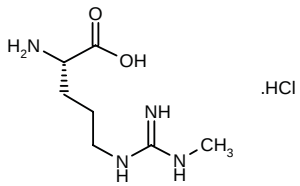
Drugs of the Future 1998, 23(2): 123-132

546C88

Nitric Oxide Synthase Inhibitor
Treatment for Septic Shock
Antimigraine

L-NMA.HCl
L-NMMA.HCl

*N*ω-Methyl-L-arginine hydrochloride



$C_7H_{16}N_4O_2.HCl$ Mol wt: 224.69

CAS: 156706-47-7
CAS: 017035-90-4 (as free base)
CAS: 053308-83-1 (as monoacetate)

EN: 250957
EN: 172859 (as free base)

Synthesis

546C88 has been obtained by several related ways: 1) The reaction of *N*-methylthiourea (I) with methyl iodide gives *N,S*-dimethylisothiouronium iodide (II), which is then condensed with L-ornithine (III) by means of NaOH (1-3) or copper acetate (4). Scheme 1.

2) The oxidation of *N*-methylthiourea (I) with peracetic acid in acetic acid gives *N*-methylamidinosulfonic acid (IV), which is then condensed with L-ornithine (III) by means of K_2CO_3 in water (5). Scheme 1.

3) The reaction of cyanogen bromide (V) with methylamine (VI) by means of Na_2CO_3 in THF gives *N*-methylcyanamide (VII), which is condensed with pyrazole (VIII) by means of HCl in refluxing dioxane to yield N^1-methylpyrazole-1-carboxamidine (IX). Finally, this compound is condensed with L-ornithine (III) by means of LiOH in water (6). Scheme 1.

4) The reaction of cellulose (X) with cyanogen bromide (V) by means of K_2CO_3 in water/DMF gives the corresponding polymeric cyanate ester (XI), which is condensed with methylamine (VI) to yield the polymeric *N*-methylamidino compound (XII). Finally, this compound is condensed with L-ornithine (III) by means of $CuCO_3$ in water (7). Scheme 1.

5) The reaction of L-ornithine (III) with benzaldehyde by means of LiOH gives N^{δ}-benzylidene-L-ornitine (XIII), which is treated first with benzyl chloroformate and NaOH and hydrolyzed with HCl to afford N^{α}-(benzyloxycarbonyl)-L-ornithine (XIV). The condensation of (XIV) with *N,S*-dimethylisothiouronium iodide (II) by means of NaOH gives N^{α}-(benzyloxycarbonyl)-N^{ω}-methyl-L-arginine (XV), whichis finally deprotected by hydrogenation with H_2 over Pd/C (8). Scheme 2.

Description

Acetate, $[\alpha]_D^{22}$ +9.5° (c 0.10, water) (1); acetate, m.p. 194-6 °C (2); hydrochloride (isomorph 1), m.p. about 205 °C (3), hydrochloride (isomorph 2), m.p. about 219 °C (3); flavinate, m.p. 252-3 °C (4); hydrochloride m.p. 219-21 °C (decomp.) (6).

