exclusion of the blood flow, the protocol of HR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through catheterized inferior vena cava. The TP levels were determined at 60th min of reoxygenation (for A, C and E groups) and at 120th min of reoxygenation (for B, D and F groups). The TP values used were adjusted for rats' mass since a powerful relation was invented between them (p-value=0.0000).

Statistical analysis

Table 1 presents the (%) decreasing influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) decreasing influence of U-74389G regarding reoxygenation time. Chi-square test was applied using the ratios which produced the (%) results per endpoint. The outcomes of chi-square tests are depicted at Table 3. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

Discussion

Barbierato et al. [3] promoted progression, morphological development and maturation of undifferentiated oligodendrocyte precursor cells into a more differentiated phenotype and TP content without affecting proliferation; although oligodendrocytes have limited ability to repair the damage to themselves or to other nerve cells, as seen in demyelinating diseases like multiple sclerosis. Tan et al. [4] ascribed the exhibited remarkable presynaptic neurotoxicity presumably to the action of neurotoxic basic phospholipases A2 in high abundance (35% of TP abundance) from a total of 11 different protein families in the venom proteome content of Sri Lankan Ilmie et al. [5] found abnormal values in TP after 28 days toxic exposure of animals at (Mitragyna speciosa Korth) ketum. Perez et al. [6] quantified butyrylcholinesterase specific activity and its inhibition by the nerve agents' sarin and VX; normalized to the TP content in a robust dried spot activity assay sample more resistant to degradation. Wang et al. [7] greatly decreased the TP expression of 5α-reductase inhibitor dutasteride mediating maximal contraction of the whole ventral prostate. Navarro et al. [8] noticed that protracted abstinence from alcohol increased the dendritic arborization within apical dendrites of pyramidal neurons; the stress sensitivity and hypothalamic-pituitary-adrenal axis dysregulation. Stress altered oligodendrocyte expression as a hypophosphorylation of the glucocorticoid receptor at Ser-232 without affecting expression of TP in rodents and promoted hypermyelination in the medial prefrontal cortex in alcohol dependent subjects. Kaur et al. [9] associated nerve tissue TP samples with chronic constriction injury of sciatic nerve induced neuropathic pain amelioration after ramipril (angiotensin-converting enzyme inhibitor) administration. Hübler et al. [10] could not correlate TP plasma concentrations and ropivacaine local total and free concentrations after psoas compartment block, especially whether combined with sciatic nerve block. Liu et al. [11] remarked the significant anti-bacterial and PO activities alterations of the TP extract after enkephalinergic neurotransmitter [Met(5)]-enkephalin incubation which firstly observed on the marginal of the dorsal half; indicated that this released by nervous system was firstly appeared, while the primitive immune defense system existed in the region of prototroch and developed maturely in trochophore, D-hinged and umbo larvae of oyster *Crassostrea gigas*. Sánchez-González et al. [12] measured plasma levels of TP in manganese (Mn) excess neurotoxicity CRF patients. Salvarani et al. [13] associated greater cerebrospinal fluid TP concentrations with bioptic diagnosis of uncommon condition (2.4 cases per 1,000,000 person-years) of primary central nervous system vasculitis in which lesions are limited to vessels of the brain and spinal cord than angiographic diagnosis. Perga et al. [14] replaced the TP content by the three protein spots method in order to subdivide the multiple sclerosis patients. Kannan et al. [15] quantified the maximum expression of a 48 kDa Pax6 transcription factor involved in vertebrate eye, brain and central nervous system formation during early embryonic and larval development or embryogenesis at 8 hours post fertilization as 179ng/embryo from the

Table 1: The (%) decreasing influence of erythropoietin in connection with reoxygenation time.

Decrease	±SD	Reperfusion time	p-values
-0.02%	±2.47%	1h	0.9904
-1.27%	±1.51%	1.5h	0.3721
-2.52%	±2.03%	2h	0.1509
+1.27%	±1.51%	Reperfusion time	0.3549
-0.68%	±2.48%	Interaction	0.4430

Table 2: The (%) decreasing influence of U-74389G in connection with reperfusion time.

Decrease	±SD	Reperfusion time	p-values
-5.48%	±2.99%	1h	0.0663
-7.34%	±1.76%	1.5h	0.0001
-9.20%	±2.16%	2h	0.0003
+1.46%	+2.12%	Reperfusion time	0.4103
-4.08%	±1.10%	Interaction	0.0005

Table 3: The U-74389G / erythropoietin decreasing efficacies ratios on TP levels after chi-square tests application.

Odds ratio	[95% Conf. Interval]		p-values	Endpoint
155.9562	153.5206	158.4305	0.0000	1h
4.397968	4.394434	4.401505	0.0000	1.5 h
2.803573	2.798201	2.808956	0.0000	2h
0.8795951	0.8766519	0.8825482	0.0000	Reperfusion time
4.518197	4.502736	4.533711	0.0000	Interaction

average TP of 9.5μg/embryo in zebra fish model system. Shibasaki et al. [16] significantly related serum TP with inpatient pre-rehabilitation FIM score and Barthel index investigating the utility of sympathetic nervous activity after treatment of acute phase illness in frail elderly patients 75 years or older. Schmid et al. [17] argued against an autochthonous production in the central nervous system due to the positive correlation between the human cerebrospinal fluid (CSF)/ serum ratios for adipsin and TP. Patients suffering from infectious diseases had higher CSF levels of adipsin than multiple sclerosis patients. Koie et al. [18] correlated the 5-year biochemical recurrence-free survival rate with preoperative serum butyrylcholinesterase - an alpha-glycoprotein found in the nervous system - levels ($P<0.001$) for prostate cancer after radical prostatectomy. Ligtenberg et al. [19] found increased salivary flow rate and protein secretion after sympathetic stimuli such as physical exercise ($p<0.01$). Yu et al. [20] associated TP with long-term prognosis in decompressive craniectomy group in severe traumatic brain injury treatment. Yao et al. [21] modulated the enteric nervous system and intestinal motility influencing malodorous flatus, irritable bowel syndrome, ulcerative colitis and prevention of colorectal cancer by harmful metabolites of increased protein fermentation due to high-TP intake or specifically, aromatic and sulphur-containing amino acids. Wang et al. [22] found that nitric oxide induces cysteine S-nitrosylation of TP which decrease the vesicular neurotransmitter uptake such as monoamines, acetylcholine and glutamate, in synaptosomes of the central nervous system. Kuznetsova et al. [23] revealed high stability of TP level in healthy children but significant individual deviations from the average TP value in children with nervous system dysfunction. Da Silva et al. [24] demonstrated significant increases in parasympathetic related indices and TP levels after 17 weeks ballet training period. Li et al. [25] manifested markedly increased levels of CSF TP and diffuse spinal Dura matter thickening in hypertrophic spinal pachymeningitis associated with rare myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA) localized exclusively in the spine.

The above biochemical capacities of U-74389G present beneficial properties such as neuroprotection, inflammatory responses counteraction; post IR cerebral edema and neuronal necrosis decline in a hippocampal subfield; apoptotic cell death and sub-sequent cortical infarction prevention, final lesion decrease; late learning impairments prevention after intrastriatal administration; anoxic terminal negativity (ATN)-latency spikes decline in hippocampal region; epileptogenic discharges reduce; hippocampal damage prevention; living pyramidal neurons increase; potential early forms of Alzheimer's disease treatment.

Epo effect was summarized in increasing glomerular filtration leakage of constant ratio both renal Epo and TP excretion reaching up to 23% of the administered high-dose rEpo due to lower kidneys maturation; considering potent neuroprotection in brain injury encephalopathy of preterm infants and improving the nutritional status (TP levels) of patients after alteration from maintenance hemodialysis (HD) to CAPD 6 months treatment program. Furthermore, Hirata et al. [26] delayed end-stage kidney disease by a single dose of epoetin β pegol (continuous erythropoietin receptor activator (CERA) given on day 1, alleviating increasing urinary TP in a rat model of chronic kidney disease. Hypoxia-inducible factors (HIFs) are transcription factors that respond to decreases in available oxygen in the cellular environment, or hypoxia [27]. In normal circumstances after injury HIF-1a is degraded by prolyl hydroxylases (PHDs). The continued up-regulation of HIF-1a via PHD inhibitors regenerates lost or damaged tissue in mammals that have a repair response; and the continued down-regulation of Hif-1a results in healing with a scarring response in mammals with a previous regenerative response to the loss of tissue. The act of regulating HIF-1a can either turn off, or turn on the key process of mammalian regeneration [28]. The HIF stabilization using an HIF prolyl-hydroxylase inhibitor enhances hippocampal memory, likely by increasing erythropoietin expression. HIF pathway activators such as ML-228 may have neuroprotective effects and are of interest as potential treatments for stroke and spinal cord injury.

As mentioned above, Table 3 shows that U-74389G has at least 4-fold hypoproteinemic capacity than Epo (p-value=0.0000). This has a dual biochemical interpretation. Epo may contribute with some of its anabolic capacities or the antioxidant capacities of U-74389G are very protein consuming. A meta-analysis of these ratios from the same experiment, for 10 other seric variables, provides comparable results (Table 4).