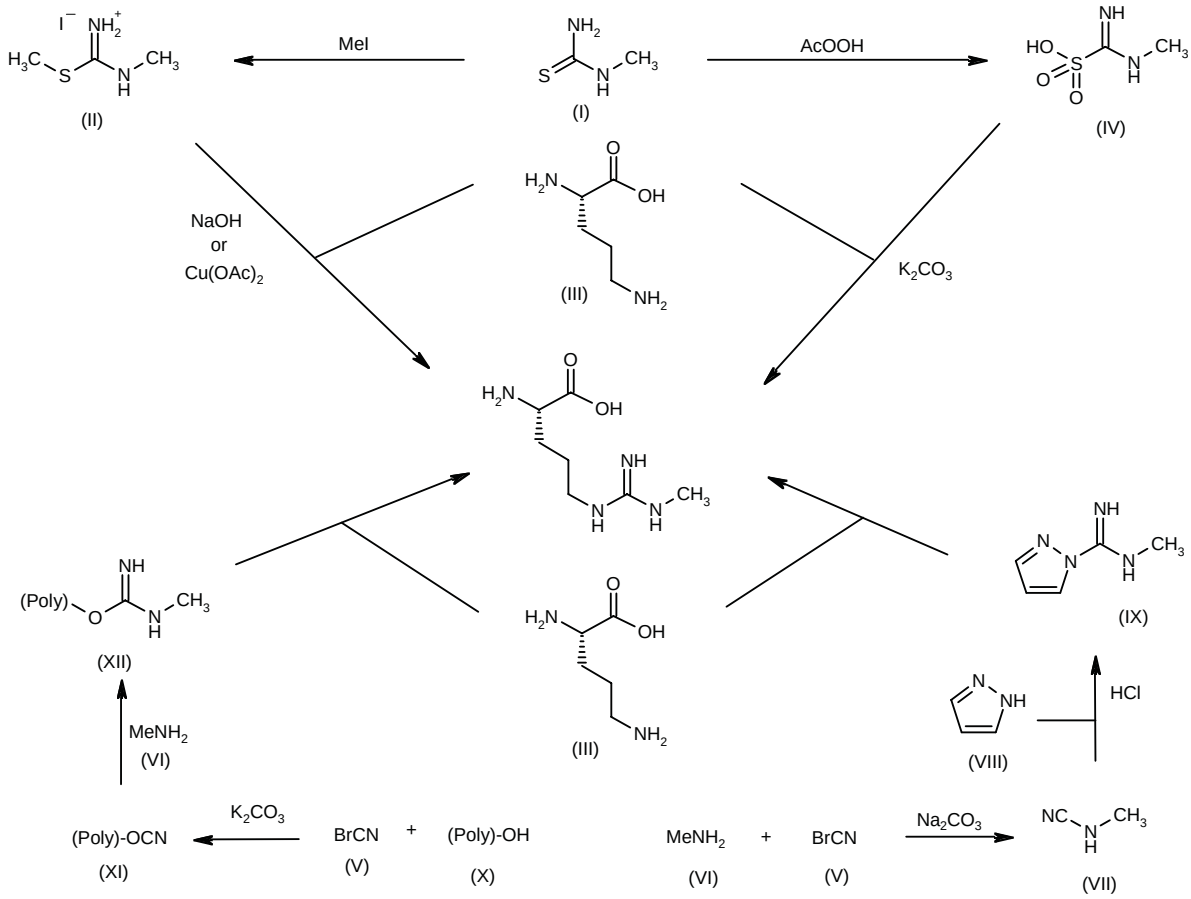
Introduction

Sepsis syndrome and septic shock both can progress to multiple organ failure (MOF), which is the most common cause of death in the intensive care unit (9, 10). The terms sepsis, septicemia and septic shock have long been used interchangeably due to the lack of precise definitions. In 1996 during a collaborative consensus conference sponsored by the American College of Chest Physicians and Society of Critical Care Medicine (ACCP/SCCM), the term systemic inflammatory response syndrome (SIRS) was introduced, recognizing the important role that endogenous mediators of systemic inflammation play in sepsis, which was no longer regarded as being caused by microbial pathogenicity factors alone (11, 12). The proposal was initially met with some opposition from European investigators (13). SIRS was defined as the systemic inflammatory response to a variety of clinical insults. Sepsis was defined as SIRS in response to infection, and septic shock as sepsis with hypotension, despite adequate fluid resuscitation.

The release of mediators in the sepsis cascade is largely responsible for the cardiovascular alterations and multiple organ dysfunction syndrome in septic shock. These mediators include proinflammatory cytokines, particularly TNF and IL-1, as well as IL-6 and IL-8, various autocoids such as eicosanoids (arachidonic acid derivatives), PAF and bradykinin, and secondary mediators such as nitric oxide and oxygen free radicals.

J.R. Prous, T. Wroblewski, J. Castañer. Prous Science, P.O. Box 540, 08080 Barcelona, Spain

Scheme 1: Synthesis of 546C88



These mediators have been proposed as investigational targets for therapeutic intervention in the management of sepsis and septic shock and for other indications as well. Table I presents investigational approaches to the management of sepsis and septic shock according to compounds described last year in patents and current literature contained in the Prous Science databases.

Nitric oxide (NO) is an endogenously produced inorganic free radical gas which is synthesized by NO synthase (NOS) [EC 1.14.23] from the amino acid L-arginine in endothelial cells, macrophages and other cell types (14). At least three isoforms of NOS are known to exist which include the two calcium-dependent constitutive isoforms termed type I or nNOS (neuronal) and type III or eNOS (endothelial), and a calcium-independent inducible isoform which is termed type II or iNOS (inducible) (14-17). iNOS is provoked by endotoxin and some proinflammatory cytokines, and has been implicated in tissue damage (18-24) and in the cardiovascular dysfunction seen in septic shock (25-28).

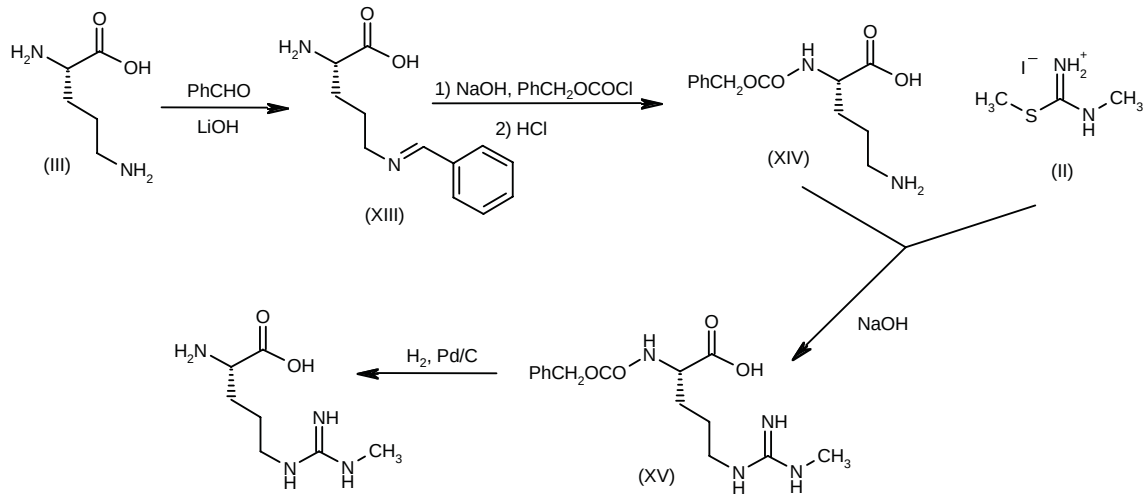
Several studies in experimental animals and in patients with sepsis have been carried out using nonselective inhibitors of the L-arginine-NO pathway which prevent the formation of NO by both cNOS (*e.g.,* in endothelial cells) and iNOS (*e.g.*, in cardiac myocytes and vascular smooth muscle cells). Administration of nonselective NOS inhibitors has been reported to cause a rapid and marked increase in systemic vascular resistance and a decrease in cardiac output in endotoxic shock in animals. Because some of these detrimental effects are due to inhibition of eNOS, another approach has been proposed: the selective inhibition of NO formed by iNOS, while maintaining physiological NO formed by the constitutive NOS in endothelial cells (28-30).

Several compounds have been reported to have greater affinity for inhibiting iNOS than cNOS, including L-canavanine (30, 31), aminoguanidine (32-34) and guanidine derivatives (35-38).

Pharmacological Actions

546C88 is a competitive inhibitor of both constitutive and inducible NOS. A comparative *in vivo* study in rats examined the effects of NO synthase inhibition by aminoguanidine, previously described as a selective

Scheme 2: Synthesis of 546C88



inhibitor of iNOS, and L-NMMA (546C88) on endotoxin-induced intestinal vascular permeability. Endotoxin (*Escherichia coli* lipopoly-saccharide; LPS) alone did not cause acute vascular leakage, nor did aminoguanidine or L-NMMA. However, administration of aminoguanidine (12.5-50 mg/kg s.c.) together with endotoxin (3 mg/kg i.v.) increased vascular leakage of radiolabeled albumin in the ileum and the colon in a dose-dependent manner after 1 h. This effect was reversed by pretreatment with L-arginine. Aminoguanidine at a dose of 50 mg/kg also elevated arterial blood pressure over the 1-h investigational period. Endotoxin administered together with L-NMMA (50 mg/kg s.c.) also induced similar potentiation of vascular injury and increased blood pressure, implying inhibition of the constitutive isoform of NO synthase. In contrast, aminoguanidine and L-NMMA given at the time of expression of the inducible isoform of NO synthase reduced the subsequent endotoxin-induced vascular leakage (39).

Another *in vivo* study evaluated the effect of NO synthase inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) and L-NMMA in a conscious rat model of vascular damage. Vascular damage was induced by LPS, and the effects of NO synthase inhibition were observed during a 5-h period. Concomitant administration of L-NAME (1-5 mg/kg s.c.) with LPS increased vascular leakage of radiolabeled albumin from intestinal tissue. Administra-tion of L-NMMA (50 mg/kg s.c.) together with LPS also increased albumin leakage, although maximum leakage occurred earlier and declined more rapidly. Administration of L-NAME (1-5 mg/kg s.c.) or L-NMMA (12.5-50 mg/kg) 3 h after LPS treatment reduced albumin leakage in a dose-dependent manner. L-arginine (300 mg/kg s.c.), given prior to administration of L-NAME (5 mg/kg s.c.) and L-NMMA (50 mg/kg s.c.), reversed either the potentiation or the inhibition of LPS-induced vascular damage. The results of the study indicate that early suppression of NO synthase aggravates acute vascular injury in the ileum and colon, suggesting a defensive role for NO. Administration of NO synthase inhibitors at the time of expression of the inducible isoform of the enzyme confers protection against the subsequent damage to the intestinal vasculature (40).

The efficacy of 546C88 was evaluated in a baboon model for the treatment of septic shock. 546C88 (5 mg/kg) was administered during a 36-h period following the induction of septic shock by i.v. infusion of live *E. coli*. The drug increased mean survival time from 45 to 107 h, with survival rates of 5/8 for the treatment group as compared to 1/8 for the placebo group. Treatment with 546C88 also lowered plasma nitrate levels, which increased following drug removal. The hemodynamic pattern was more stable in the treatment group reflecting the effects of NO synthase inhibition. The placebo group required more fluid support than the treatment group, indicating more extensive vascular leakage. The study suggests that NO synthase inhibition with 546C88 has therapeutic value in this baboon model (41).

A murine model of pneumococcal pneumonia was used to study the effects of L-NMMA on the pathogenesis of pneumonia. Intranasal inoculation of CD1 mice with *Streptococcus pneumoniae* resulted in a 100% mortality rate over a period of 96 h. A 4-day twice-daily treatment with L-NMMA (3 mg/kg s.c.), initiated immediately before infection, increased survival rate by 24% over the 4-day period. Preservation of alveolar spaces was better in animals treated with L-NMMA than in controls, as observed by histological examination of lung tissue. These findings imply that L-NMMA treatment profoundly affects cellular and inflammatory response, tissue integrity and death rate (42).

Table I: Investigational approaches to the management of sepsis and septic shock compiled from Prous Science Daily Essentials and Ensemble databases.

Compound	Source	Status
TARGETING MEDIATORS OF INFLAMMATION		
Cytokine modulators		
Anti-TNF-α antibodies		
D2E7	BASF	Biological testing
CDP-571 (Bay-10-3356)[1]	Bayer/Celltech	Phase II
CytoTAb[2]	Therapeutic Antibodies	Phase IIB
Bay-x-1351[3]	Bayer/Celltech	Phase III
Cytokine (TNF-α and/or IL-1)production/release inhibitors		
CNI-1493	Picower Inst. Med. Res	Preclinical
FR-133605	Fujisawa	Preclinical
FR-167653	Fujisawa	Preclinical
KB-R7785	Kanebo	Preclinical
Neu-Sensamide™	OXiGene	Preclinical
OXi-104	OXiGene	Preclinical
SDZ-MRL-953[4]	Novartis	Phase I
Carbocyclic nucleosides	Hoechst Marion Roussel	Biological testing
Diaryl compounds	Celgene	Biological testing
Thalidomide derivatives	Massachusetts Inst. Technol.	Biological testing
EI-1941-1 (EP 768306)	Kyowa Hakko	Biological testing
EP 720983	Nishin Flour Milling	Biological testing
EP 761680	Ono	Biological testing
JP 96119987	Sankyo	Biological testing
JP 97071581	Nishin Flour Milling	Biological testing
US 5550132	Macronex; Univ. North Carolina	Biological testing
US 5627173	Hoechst Marion Roussel	Biological testing
US 5641751	Centocor	Biological testing
US 5643893	Macronex; Univ. North Carolina	Biological testing
WO 9627583[5]	Pfizer	Biological testing
WO 9631476[6]	Rhône-Poulenc Rorer	Biological testing
WO 9633172[5]	Pfizer	Biological testing
WO 9633968	Fuji Yakuhin	Biological testing
WO 9636611	Chiroscience	Biological testing
WO 9636638[6]	Chiroscience	Biological testing
WO 9639408[5]	Pfizer	Biological testing
WO 9640143	SmithKline Beecham	Biological testing
WO 9640636[6]	Pfizer	Biological testing
WO 9703945[6]	SmithKline Beecham	Biological testing
WO 9705105[6]	Pfizer	Biological testing
WO 9705877	Merck & Co.	Biological testing
WO 9705878	Merck & Co.	Biological testing
WO 9718188[5]	Abbott	Biological testing
WO 9719053[5]	British Biotech	Biological testing
WO 9724355[6]	Fujisawa	Biological testing
WO 9725312[6]	Schering AG	Biological testing
TNF-α neutralizing agents		
Lenercept[7]	Roche	Phase II
IL-1 receptor antagonists		
WO 9629088	Hoechst Marion Roussel; Affymax	Biological testing
IL-6 receptor antagonists		
US 5639455	Ajinomoto	Biological testing
IL-8β receptor atagonists		
WO 9625157	SmithKline Beecham	Biological testing
Anticytokine antisense peptides		
WO 9634887	Imperial Coll. of Sci., Technol. & Med. London Sch. of Hyg. and Trop. Med.	Biological testing

(Continued)

Table I: Continued.

Compound	Source	Status
Phospholipase A_2 inhibitors		
WO 9603120	Lilly; Shionogi	Biological testing
WO 9603383	Lilly; Shionogi	Biological testing
WO 9702242	SmithKline Beecham	Biological testing
WO 9710247	SmithKline Beecham	Biological testing
WO 9741099	SmithKline Beecham	Biological testing
PAF antagonists		
US 5700817	Pohang Iron and Steel; Res. Inst. Ind. Sci. Technol	Biological testing
WO 9614317	Uriach	Biological testing
Cyclooxygenase inhibitors		
WO 9716405	NiCOx	Biological testing
Endotoxin modulators		
Endotoxin production inhibitors		
L-161240	Merck & Co.	Preclinical
L-573655	Merck & Co.	Preclinical
MAb to endotoxin		
Edobacomab[8]	Xoma; Pfizer	Phase III
US 5593969	IGEN	Biological testing
Endotoxin antagonists		
E-5531	Eisai	Phase II
Kinase inhibitors		
CHUK inhibitors[9]	Tularik	Biological testing
SAP kinase inhibitors[10]	Mercury Therapeutics	Biological testing
Bradykinin B_2 antagonists		
Deltibant (Bradycor)[11]	Cortech; SmithKline Beecham	Phase II
EP 808838	Hoechst Marion Roussel	Biological testing
Other inflammation inhibitors		
rBPI21/Neuprex[12]	Xoma	Phase II
TARGETING ADHESION MOLECULES		
ISIS-4730[13]	ISIS Pharmaceuticals	Preclinical
EP 771795	Hoechst Marion Roussel	Biological testing
US 5596090[13]	US Navy	Biological testing
US 5602230	Centocor	Biological testing
US 5618785	Centocor	Biological testing
US 5622701	Protein Design Labs	Biological testing
WO 9701335	Texas Biotechnology	Biological testing
WO 9719104	Novartis	Biological testing
TARGETING NITRIC OXIDE (NO)		
NO production inhibitors		
546C88	Glaxo Wellcome	Phase III
JP 96041008	Ono	Biological testing
WO 9601825	Fujisawa	Biological testing
WO 9630350	Fujisawa	Biological testing
WO 9703678[14]	SCRAS	Biological testing
WO 9716430	Merck & Co.	Biological testing
WO 9736871	Pfizer	Biological testing

(Continued)

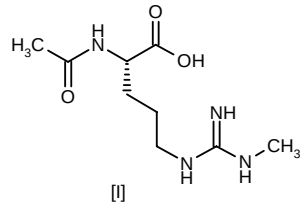
Table I: Continued.

Compound	Source	Status
REPLACEMENT ANTICOAGULANT THERAPY		
Activated protein C	Lilly	Phase I
HEMOREGULATORY AGENTS		
PHP[15]	Apex Bioscience	Phase I/II
Glaspimod[8]	Nycomed Amersham; SmithKline Beecham	Phase II
WO 971850	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717851	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717957	SmithKline Beecham	Biological testing
WO 9717958	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717959	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717961	SmithKline Beecham	Biological testing
WO 9717963	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717964	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717965	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9717973	SmithKline Beecham	Biological testing
WO 9717985	SmithKline Beecham; Nycomed Amersham	Biological testing
WO 9718210	SmithKline Beecham	Biological testing
WO 9718213	SmithKline Beecham	Biological testing
WO 9718214	SmithKline Beecham	Biological testing

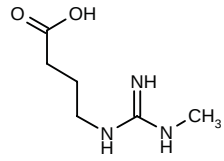
[1]No longer in active development for this indication. [2]Anti-TNF polyclonal antibody. [3]Not effective in reducing mortality. [4]Lipid A analog. [5]Also matrix metalloproteinase (including TNF-α-converting enzyme) inhibitors. [6]Also phosphodiesterase IV inhibitors. [7]Recombinant TNF receptor fusion protein. [8]Discontinued due to insufficient efficacy. [9]Conserved Helix-loop-helix Ubiquitous Kinase inhibitors. [10]Stress Activated Protein kinase inhibitors. [11]Improvement in mortality not demonstrated in SIRS. [12]Recombinant bactericidal/permeability increasing factor. [13]Antisense oligonucleotide. [14]Also cyclooxygenase inhibitor. [15]Pyridoxalated hemoglobin polyoxyethylene; treatment of hypotension.

Pharmacokinetics and Metabolism

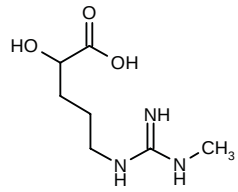
The pharmacokinetics, disposition and metabolism of radiolabeled 546C88 were evaluated in rats and dogs following a 5-min infusion of 1.7 mg/kg (20 mg/kg/h) of title compound. The parent compound exhibited rapid distribution and elimination from plasma; peak plasma concentrations were reached immediately after administration and declined in a biphasic manner. Initial and terminal elimination was rapid, with a $t_{1/2}$ of approximately 2-4 min and $t_{1/2b}$ of 1-2 h. AUC during the 48-h sampling period was approximately 19-fold higher than $AUC_{(0-\infty)}$. Drug-derived radioactivity was eliminated slowly, with up to 39% present in the carcasses at the termination of the 7-day collection period. The drug was excreted mainly as CO_2 in expired air, while less than 8 and 5% was excreted in urine and feces, respectively. Radioactivity was widely distributed throughout the body with the highest concentrations present in those tissues with high protein turnover, such as liver and glandular tissue. 546C88 was eliminated mainly via metabolism and putative amino acid catabolism, which would account for its distribution patterns and route of excretion. The study shows that 546C88 is a drug with rapid and wide distribution into tissues and is probably eliminated by metabolism to citrulline, and perhaps also ornithine, and subsequent catabolism in the urea and citric acid cycles. Three metabolites were identified in urine following administration to rats [I and II] and dogs [II and III] (43).



[I]



[II]



[III]

The use of the system automated sequential trace enrichment of dialysates (ASTED) to prepare plasma samples for the estimation of 546C88 has been described (44).

Clinical Studies

The effects of NO synthase inhibition by L-NMMA and L-NAME were evaluated in 2 patients with life-threatening septic shock whose blood pressure was not restored by

conventional treatment. L-NMMA (0.3-1 mg/kg i.v.) rapidly increased systolic, diastolic and mean arterial blood pressure in a dose-dependent manner and elevated vascular resistance in both patients. L-NMMA (0.3 mg/kg i.v.) raised mean arterial blood pressure from 63.5 ± 2.5 to 76.2 ± 4.2 mmHg within 2 min, and was maintained for less than 15 min. However, in the first patient, who had impaired renal function, the effect of 1 mg/kg L-NMMA lasted for almost 10 h. In the second patient with normal renal function, increased arterial blood pressure lasted for 10-15 min after 1 mg/kg L-NMMA, and a second injection (150 mcg/kg bolus i.v.) raised the mean arterial blood pressure from 84 to 102 mmHg during 10-15 min, indicating elevated systemic vascular resistance. These observations suggest that application of NO synthase inhibitors may be a valuable treatment for refractory hypotension of septic shock when other conventional interventions fail (45).

A randomized, double-blind, placebo-controlled study assessed the effects of NO synthase inhibition with L-NMMA. Twelve patients with hypotension as a result of severe septic shock were given a bolus i.v. injection of L-NMMA (0.3 mg/kg) followed by a second injection of 1 mg/kg bolus i.v. and finally an infusion of 1 mg/kg/h during a period of 6 h. The treatment induced a dose-dependent elevation of mean arterial blood pressure (from 80.9 ±0 2.9 to 100.5 ± 6.1 mmHg) and systemic vascular resistance (from 547 ± 92 to 889 ± 143 dyne.s./cm^5). Pulmonary vascular resistance, central venous pressure and pulmonary artery occlusion pressure also increased. Cardiac output decreased from 11.2 ± 2.1 to 8.9 ± 1.9 l/min, as did heart rate, although the decrease in heart rate was not statistically significant. Platelet count decreased in L-NMMA-treated subjects as well as in those administered placebo and did not differ significantly between groups. This study demonstrates the importance of NO in the mechanism of vasodilatation and hypotension and indicates that NO synthase inhibitors such as L-NMMA effectively increase blood pressure in patients with hypotension as a result of septic shock (46).

The efficacy of 546C88 was evaluated in 312 patients with septic shock, defined as severe sepsis complicated either by sustained hypotension or by the need for vasopressor support in order to maintain MAP at 70-90 mmHg. The compound was administered at 0-20 mg/kg/h, titrated to maintain MAP3 at 70 mmHg while attempting to discontinue current vasopressors. Resolution of shock at 72 h, defined as MAP3 of 70 mmHg without conventional vasopressors, was the primary endpoint. Laboratory confirmed bacterial infections were classified as Gram-positive, Gram-negative, mixed or other. In this patient study, the efficacy of the NO synthase inhibitor 546C88 in septic shock varied depending upon the underlying infection. The 546C88-treated group had a total of 77 overall and 28 serious disease-related events compared to 85 and 37, respectively, in the placebo group. Total number of adverse effects recorded in the treatment group was 74, as compared to 71 in the placebo group. The results indicated that treatment with 546C88 did not raise any pertinent safety issues and the drug was deemed to have a satisfactory safety profile worthy of further clinical investigation (47-51).

Another double-blind, randomized, placebo-controlled trial evaluated 546C88 in 15 patients suffering from migraine without aura. 546C88 (6 mg/kg i.v. 15-min infusion) produced headache relief in 10 patients within 2 h after the initiation of treatment, as compared to 2/14 in the placebo group. Other symptoms such as phonophobia and photophobia were also efficiently eliminated in the active treatment group. A nonsignificant trend for improvement in nausea was also observed. The results from this clinical trial indicate that NO is involved in both initiation and continuation of migraine attacks, and that synthase inhibitors such as 546C88 may have an important role in the treatment of migraine (52, 53).

In another study in humans, the importance of NO in the circulation was evaluated using L-NMMA. Administration of L-NMMA (1, 3 and 10 mg/kg by i.v. bolus) reduced both mean common carotid artery (CCA) flow and internal carotid artery (ICA) flow, effects which were reversed by L-arginine. Systemic blood pressure increased, but no effect was observed on middle cerebral artery (MCA) flow velocity. Administration of carbon dioxide (6% or 8%) after L-NMMA did not affect cerebral blood flow. Noradrenaline induced a slightly smaller decrease in basal CCA flow and had a similar effect on the hypercapnic response. This study shows that NOS inhibition with L-NMMA results in reduced cerebral blood flow but does not inhibit the hypercapnic hyperemic response. It is suggested that neuronal NOS inhibition in humans may confer protective effects in stroke, although nonselective NOS inhibitors such as L-NMMA should be employed with care since they diminish cerebral blood flow (54).

546C88 is currently undergoing evaluation in a large, international, phase III efficacy study for the inidication of septic shock (55).

Manufacturer

Glaxo Wellcome, Inc. (GB).

References

1. Zhang, W., Hershline, R., Dowd, P. *A highly efficient preparation of N^G-methyl-L-arginine and N^G-methyl-D-arginine*. Synth Commun 1994, 24: 2789-92.

2. Patthy, A., Bajusz, S., Patthy, L. *Preparation and characterization of N^G-mono-, di- and trimethylated arginines.* Acta Biochim Biophys Acad Sci Hung 1977, 12: 191-6.

3. Hodson, H.F. (The Wellcome Found., Ltd.). *N^G-Monomethyl-L-arginine hydrochloride derivs. and their use in the treatment of septic shock*. EP 651741, JP 95509239, WO 9402453.

4. Corbin, J.L., Reporter, M. *N^G-Methylated arginines: A convenient preparation of N^G-methylarginine*. Anal Biochem 1974, 57: 310-2.

5. Patel, R., Mahal, A.S., Burford, D.L.W. (The Wellcome Found., Ltd.). *Process for preparing N^G-monoalkyl-L-arginine and related cpds.* WO 9426701.

6. Gillies, I. (Glaxo Group, Ltd.). *Process for the preparation of N^G-monomethyl-L-arginine hydrochloride*. WO 9730972.

7. Pundak, S., Wilchek, M. S*ynthesis of guanidino-N-alkylarginines by the use of polymeric pseudoureas.* J Org Chem 1981, 46: 808-9.

8. Ferrario, F., Levi, S., Sala, A., Trupiano, F. *Multigram synthesis of N^G-methyl-(L-)-arginine and its analytical characterization.* Synth Commun 1991, 21: 99-105.

9. Young, L.S., Martin, W.J., Meyer, R.D., Weinstein, R.J., Anderson, E.T. *Gram-negative rod bacteremia: Microbiologic, immunologic, and therapeutic considerations*. Ann Intern Med 1977, 86: 456-71.

10. Perlino, C.A., Rimland, D. *Alcoholism, leukopenia and pneumococcal sepsis.* Amer Rev Respir Dis 1985, 132: 757-60.

11. Bone, R.C., Balk, R.A., Cerra, F.B., Dellinger, R.P., Fein, A.M., Knaus, W.A., Schein, R.M., Sibbald, W.J. *Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. ACCP/SCCM Consensus Conference Committee.* Chest 1992, 101: 1644-55.

12. Nyström, P.-O. *The systemic inflammatory response syndrome: Definitions of aetiology.* J Antimicrob Chemother 1998, 41(Suppl. A): 1-7.

13. Sibbald, W.J., Doig, G., Inman, K.J. *Sepsis, SIRS and infection*. Intensive Care Med 1995, 21: 299-301.

14. Moncada, S., Palmer, R.M.J., Higgs, E.A. *Nitric oxide: Physiology, pathophysiology, and pharmacology*. Pharmacol Rev 1991, 43: 109-42.

15. Kerwin, J.F. Jr., Heller, M. *The arginine-nitric oxide pathway: A target for new drugs.* Med Res Rev 1994, 14: 23-74.

16. Griffith, O.W., Stuehr, D.J. *Nitric oxide synthases: Properties and catalytic mechanism*. Annu Rev Physiol 1995, 57: 707-36.

17. Marletta, M.A. *Nitric oxide synthase structure and mechanism*. J Biol Chem 1993, 268: 12231-4.

18. Palmer, R.M.J., Bridge, L., Foxwell, N.A., Moncada, S. *The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids.* Brit J Pharmacol 1992, 105: 11-2.

19. O'Connor, K.J., Moncada, S. *Glucocorticoids inhibit the induction of nitric oxide synthase and the related cell damage in adenocarcinoma cells*. Biochim Biophys Acta 1991, 1097: 227-31.

20. Boughton-Smith, N.K., Evans, S.M., Laszlo, F., Whittle, B.J.R., Moncada, S. *The induction of nitric oxide synthase and intestinal vascular permeability by endotoxin in the rat.* Brit J Pharmacol 1993, 110: 1189-95.

21. Boughton-Smith, N.K., Evans, S.M., Hawkey, C.J., Cole, A.T., Balsitis, M., Whittle, B.J.R., *Moncada, S. Nitric oxide synthase activity in ulcerative colitis and Crohn's disease.* Lancet 1993, 342: 338-40.

22. Middleton, S.J., Shorthouse, M., Hunter, J.O. *Increased nitric oxide synthesis in ulcerative colitis*. Lancet 1993, 341: 465-6.

23. Miller, M.J., Zhang, X.-J., Sadowska-Krowicka, H., Chotinaruemol, S., McIntyre, J.A., Clark, D.A., Bustamante, S.A. *Nitric oxide release in response to gut injury.* Scand J Gastroenterol 1993, 28: 149-54.

24. Miller, M.J., Sadowska-Krowicka, S., Chotinaruemol, S., Kakkis, J.L., Clark, D.A. *Amelioration of chronic ileitis by nitric oxide synthase inhibition.* J Pharmacol Exp Ther 1993, 264: 11-6.

25. Joly, G.A., Ayres, M., Chelly, F., Kilbourn, R.G. *Effects of N^G-methyl-L-arginine, N^G-nitro-L-arginine, and aminoguanidine on constitutive and inducible nitric oxide synthase in rat aorta.* Biochem Biophys Res Commun 1994, 199: 147-54.

26. Nava, E., Palmer, R.M.J., Moncada, S. *Inhibition of nitric oxide synthesis in septic shock: How much is beneficial?* Lancet 1991, 338: 1555-7.

27. Wright, C.E., Rees, D.D., Moncada, S. *Protective and pathological roles of nitric oxide in endotoxin shock.* Cardiovasc Res 1992, 26: 48-57.

28. Parratt, J.R. *Nitric oxide in sepsis and endotoxaemia*. J Antimicrob Chemother 1998, 41(Suppl. A): 31-9.

29. Wu, C.C., Chen, S.J., Szabó, C., Thiemermann, C., Vane, J.R. *Inhibition of nitric oxide formation with aminoguanidine attenuates the delayed circulatory failure in endotoxic shock in the rat.* Brit J Pharmacol 1994, 112(Suppl.): Abst 221P.

30. Cai, M., Sakamoto, A., Ogawa, R. *Inhibition of nitric oxide formation with L-canavanine attenuates endotoxin-induced vascular hyporeactivity in the rat.* Eur J Pharmacol 1996, 295: 215-20.

31. Fatehi-Hassanabad, Z., Burns, H., Augley, E.A., Paul, A., Plevin, R., Parrat, J.R., Furman, B.L. *Effects of L-canavanine, an inhibitor of inducible nitric oxide synthase, on endotoxin mediated shock in rats*. Shock 1996, 6: 194-200.

32. Griffiths, M.J.D., Messent, M., MacAllister, R.J., Evans, T.W. *Aminoguanidine selectively inhibits inducible nitric oxide synthase. Brit J Pharmacol 1993, 110: 963-8.*

33. Misko, T.P., Moore, W.M., Kasten, T.P., Nickols, G.A., Corbett, J.A., Tilton, R.G., McDaniel, M.L., Williamson, J.R., Currie, M.G. *Selective inhibition of the inducible nitric oxide synthase by aminoguanidine*. Eur J Pharmacol 1993, 233: 119-25.

34. Wu, C.-C., Ruetten, H., Thiemermann, C. *Comparison of the effects of aminoguanidine and $N^{(\omega)}$-nitro-L-arginine methyl ester on the multiple organ dysfunction caused by endotoxaemia in the rat.* Eur J Pharmacol 1996, 300: 99-104.

35. Southan, G.J., Szabó, C., Thiemermann, C. *Isothioureas: Potent inhibitors of nitric oxide synthases with variable isoform selectivity*. Brit J Pharmacol 1995, 114: 510-6.

36. Thiemermann, C., Ruetten, H., Wu, C.-C., Vane, J.R. *The multiple organ dysfunction syndrome caused by endotoxin in the rat: Attenuation of liver dysfunction by inhibitors of nitric oxide synthase.* Brit J Pharmacol 1995, 116: 2845-51.

37. Hasan, K., Heesen, B.-J., Corbett, J.A., McDaniel, M.L., Chang, K., Allison, W., Wolffenbuttel, B.H.R., Williamson, J.R., Tilton, R.G. *Inhibition of nitric oxide formation by guanidines.* Eur J Pharmacol 1993, 249: 101-6.

38. Ruetten, H., Southan, G.J., Abate, A., Thiemermann, C. *Attenuation of endotoxin-induced multiple organ dysfunction by 1-amino-2-hydroxy-guanidine, a potent inhibitor of inducible nitric oxide synthase*. Brit J Pharmacol 1996, 118: 261-70.

39. Laszlo, F., Evans, S.M., Whittle, B.J.R. *Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase iso-*

forms in rat intestinal microvasculature in vivo. Eur J Pharmacol 1995, 272: 169-75.

40. Laszlo, F., Whittle, B.J.R., Moncada, S. *Time-dependent enhancement or inhibition of endotoxin-induced vascular injury in rat intestine by nitric oxide synthase inhibitors*. Brit J Pharmacol 1994, 111: 1309-15.

41. Schlag, G., Redl, H., Gasser, H., Davies, J., Rees, D., Grover, R. *Delayed treatment with the NO-synthase inhibitor 546C88 in a baboon model of septic shock*. Amer J Respir Crit Care Med 1997, 155(4, Part 2): A263.

42. Ouellet, N., Bergeron, Y., Simard, M., Duong, M., Olivier, M., Beauchamp, D., Bergeron, M.G. *Beneficial immunomodulation of pneumococcal pulmonary infection with N^G-monomethyl-L-arginine*. 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst G-56.

43. Schwartz, S., Clare, R., Devereux, K., Sheung, C.F. *Pharmacokinetics, disposition and metabolism of 546C88 (L-N^G-methylarginine hydrochloride) in rat and dog.* Xenobiotica 1997, 27: 1259-71.

44. Cooper, J.D.H., Shearsby, N., Sheung, C.T.C.F. *Use of the ASTED system to determine L-N^G-monomethylarginine (546C88) in human plasma by pre-column o-phthalaldehyde derivatisation and high-performance liquid chromatography*. J Chromatogr B 1997, 696: 117-22.

45. Petros, A., Bennett, D., Vallance, P. *Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock.* Lancet 1991, 338: 1557-8.

46. Petros, A., Lamb, G., Leone, A., Moncada, S., Bennett, D., Vallance, P. *Effects of a nitric oxide synthase inhibitor in humans with septic shock.* Cardiovasc Res 1994, 28: 34-9.

47. Andersson, J., Watson, D., Donaldson, J., Grossman, S., Smithies, M., Le Gall, J.-R. *Multicentre, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88 in patients with septic shock: Effect in patients with confirmed bacterial infection.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst G-48b.

48. Watson, D., Beerahee, M., Holzapfel, L., Lodato, R., Grover, R., Andersson, J. *Multicentre, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88 in patients with septic shock: Effect on plasma nitrate profile.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst G-40.

49. Anzueto, A., McLuckie, A., Holzapfel, L., Grove, G., Mottola, D., Takala, J. *Multicentre, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88 in patients with septic shock: Safety profile.* Chest 1997, 112(3): 88S.

50. Anzueto, A., Lodato, R., Lorente, J., Holzapfel, L., Grover, R., Takala, J. *Multicenter placebo-controlled, double-blind trial of the nitric oxide synthase inhibitor 546C88 in patients with septic shock: Acute hemodynamic effects.* Amer J Respir Crit Care Med 1997, 155(4, Part 2): A263.

51. Bakker, J., Lodato, B., Donaldson, J., Grover, R., Guntupalli, K., Takala, J. *Multicenter, placebo-controlled double-blind trial of the nitric oxide synthase inhibitor 546C88 in patients with septic shock: Acute effects on the pulmonary circulation*. Amer J Respir Crit Care Med 1997, 155(4, Part 2): A702.

52. Lassen, L.H., Ashina, M., Christiansen, I., Ulrich, V., Olesen, J. *Nitric oxide synthase inhibition in migraine*. Lancet 1997, 349: 401-2.

53. Lassen, L.H., Ashina, M., Christiansen, I., Ulrich, V., Grover, R., Donaldson, J., Olesen, J. *Nitric oxide synthase inhibition: A new principle in the treatment of migraine attacks.* Cephalalgia 1997, 17(3): 423.

54. White, R.P., Deane, C., Vallance, P., Markus, H.S. *Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia*. Stroke 1998, 29: 467-72.

55. *546C88 development status.* Glaxo Wellcome Company Communication 1997, December 1.

Additional References

Peterson, D.A., Peterson, D.C., Archer, S., Weir, E.K. *The non specificity of specific nitric oxide synthase inhibitors*. Biochem Biophys Res Commun 1992, 187: 797-801.

Thomas, G., Myers, A., Farhat, M., Cathapermal, S., Ramwell, P.W. *Effect of N-substituted arginine compounds on blood pressure in anesthetized rats.* J Pharmacol Exp Ther 1992, 261: 875-8.

Oyanagui, Y., Sato, S. *Superoxide dismutases and anti-oxidants protected mice from no-reflow and necrotic damage induced by ischemia.* Free Radic Res Commun 1993, 18: 147-57.

Cappendijk, S.L.T., de Vries, R., Dzoljic, M.R. *Inhibitory effect of nitric oxide (NO) synthase inhibitors on naloxone-precipitated withdrawal syndrome in morphine-dependent mice.* Neurosci Lett 1993, 162: 97-100.

Stefanovic-Racic, M., Myers, K., Meschter, C., Coffey, J.W., Hoffman, R., Evans, C.H. *N-Monomethylarginine, an inhibitor of nitric oxide synthase, suppresses the onset of adjuvant arthritis in rats*. 55th Annu Meet Amer Coll Rheumatol (Nov 7-11, San Antonio) 1993, Abst 36.

Evans, C.H., Coffey, J.W., Hoffman, R., Meschter, C., Meyers, K., Stefanovic-Racic, M. *N-Monomethylarginine, an inhibitor of nitric oxide synthase suppresses the onset of adjuvant arthritis in rats.* IAIS World Cong Inflamm (Oct 10-15, Vienna) 1993, Abst 149.

Alak, A.M., Moy, S. *Determination of N-monomethyl-L-arginine in biological samples by high performance liquid chromatograph.* Pharm Res 1993, 10(10, Suppl.): Abst APQ 1120.

Rongen, G.A., Smits, P., Thien, T. *L-NMMA attenuates the forearm vasodilator response to acetylcholine but not to adenosine-5'-triphosphate in man.* Pharm World Sci 1994, 16: D7.

Kikkawa, K., Yamauchi, R., Hoshino, T., Murata, S. *Characteristics of selective vasoconstriction by N^G-monomethyl-L-arginine in the isolated intracranial artery of dog.* Jpn J Pharmacol 1994, 64(Suppl. 1): Abst O-94.

Freitag, A., Reimann, A., Wessler, I., Rack,, K. *Effects of dexamethasone and N^G-monomethyl-L-arginine (L-NMMA) on morphological changes induced by bacterial lipopolysaccharides*

(LPS) and tumor necrosis factor α (TNFα) on rat tracheal epithelial cells in culture. Naunyn-Schmied Arch Pharmacol 1994, 350(Suppl.): Abst 14.

Akarid, K., Levacher, M., Sinet, M., Chau, F., Desforges, B., Pocidalo, M.A. *Effect of in vivo administration of NO synthase inhibitor on immune abnormalities observed in murine AIDS.* 34th Intersci Conf Antimicrob Agents Chemother (Oct 4-7, Orlando) 1994, Abst I106.

Ansite, J.D., Stevens, R.B., Brown, R.R., Field, M.J., Mills, C.D., Sutherland, D.E.R. *Administration of L-N^G-monomethyl-L-arginine in a marginal renal subcapsular mouse islet transplant model*. 15th World Cong Transplant Soc (Aug 28-Sept 2, Kyoto) 1994, Abst 392.

Khraibi, A., Bracamonte, M. *Role of prostaglandins in the natriuresis of L-NMMA infusion in SHR and WKY rats.* FASEB J 1994, 8(5, Part 2): Abst 4840.

Hansen, P.R., Haunso, S. *Effects of nitric oxide synthase inhibition on myocardial capillary permeability and reactive hyperaemic response*. Eur Heart J 1994, 15(Suppl.): Abst 1846.

Oddis, C.V., Hattler, B.G., Moorer, G., Simmons, R.L., Finkel, M.S. *The nitric oxide inhibitor, L-NMMA, alters E-C coupling in the heart*. J Amer Coll Cardiol 1994, Suppl.: Abst 917-67.

O'Kane, K.P.J., Webb, D.J., Collier, J.G., Vallance, P.J.T. *Local L-N^G-monomethyl-arginine attenuates the vasodilator action of bradykinin in the human forearm.* Brit J Clin Pharmacol 1994, 38: 311-5.

Cockcroft, J.R., Chowienczyk, P.J., Brett, S.E., Ritter, J.M. *Effect of N^G-monomethyl-L-arginine on kinin-induced vasodilation in the human forearm.* Brit J Clin Pharmacol 1994, 38: 307-10.

Uchida, Y., Tsukahara, F., Irie, K., Nomoto, T., Muraki, T. *Possible involvement of L-arginine-nitric oxide pathway in modulating regional blood flow to brown adipose tissue of rats.* Naunyn-Schmied Arch Pharmacol 1994, 349: 188-93.

Cordero, J.J., González, J., Feria, M. *Effects of N^{ω}-monomethyl-L-arginine on short-term RR interval and systolic blood pressure oscillations.* J Cardiovasc Pharmacol 1994, 24: 323-7.

Gilkeson, G.S., Pisetsky, D.S., Coffman, T.M., Spurney, R.F., Best, C., Weinberg, J.B. *Chronic blockage of nitric oxide overproduction in MRL-lpr mice by oral N^G-monomethyl L-arginine (NMMA) prevents inflammatory renal disease and enhances renal blood flow.* Clin Res 1994, 42(2): 139A.

Tracey, W.R., Nakane, M., Kuk, J., Budzik, G., Klinghofer, V., Harris, R., Carter, G. *The nitric oxide synthase inhibitor, L-N^G-monomethylarginine, reduces carrageenan-induced pleurisy in the rat.* J Pharmacol Exp Ther 1995, 273: 1295-9.

Noon, J.P., Shore, A.C., Haynes, W.G., Walker, B.R., Webb, D.J. *The effects of brachial artery infusion of N^G-monomethyl-L-arginine on microvascular blood flow in healthy volunteers.* Brit J Clin Pharmacol 1995, 39: 92P.

Schedin, U., Albert, J., Hjemdahl, P., Lindqvist, M., Melcher, A., Frostell, C. *Infusion of L-NMMA reduces human nasal nitric oxide levels.* Brit J Anaesth 1996, 76(Suppl. 2): Abst A.365.

Fonseca, G.A., Griffith, O.W., Jones, E., Kilbourn, R.G. *Interleukin-2 mediated hypotension in humans is reversed by N^G-monomethyl-L-arginine, an inhibitor of nitric oxide production.* Proc Amer Assoc Cancer Res 1994, 35: Abst 1500.

Krejcy, K., Schmetterer, L., Kastner, J., Eichler, H.G., Kyrle, P.A. *Effects of nitric oxide (NO) - Inhalation or inhibition of NO synthesis by infusion of N^G-monomethyl-L-arginine (L-NMMA) on hemostatic system activation in vivo in man*. Thromb Haemost 1995, 73(6): Abst 67.

Nishida, K., Chung, D.C., Kikuchi, Y., Booke, M., Traber, L.D., Traber, D.L. *Effects of nitric oxide synthase inhibitor on left ventricular end-systolic pressure-diameter relationship in healthy sheep.* FASEB J 1995, 9(3, Part 1): Abst 3231.

Patch, D., McCormick, P.A., Greenslade, L., Dick, R., Burroughs, A.K. *The effect of a nitric oxide synthase inhibitor on system haemodynamics in cirrhotic patients.* Gastroenterology 1995, 108(4, Suppl.): A1144.

Tousoulis, D., Tentolouris, C., Crake, T., Gialafos, J., Davies, G., Toutouzas, P. *Inhibition of nitric oxide synthesis in coronary arteries.* Int Angiol 1995, 14(1, Suppl. 1): Abst 3.01.

Albert, J., Frostell, C., Schedin, U., Lindqvist, M., Melcher, A., Hjemdahl, P. *Infusion during 60 min of L-NMMA to healthy volunteers.* Brit J Anaesth 1996, 76(Suppl. 2): Abst A.274.