Conclusion

The neurologist must be informed about the hypoproteinemic effects whether treats patients receiving Epo but mainly U-74389G. Epo expression enhances hippocampal memory; whereas HIF

Table 4: A U-74389G / erythropoietin efficacies ratios meta-analysis on 10 hematologic variableç (4 variables with balancing efficacies and 2 variables with opposite efficacies) [29].

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
WBC	0.957451	0.3782	1.396122	0.0000	1.918237	0.0000	1.71622	0.0000	1.601887	0.0000
Hematocrit	38.424	0.0000	9.076658	0.0000	6.222898	0.0000	1.001356	0.2184	12.66419	0.0000
Hemoglobin	1.268689	0.0000	1.839035	0.0000	13.1658	0.0000	1.252422	0.0000	1.94889	0.0000
RBC count	0.961059	0.0000	1.733395	0.0000	6.519657	0.0000	1.039524	0.0000	1.309673	0.0000
Platelet count	2.42839	0.0000	6.00238	0.0000	6.1333429	0.0000	3.939027	0.0000	37.62979	0.0000
MPV	145.8532	0.0000	4.053619	0.0000	2.603947	0.0000	1.2334644	0.0000	4.164431	0.0000
Platelet DW	0.6940233	0.0000	1.319118	0.0000	2.206972	0.0000	2.2484006	0.0000	2.458888	0.0000
Creatinine	168.9034	0.0000	4.872332	0.0000	3.039572	0.0000	1.0262016	0.0000	5.005523	0.0000
Mean	6.07797485	0.0472	2.98583275	0.0000	4.26641598	0.0000	1.49596378	.0273	4.21627953	0.0000
Mean corpuscular hemoglobin concentrations	-0.2774225	0.0000	-0.5504722	0.0000	-0.8522433	0.0000	+3.044774	0.0000	-0.7793243	0.0000
Platelet crit	-0.2312044	0.0000	-0.6719365	0.0000	-1.330756	0.0886	5.620077	0.0000	-0.9771515	0.0000
Mean	-0,2532076	0.0000	-0,6081795	0.0000	-1,0649544	0.0443	4,1366488	0.0000	-0,8726499	0.0000

pathway activators such as ML-228 may have neuroprotective effects and are of interest as potential treatments for stroke and spinal cord injury.

References

1. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A (2015) The effect of the antioxidant drug "*U-74389G*" on total protein levels during ischemia reperfusion injury in rats. Romanian J Neurol 15: 30-41.
2. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A (2015) The Effect of Erythropoietin on Total Protein Levels during Ischemia Reperfusion Injury in Rats. Int J Neurorehabilitation 2:146. doi:10.4172/2376-0281.1000146
3. Barbierato M, Facci L, Marinelli C, Zusso M, Argentini C, et al. (2015) Co-ultramicronized Palmitoylethanolamide/Luteolin Promotes the Maturation of Oligodendrocyte Precursor Cells. Sci Rep 5: 16676.
4. Tan NH, Fung SY, Tan KY, Yap MK, Gnanathasan CA, et al. (2015) Functional venomics of the Sri Lankan Russell's viper (Daboia russelii) and its toxinological correlations. J Proteomics 128: 403-423.
5. Ilmie MU, Jaafar H, Mansor SM, Abdullah JM (2015) Subchronic toxicity study of standardized methanolic extract of Mitragyna speciosa Korth in Sprague-Dawley Rats. Front Neurosci 9: 189.
6. Perez JW, Pantazides BG, Watson CM, Thomas JD, Blake TA, et al. (2015) Enhanced stability of blood matrices using a dried sample spot assay to measure human butyrylcholinesterase activity and nerve agent adducts. Anal Chem 87: 5723-5729.
7. Wang D, Zha X, Nagase K, Akino H, Muramatsu I, et al. (2015) Effects of the 5α-reductase inhibitor dutasteride on rat prostate α1A-adrenergic receptor and its mediated contractility. Urology 85: 704.e9-14.
8. Navarro AI, Mandyam CD (2015) Protracted abstinence from chronic ethanol exposure alters the structure of neurons and expression of oligodendrocytes and myelin in the medial prefrontal cortex. Neuroscience 293: 35-44.
9. Kaur P, Muthuraman A, Kaur J (2015) Ameliorative potential of angiotensin-converting enzyme inhibitor (ramipril) on chronic constriction injury of sciatic nerve induced neuropathic pain in mice. J Renin Angiotensin Aldosterone Syst 16: 103-112.
10. Hübler M, Planitz MC, Vicent O (2015) Early pharmacokinetic of ropivacaine without epinephrine after injection into the psoas compartment. Br J Anaesth 114: 130-135.
11. Liu Z, Zhou Z, Wang L, Song X, Chen H, et al. (2015) The enkephalinergic nervous system and its immunomodulation on the developing immune system during the ontogenesis of oyster Crassostrea gigas. Fish Shellfish Immunol 45: 250-259.
12. Sánchez-González C, López-Chaves C, Gómez-Aracena J, Galindo P, Aranda P, et al. (2015) Association of plasma manganese levels with chronic renal failure. J Trace Elem Med Biol 31: 78-84.
13. Salvarani C, Brown RD Jr, Christianson T, Miller DV, Giannini C, et al. (2015) An update of the Mayo Clinic cohort of patients with adult primary central nervous system vasculitis: description of 163 patients. Medicine (Baltimore) 94: e738.
14. Perga S, Giuliano Albo A, Lis K, Minari N, Falvo S, et al. (2015) Vitamin D Binding Protein Isoforms and Apolipoprotein E in Cerebrospinal Fluid as Prognostic Biomarkers of Multiple Sclerosis. PLoS One 10: 0129291.
15. Kannan RR, Vincent SG (2015) ELISA based quantification of Pax6 expression in the developing Zebrafish embryos. Ann Neurosci 22: 171-175.
16. Shibasaki K, Ogawa S, Yamada S, Iijima K, Eto M, et al. (2015) Favorable effect of sympathetic nervous activity on rehabilitation outcomes in frail elderly. J Am Med Dir Assoc 16: 799.e7-799.e12.
17. Schmid A, Hochberg A, Berghoff M, Schlegel J, Karrasch T, et al. (2015) Quantification and regulation of adipsin in human cerebrospinal fluid (CSF). Clin Endocrinol (Oxf) 84: 194-202.
18. Koie T, Ohyama C, Hatakeyama S, Imai A, Yoneyama T, et al. (2015) Significance of preoperative butyrylcholinesterase as an independent predictor of biochemical recurrence-free survival in patients with prostate cancer treated with radical prostatectomy. Int J Clin Oncol 21: 379-383.
19. Ligtenberg AJ, Brand HS, van den Keijbus PA, Veerman EC (2015) The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme. Arch Oral Biol 60: 1639-1644.
20. Yu P, Tian Q, Wen X, Zhang Z, Jiang R (2015) Analysis of Long-Term Prognosis and Prognostic Predictors in Severe Brain Injury Patients Undergoing Decompressive Craniectomy and Standard Care. J Craniofac Surg 26: e635-41.
21. Yao CK, Muir JG, Gibson PR (2016) Review article: insights into colonic protein fermentation, its modulation and potential health implications. Aliment Pharmacol Ther 43: 181-196.
22. Wang Y, Zhou Z, Leylek T, Tan H, Sun Y, et al. (2015) Protein cysteine S-nitrosylation inhibits vesicular uptake of neurotransmitters. Neuroscience 311: 374-381.
23. Kuznetsova AA, Shakhmatova EI (2015) Study of the Efficacy of Regulation of the Blood Serum Physicochemical Parameters in Children with Dysfunction of Physiological Systems. Zh Evol Biokhim Fiziol 51: 276-282.
24. da Silva CC, Goldberg TB, Soares-Caldeira LF, Dos Santos Oliveira R, de Paula Ramos S, et al. (2015) The Effects of 17 Weeks of Ballet Training on the Autonomic Modulation, Hormonal and General Biochemical Profile of Female Adolescents. J Hum Kinet 47: 61-71.
25. Li X, Zhao J, Wang Q, Fei Y, Zhao Y (2015) ANCA-Associated Systemic Vasculitis Presenting With Hypertrophic Spinal Pachymeningitis: A Report of 2 Cases and Review of Literature. Medicine (Baltimore) 94: e2053.
26. Hirata M, Tashiro Y, Aizawa K, Kawasaki R, Shimonaka Y, et al. (2015) Epoetin beta pegol alleviates oxidative stress and exacerbation of renal damage from iron deposition, thereby delaying CKD progression in progressive glomerulonephritis rats. Physiol Rep 3: e12637.
27. Xing J, Lu J (2016) HIF-1α Activation Attenuates IL-6 and TNF-α Pathways in Hippocampus of Rats Following Transient Global Ischemia. Cell Physiol Biochem 39: 511-520.
28. Chen H, Li J, Liang S, Lin B, Peng Q, et al. (2017) Effect of hypoxia-inducible factor-1/vascular endothelial growth factor signaling pathway on spinal cord injury in rats. Exp Ther Med 13: 861-866.
29. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos CG, et al. (2017) Comparison of the Hypervolemic Capacities of Erythropoietin and U-74389G concerning Mean Platelet Volumes Levels. IJMRHS 2: 5-9.

Author Affiliations

Top

[1]*Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios" Thessaloniki, Greece*

[2]*Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Greece*

[3]*Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Greece*

[4]*Department of Biologic Chemistry, Athens University, Athens, Attiki, Greece*

[5]*Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece*