

Endothelial dysfunction in heart failure and ischemic heart disease: rationale for the clinical use of mononitrates

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ABSTRACT: *The clinical observation that heart failure can progress independently of the patient's hemodynamic status has focused interest on new, potential mechanisms underlying the progression of cardiac insufficiency. It has been shown that in both ischemic and non-ischemic heart failure, endothelial dysfunction and reduced bioavailability of NO can contribute to the process of left ventricular remodelling. The endothelial dysfunction contributes to increasing peripheral vascular resistance, limiting blood flow to skeletal muscles, particularly during exercise, and compromising the regulation of pulmonary blood flow, leading to a flow-perfusion mismatch. The nitroderivates are a heterogeneous class of compounds that have in common their pharmacodynamic action, which is mediated through the production of NO.*

Isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) are the two main metabolites of isosorbide dinitrate (ISDN) and both act on the smooth muscle cells of vessel walls, causing generalized peripheral vasodilation, which is more marked in the venous system, reduced venous return to the heart and a reduction in peripheral resistance.

The shorter half-life of IS-2-MN compared to that of IS-5-MN allows greater fluctuation of the blood levels of the drug; it can, therefore, be hypothesized that tolerance would develop more slowly.

In the light of the important role of oxidative stress in the progression of heart failure, reduced occupation of the receptors for NO by IS-2-MN could have important implications for the bioavailability of the sulphhydryl groups otherwise involved in the denitrication of the organic nitrates. (Heart International 2007; 3: 86-97)

KEY WORDS: *Nitrates, Endothelial dysfunction, Heart failure, Ischemic heart disease*

THE PHYSIOLOGICAL ROLE OF NITRIC OXIDE

Nitric oxide (NO) is one of the most important factors that regulate the homeostasis of the vessel wall (1). It is an electrically neutral, gaseous molecule with an odd number of electrons. The unpaired electron in a high energy orbit confers NO the property of being a chemically reactive radical. Given its reactivity, NO is a very labile molecule with a short half-life.

In physiological conditions, NO is produced by various cell types in the body (endothelial cells, white blood cells, nervous system cells, muscle cells, etc). The cells

synthesize NO from the nitrogen atoms of the guanidine terminals of L-arginine through the effect of a group of enzymes known as nitric oxide synthases (NOS). There are three isoforms of NOS: the endothelial form (eNOS), the neuronal form (nNOS) and the inducible form (iNOS). These can be expressed in different proportions by the cells that produce NO. The NOS have a complex molecular structure, similar to that of cytochrome P450. They have a haem group, with one oxidizing and one reducing domain, and their action is influenced by various co-factors that regulate their redox state, including nicotinamide adenine dinucleotide phosphate (NADPH),

flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and tetrahydrobiopterin.

In the vessel wall, NO diffuses through the endothelial cell membrane, with a half-life of 3-5 seconds, and enters the smooth muscle cells, where it activates soluble guanylate cyclase. This enzyme catalyzes the formation of cyclic guanosine 3',5-monophosphate (cGMP), which, in its turn, acts as a second messenger inducing relaxation of myofibrils (probably through the reduction of free calcium in the cytoplasm) and inhibition of platelet adhesion and aggregation.

Furthermore, NO forms stable compounds that bind to the heme group of deoxygenated hemoglobin, forming hemoglobin iron nitrosyl (HbFeNO), which is transformed during the oxygenation process in the lungs into S-nitrosohemoglobin (SNO-Hb). This latter compound is able to carry NO and, in conditions of hypoxia, to release it in the peripheral microcirculation, where the vasculature does not produce this substance.

CHANGES IN THE NO METABOLIC PATHWAY IN CARDIOVASCULAR DISEASES

Under physiological conditions, the endothelium releases NO which, in its turn, regulates vascular tone by diffusing into the underlying smooth muscle cells.

The main cardiovascular risk factors (diabetes, hypertension, dyslipidemia, smoking, hyperhomocysteinemia) alter the protective role of the endothelium, producing a set of changes collectively known as endothelial dysfunction. Experimental studies have demonstrated that endothelial dysfunction is associated with reduced bioavailability of endogenous NO as a consequence of both impaired production and accelerated destruction by free radicals; this leads to a change in the regulation of endothelial-dependent vascular tone, with the development of paradoxical vasoconstriction in response to stimuli that normally cause vasodilation (acetylcholine, serotonin) (2). Disturbed vasoregulation of the coronary circulation is one of the mechanisms hypothesized to underlie acute ischemic syndromes, such as unstable angina and variant angina. Reactive oxygen species (ROS), including hydrogen peroxide, superoxide anions and hydroxyl radicals, play a central role in modulating the NO pathway by causing a state of oxidative stress. Superoxide anions in excess react direct-

ly with NO to form other reactive and toxic molecules such as peroxyxynitrite; furthermore, they interfere with the process of S-nitrosylation of cysteine residues, which are key structures for correct cell function since they act as calcium channels, thus preventing signal transduction (3).

ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS AS MECHANISMS UNDERLYING THE PROGRESSION OF THE CARDIAC DISEASES

Endothelial dysfunction. The clinical observation that heart failure can progress independently of the patient's hemodynamic status has focused interest on new, potential mechanisms underlying the progression of cardiac insufficiency. As mentioned above, abnormal generation of ROS, which is not only responsible for triggering myocyte apoptosis, but also contributes to endothelial dysfunction (4, 5), has been proposed as the mechanism underlying impaired vasodilation in the coronary, pulmonary and peripheral circulation. In fact, it has been demonstrated that endothelial-dependent vasodilation is altered in patients with chronic heart failure, independently of whether the aetiology of the cardiac pathology is ischemic or non-ischemic (6, 7). Endothelial dysfunction contributes to increasing peripheral vascular resistance, limiting blood flow to skeletal muscles, particularly during exercise, and compromising the regulation of pulmonary blood flow, leading to a flow-perfusion mismatch. The most important abnormality occurs in the regulation of myocardial blood flow, leading to a possible mismatch between metabolic supply and demand, and subsequent myocardial ischemia even in the presence of coronary vessels free of coronarographically detectable lesions.

Ten years ago, using positron emission tomography, our group demonstrated that the vasodilation of coronary vessels in subjects with idiopathic dilated cardiomyopathy and mild-moderate myocardial dysfunction was substantially compromised. We hypothesized that this was due to functional alterations in the coronary microcirculation (8). These perfusion abnormalities, both at baseline and at maximum vasodilation, were not correlated with either functional class or degree of hemodynamic compromise, suggesting a primary involvement of the coronary resistance vessels.

These findings, subsequently confirmed by other studies, stimulated new approaches to the study of microcirculatory function in patients with heart failure, with important pathophysiological and prognostic implications (9). The possibility that abnormalities in vasodilation, due to the mechanisms described above, could cause progression of myocardial dysfunction towards heart failure opens up new prospects for the prevention and treatment of heart failure. At the same time, new, non-invasive techniques for evaluating both endothelial-dependent and endothelial-independent vascular reactivity can help to clarify the pathophysiological mechanisms underlying abnormalities of the microcirculation and can also be used to evaluate the efficacy of treatments with vasodilators, such as NO donors, in slowing the progression of these abnormalities.

Oxidative stress. One hypothesis that has been gaining ever more consensus is that oxidative stress is involved in the progression of heart failure (10, 11).

The most widely accepted mechanism of endothelial dysfunction in heart failure is abnormal generation of ROS, which is responsible for reducing the bioavailability of NO and increasing local oxidative stress by reacting directly with NO to form peroxynitrites, which cause further oxidative damage to the endothelium. In vitro and in vivo research using animal models has demonstrated that increased production of free radicals or a decrease in antioxidant defenses can affect various functions of myocytes, reduce myocardial contractility, cause damage to cardiac tissue and induce apoptosis (12). There are various sources of excessive production of free radicals, including the activation of xanthine oxidases during ischemia-reperfusion phenomena, auto-oxidation of catecholamines, and cytokine-mediated activation of NO synthase (13, 14).

The increased production of pro-inflammatory cytokines and neutrophil activation seen in heart failure seems to be involved in endothelial dysfunction, through an increase in the capacity to generate ROS and altered regulation of eNOS expression (15).

It has been shown that in both ischemic and non-ischemic heart failure, endothelial dysfunction and reduced bioavailability of NO can contribute to the process of left ventricular remodelling; in fact, the impaired production of NO seems to be involved in the depressed contractility and apoptosis responsible for the progressive deterioration of ventricular function (16),

and in the altered vascular response to circulating vasoactive substances.

Furthermore, NO is able to regulate the myocardial use of substrates and the mitochondrial respiratory chain (17). In clinical models of heart failure, it was shown that there is reduced release of NO under basal conditions, decreased sensitivity to the inhibition of NOS, alterations of NO-mediated processes and an increase in oxidative stress. The autoregulatory mechanism of peripheral vasodilatation activated by hypoxia during low output states in patients with heart failure is altered because of the reduced release of NO from S-nitrosohemoglobin (18).

Amino thiols play an important role in counteracting oxidative stress since they constitute both an intracellular buffer and an extracellular redox system; in particular, glutathione and its metabolite cysteinylglycine have marked antioxidant properties. Patients with heart failure have been found to have a reduction in the plasma thiol pool, evaluated as total sulphhydryl groups; this has been interpreted as being secondary to an increased interaction between free radicals and membrane proteins. Contrasting data, in large part due to the use of inaccurate biochemical methods, have been reported concerning the levels of red cell glutathione in patients with heart failure. It is not, therefore, currently possible to interpret the role of this scavenger in the antioxidant defense system in heart failure (10, 19).

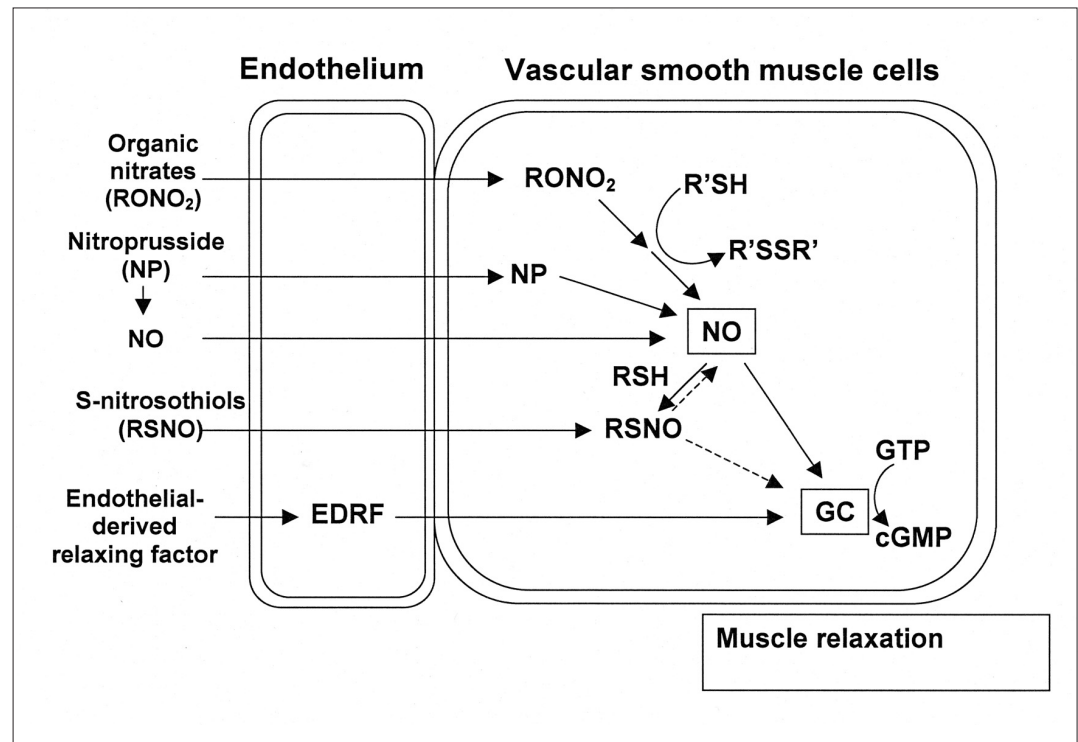
NITRODERIVATES

There are drugs, such as the nitroderivates, which are able to improve the NO balance through an exogenous supply of the molecule, and to “pseudonormalize” the endothelial-dependent response, restoring the effect of physiological vasodilation instead of vasoconstriction, even in the presence of endothelial dysfunction (20). The extent of vasodilation is related to the amount of NO released during the biotransformation of the nitroderivate.

The nitroderivates are a heterogeneous class of compounds that have in common their pharmacodynamic action, which is mediated through the production of NO. From a metabolic point of view, the nitroderivates can be divided into two classes:

1. Nitroderivates that need specific co-factors in order to release NO:

Fig. 1



- organic nitrates such as nitroglycerin (NTG), isosorbide dinitrate (ISDN), isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN)
 - organic nitrites (S-nitroso-N-acetyl-DL-penicillamine).
2. Nitroderivates that do not need specific co-factors in order to release NO:
- sodium nitroprusside
 - nitrites
 - S-nitrosothiols.

The molecules most widely used in clinical practice are the organic nitrates.

Experimental data indicate that after having penetrated into the vascular wall smooth muscle cells, the nitrates react with tissue thiols to generate free oxygen radicals, such as NO and S-nitrosothiols (Fig. 1). These, in turn, activate intracellular guanylate cyclase leading to the production of cGMP (21), which plays a fundamental role in the relaxation of the vascular smooth muscle through the activation of a cGMP-dependent protein kinase that causes dephosphorylation of the light chain of myosin. This dephosphorylation blocks the interaction between myosin and actin, facilitating smooth muscle relaxation and vasodilation. It is thought that the same

cGMP-dependent protein kinase activates a calcium-dependent ATPase that reduces the intracellular concentration of calcium and the activity of the myosin light chain kinase. Suppliers of sulphhydryl groups (thiols), such as cysteine and glutathione, are necessary for the formation of NO and for the activation of guanylate cyclase.

From the above considerations it can be deduced that nitrates are potent coronary artery dilators that can mimic the local effects of NO in the presence of endothelial dysfunction. Therefore, there are solid biochemical bases for using nitrates in the treatment of ischemic heart disease. It is important to emphasize that the administration of nitroderivates does not normalize endothelial function, but compensates for the critical reduction in the bioavailability of NO.

PHARMACOLOGICAL EFFECTS OF THE NITRATES

Hemodynamic effects. Nitrates induce a non-uniform, but dose-dependent vasodilation in many vascular districts (22). In fact, at low concentrations they cause dilation of the peripheral capacitance veins, with a redistrib-

ution of circulating blood from the center (heart, lungs) to the splanchnic and mesenteric districts, with a consequent decrease in preload, cardiac output, and myocardial oxygen requirements. At higher concentrations, nitrates also induce dilation of the arterial compartment. In the coronary circulation (epicardial vessels, arterioles and collateral vessels), vasodilation leads to an increased supply of oxygen to the myocardium. Dilation of peripheral arteries causes a reduction in afterload, an increase in cardiac output and a further decrease in myocardial oxygen demand.

Antithrombotic effects. Nitrates also have important antiplatelet properties (23). Although the clinical relevance of this pharmacological effect, which has been documented in vitro, is still being debated, many studies suggest that at conventional doses nitrates can inhibit platelet activation. In vitro, nitrates increase the concentration of guanylate cyclase within platelets; this leads to an increase in the concentration of cGMP, thus inhibiting the intracellular transport of calcium and reducing the binding affinity between fibrinogen and the glycoprotein IIb/IIIa receptor.

NITRATES IN CHRONIC HEART FAILURE

Nitroderivates, drugs that have been used for over a century in the treatment of myocardial ischemia, have numerous mechanisms of action that can have favourable effects also in heart failure.

The theoretical rationale for using nitroderivates as vasodilators in the treatment of heart failure is the ability of these drugs to correct the hemodynamic changes that occur in this disease. Venous dilation is the dominant effect of nitroderivates at usual doses. Given the marked compliance of the venous system, even small decreases in tone cause peripheral pooling of large amounts of blood, and reductions in venous tone, preload and pressures in the right atrium, pulmonary artery, pulmonary capillaries and left atrium.

In addition to this rationale, the discovery that the NO pathway is altered in heart failure opened up new perspectives for the use of nitrates in this condition. By acting as NO-donors, nitroderivates increase the bioavailability of NO and therefore, their use in chronic heart failure could have favourable effects, beyond purely hemodynamic changes, on ventricular remodelling and

vascular tone.

At the present state of knowledge it can be stated that nitrates could, theoretically, favourably influence the pathophysiological mechanisms of cardiocirculatory dysfunction, although when leaving the realm of theoretical considerations, clinical evidence of a real benefit has only been shown in blacks (24-32).

The real limitations of nitrate therapy seem to be the total lack of effects on activation of the sympathetic and renin-angiotensin-aldosterone systems and on the production of arginine-vasopressin, cytokines and natriuretic peptides, which are increasingly reported to be important in the pathogenesis and progression of heart failure.

Another indication for nitroderivates is heart failure due to diastolic dysfunction in which the aim of treatment is to reduce symptoms by lowering ventricular filling pressures, without significantly reducing cardiac output; this aim seems to be achievable with the careful use of diuretics and nitroderivates. Various studies have confirmed the favourable effects of nitrates in elderly heart failure patients in whom diastolic dysfunction is predominant and in whom a greater reduction in left ventricular end diastolic pressure has been observed compared to that in younger patients.

Although therapy guidelines indicate the addition of a nitroderivative in the treatment scheme for heart failure in the case of intolerance to an ACE-inhibitor, or in the presence of anginal symptoms, nitrates are still very widely used in patients with this disease: a recent survey among members of the American Heart Failure Society found that 90% of the cardiologists questioned use nitroderivates in patients with heart failure of ischemic origin and 81% in heart failure of non-ischemic origin (33). The main reasons were to control symptoms (96%), to improve hemodynamic status (74%) and to improve exercise tolerance (65%). Nitroderivates were prescribed in combination with hydralazine in only 25% of the cases.

TOLERANCE TO NITRODERIVATES

A significant problem related to the use of nitrates is that over time the same dose of drug becomes clinically and hemodynamically less effective, a phenomenon that has been termed *tolerance*. This problem has been

observed with all the drugs and all methods of administration that provide relatively stable and continuous blood levels: it is estimated that about 50-60% of patients treated are affected (34).

The specific mechanisms responsible for the tolerance phenomenon are not yet clear. In fact, while some researchers have demonstrated that the favourable hemodynamic effect of nitrates persist for 4-6 hours with particularly important results in patients with systolic dysfunction, cardiomegaly, and ischemic cardiomyopathy, others (35) have reported good results in the acute phase without long-term benefits. These conflicting results have been explained in different ways (36). Cowan affirmed that the different mechanisms of action of nitrates (on the venous, systemic and coronary vessels) are exploited diversely in patients with heart failure, with differences between one patient and another, and in the same patient depending on the phase of the disease (37). Leier found that, during long-term treatment, tolerance developed in relation to systemic vascular alterations, whereas drug effects on the venous system persisted (25).

The most widely accepted explanation of tolerance is that there is a depletion of intracellular co-factors, represented by the sulphhydryl groups, or more precisely, of a crucial component in the metabolic conversion of nitrates into NO. In fact, the sulphhydryl pool diminishes after chronic exposure to nitrates: the tissue concentrations of cysteine and glutathione are reduced by about 30% in the arteries that show the tolerance effect, compared to those that do not show this effect. An infusion of N-acetylcysteine (NAC) or methionine (both thiol donors) can counteract the onset of tolerance by facilitating the formation of -SH groups, thereby increasing the intracellular synthesis of S-nitrosothiols, and by promoting the extracellular formation of thiols able to enter vascular cells (38). This effect of NAC occurs if the compound is administered before tolerance develops, but not when it is already established, indicating that it is predominantly an extracellular effect.

The administration of -SH group donors is, however, able to resolve only part of the problem of tolerance, so that it is reasonable to hypothesize that the phenomenon is also related to extracellular mechanisms.

According to the hypothesis of the involvement of neurohormonal factors, the administration of nitroderivates is associated with the reflex release of various vasocon-

strictive hormones to compensate for the vasodilating effect of the drugs. Parker et al demonstrated that the chronic administration of nitroderivates to healthy volunteers was able to cause a progressive neurohormonal response characterized by increases in the plasma levels of noradrenaline, renin, and arginine-vasopressin (39). These hormonal changes, with hypersecretion of aldosterone, lead to the retention of water and salt responsible for the increase in intravascular volume and a state of generalized vasoconstriction. This type of tolerance is defined pseudotolerance and develops early, within the first 24 hours after starting chronic treatment (40). Pseudotolerance, related to the reflex mechanisms, occurs at the start of treatment, with the activation of the renin-angiotensin-aldosterone axis secondary to vasodilation of the resistance arteries. The consequent expansion of plasma volume counteracts the vasodilatory effect of NTG. The hypotensive effect of nitrates does, therefore, tend to diminish rapidly.

Preliminary studies seem to indicate that the neurohormonal blockade produced by the combination of ACE-inhibitors with NTG can counterbalance these reflex effects, thus reducing tolerance. Furthermore, it has been observed that intravenous administration of NTG in heart failure induces a redistribution of body fluids, with an increase in intravascular volume and a decrease in haematocrit, without changes in the water-salt balance. In addition, the use of diuretics can reduce tolerance to nitroderivates, with the possible exception of ethacrynic acid, which has documented a direct effect of inactivating sulphhydryl groups.

In contrast, long-term treatment (72 hours) causes a reduction in vasodilation in response to NTG through an intrinsic mechanism of the vascular smooth muscle cells which increase local production of superoxide and endothelin as a result of activation of the vascular oxidases. The superoxide anion binds easily to NO, forming peroxynitrite, which has less capacity to stimulate guanylate cyclase; by activating a protein kinase, it also increases the sensitivity of the endothelium to vasoconstrictive substances, thus counteracting the vasodilating effect of the nitrates. This mechanism has been confirmed in two clinical studies, limited to a few patients, which showed that the administration of antioxidant vitamins (vitamins C and E) (41, 42), reduces tolerance in patients with chronic ischemic heart disease.

The loss of the anti-ischemic effect is the main problem

connected to the use of repeated doses of nitrates. Fluctuations in the circulating levels of the drug and the peak plasma concentration are the major determinants of the development of tolerance. Possible strategies for the prevention of tolerance to nitroderivates include the administration of the lowest effective doses of the drug, the use of compounds with a shorter duration of action, and leaving a drug-free interval (therapeutic window). This concept is not valid for the transdermal formulations that, although administered at lower doses, are more able to cause tolerance. The only strategy that has so far been demonstrated effective in preventing tolerance is the *asymmetric* administration of nitroderivates in order to cause a drop in plasma levels of these drugs and to restore the response to them. The recommended interval (or therapeutic window) between administrations is about 12-14 hrs.

Treatment at intervals, however, carries the risk of triggering rebound angina. The mechanism of the rebound effect is related to the sudden rise in systemic and pulmonary arterial blood pressures after the removal of the transdermal NTG release system. There are also reports of negative effects on physical performance and exercise duration during the nitrate-free period. The explanation for this phenomenon during the administration of a nitroderivate could be increased sensitivity to vasoconstrictive stimuli, perhaps associated with greater local production of endothelin. It has been documented that combining the nitroderivate with a beta-blocker, which counteracts the reflex sympathetic activation, reduces the risk of ischemic rebound compared to that occurring with nitrate monotherapy (43).

AREAS TO EVALUATE

Therapeutic prospects in the field of nitroderivates are:

- Methods to prevent tolerance: treatment at intervals, rapidly rising plasma concentrations, dose increases to a maximum dose, -SH group donors.
- New drugs in the class with better bioavailability, such as IS-2-MN.
- Greater sparing of the intracellular sulphhydryl groups to enable more efficient and prolonged denitration of the organic nitrates.
- NO donors that do not need intracellular conversion by sulphhydryl groups, such as S-nitrosothiols (44).

ORGANIC NITRATES

On the basis of their duration of action, the organic nitrates are classified into:

1. Short-acting nitroderivates (NTG via the sublingual or intravenous route and amylnitrite, in ampoules for inhalation). This group of nitrates causes a very intense but brief dilation of the coronary arteries and is used in the treatment of attacks of angina pectoris.
2. Long-acting nitroderivates, related to their absorption, diffusion and elimination characteristics (erythritol tetranitrate, pentaerythritol tetranitrate, mannitol hexanitrate, inositol hexanitrate, trolnitrate phosphate, ISDN, IS-2-MN and IS-5-MN, pentaerythrylnitrate, tenitramine).

NTG is absorbed through the skin and oral mucosa and less rapidly through the intestine, while the other organic nitrates (erythritol tetranitrate, pentaerythritol tetranitrate, pentaerythrylnitrate, ISDN, IS-2-MN, IS-5-MN, mannitol hexanitrate, inositol hexanitrate, and trolnitrate phosphate) are absorbed more slowly than NTG. The common step in the metabolism of nitrates is a denitration reaction catalyzed by a glutathione-dependent reductase of organic nitrates. The mono- and di-nitrate metabolites of NTG are less active than their precursor.

ISDN administered *per os* is absorbed rapidly and metabolized into active catabolites, including IS-2-MN and IS-5-MN (Tab. I).

IS-2-MN is, like IS-5-MN, a catabolite of ISDN, with the

TABLE I - THE MAIN PHARMACOKINETIC CHARACTERISTICS OF NTG, ISDN AND IS-5-MN

	NTG	ISDN	IS-5-MN
Protein binding (%)	-	28	-
Clearance (ml/min/kg)	230 ± 90	25 ± 20	1.8 ± 0.26
Half-life	2.3 min	0.8 hours	4.4 hours
Bioavailability (%)			
Oral	0	22	93
Sublingual	38	59	-
Cutaneous	-	33	-
Urinary excretion (%)	<1	<1	<5
Altered metabolism			
Liver disease	Yes	Yes	No
Renal impairment	No	No	No
Active metabolites	Yes	Yes	No

(modified from: Ferrari M, Padriani R. Farmacologia Clinica Cardiovascolare, Piccin Editore, 1996, 4th edition, with permission)

same mechanism of action: it increases the synthesis of NO which, by stimulating guanylate cyclase, induces an increase in the synthesis of cGMP and thus vasodilation through a reduction of the concentration of calcium in the vascular smooth muscle cells. This reduction is the result of both inhibition of the entry of calcium into the cells and an increase in their exit.

Pharmacokinetic aspects

The main studies carried out in humans have been predominantly pharmacokinetic and the method used for determining plasma concentrations of IS-2-MN was, in most cases, gas chromatography. These studies have shown that IS-2-MN has a high bioavailability (almost 100%) because it does not undergo like IS-5-MN, hepatic first-pass metabolism. Oral doses between 5 and 20 mg are rapidly and completely absorbed and the plasma concentrations of the drug reach maximum values, of 400-500 ng/ml, within 20-30 min after the administration of a single dose of 20 mg.

The drug is absorbed very quickly, with minimum interindividual differences in plasma concentrations and time to peak plasma levels.

Tables II and III present the results, obtained in various different studies, of some pharmacokinetic parameters of IS-2-MN and IS-5-MN administered either orally or intravenously.

It can be seen that the half-life ($T_{1/2}$) of IS-2-MN (1.7-1.9 h) is about half that of IS-5-MN. The clearance of IS-2-MN (21.7-23.2 l/h) is higher than that of IS-5-MN (6.5-8.5 l/h) and the volume of distribution of the two drugs is basically the same. The pharmacokinetics of IS-2-MN have been evaluated in healthy volunteers both after oral administration (20 mg) and after intravenous injection (5 mg). Following oral administration, the peak plasma concentration is reached after about 25 minutes and the drug's half-life is 2.1 h. Like IS-5-MN, IS-2-MN has a relatively simple pharmacokinetic behaviour in young subjects, which can be represented by a two-compartment system (45).

The systemic bioavailability of IS-2-MN is almost 100%, indicating fast and complete intestinal absorption. Unlike the original compound (ISDN), IS-2-MN does not undergo hepatic first-pass metabolism; indeed, the fact that concentration-time curves show a second peak suggests that compound probably enters an entero-he-

patic circulation. The clearance of IS-2-MN is one-quarter of that calculated for ISDN (92.4 L/h). These data allow the distribution of the compound in the body to be calculated as 70-80%, predominantly in the various aqueous compartments (the volume of distribution of IS-2-MN is 44.5 L). The combination of a much lower clearance and a volume of distribution only slightly smaller than that of ISDN explains the significant prolongation of the half-life. IS-2-MN does not show accumulation phenomena in the body and its pharmacokinetic profile does not appear to be significantly altered in patients with renal impairment (46).

For the most part, this product of the biotransformation of ISDN is metabolized in the liver, through redox reactions. Urinary elimination occurs in the form of the unmodified drug (1-5%) and in the form of various compounds (glucuronic esters, etc.) which are all pharmacologically inactive. The lack of active metabolites (another important difference from that of the other currently used nitroderivates) can be considered an advan-

TABLE II - IS-5-MN

Author	$T_{1/2}$ (h)	Clearance (L/h)	Volume of distribution (L)
Taylor et al	4.2	7.9	48.4
Bonn	4.2	7.69	-
Steudel et al	6.1	6.48	-
Abshagen et al	4.6	6.69	45.6*
Laufen et al	4.2	5.87	34.4
Straehl et al	4.15	8.5	48.2
	4.57±0.76	7.19±0.99	

* calculated according to a single compartment model. From Straehl P, et al (45); Adapted by permission from Macmillan Publisher Ltd: Clinical Pharmacology & Therapeutics 1984; 4 (36): 485-492. ©1984

TABLE III - IS-2-MN

Author	$T_{1/2}$ (h)	Clearance (L/h)	Volume of distribution (L)
Taylor et al	1.8	21.7	55.1
Bonn	1.8	21.7	-
Laufen et al	1.7	17.1	44.5
Straehl et al	1.93	23.2	54.4
	1.81±0.09	20.92±2.64	

From Straehl P et al (45); Adapted by permission from Macmillan Publisher Ltd: Clinical Pharmacology & Therapeutics 1984; 4 (36): 485-492. ©1984

tageous property for oral therapy in that it results in a greater correspondence between dose administered and therapeutic effect.

PHARMACOLOGICAL PROSPECTS

IS-2-MN and IS-5-MN are the two main metabolites released by the hepatic biotransformation of ISDN under the effect of glutathione-dependent nitrate reductases. Both act on the smooth muscle cells of vessel walls, causing generalized peripheral vasodilation, which is more marked in the venous system, reduced venous return to the heart and a reduction in peripheral resistance.

The main differences between IS-2-MN and IS-5-MN lie in their pharmacokinetic characteristics and potency of effect. In fact, the potency of IS-2-MN is about 4-10 times greater than that of IS-5-MN; in other words, considerably lower plasma levels of the former are needed to obtain the same therapeutic result (48).

With regards to the pharmacokinetic profile, the plasma half-life of IS-2-MN is about 2 hours, being much shorter than the 4-5 hours of IS-5-MN. The short plasma half-life of IS-2-MN could be an advantage in preventing the development of tolerance. A delayed release preparation of IS-2-MN would guarantee therapeutically active blood levels for several hours, whereas the short half-life would rapidly free the receptors, thus restoring the previously used -SH groups (47, 48).

Summary of experimental studies. The pharmacological effects of IS-2-MN and IS-5-MN were compared with those of ISDN and NTG by Wendt (52), who demonstrated that all four nitrates caused significant reductions in systolic and diastolic blood pressures. Furthermore, early and transient increases in cardiac output, coronary flow and heart rate were seen after the administration of NTG, ISDN and IS-2-MN. With regards to their vasodilating effects on the systemic and coronary vasculature, the potency of the drugs, in decreasing order of power, is: NTG, ISDN, IS-2-MN, IS-5-MN.

Further pharmacological studies confirmed that the pharmacodynamic effects of IS-2-MN lie between those of ISDN and IS-5-MN (48-51). Atanasova et al (53) studied the pharmacodynamic effects of IS-2MN *in vivo* and its pharmacobiochemical effects *in vitro*.

In vivo, the administration of IS-2-MN causes reduc-

tions in systolic and diastolic arterial pressures, left ventricular end-diastolic pressure, and peripheral resistance, and an increase in the oxygen content of venous blood. Furthermore, IS-2-MN reduces both preload and afterload and does not cause an increase in myocardial oxygen consumption (49-51). Pharmacobiochemical studies have shown that ATP-ase activity is not modified by the administration of IS-2-MN and, therefore, the cardiovascular effects induced are probably not related to the drug affecting bioenergetic processes of the myocardium.

FUTURE PROSPECTS FOR IS-2-MN

The pharmacodynamic and pharmacokinetic characteristics of this drug suggest that it would have good activity in angina pectoris, both as a treatment for an acute attack and as prophylaxis against attacks. Its good bioavailability and lack of first pass hepatic metabolism raise the possibility that IS-2-MN could cause less tolerance than the other nitrates and have a better tolerability in patients with liver disease or congestive heart failure with hepatic damage. The shorter half-life of IS-2-MN compared to that of IS-5-MN allows greater fluctuation of the blood levels of the drug, and it can, therefore, be hypothesized that tolerance would develop more slowly.

Finally, in the light of the important role of oxidative stress in the progression of heart failure, reduced occupation of the receptors for NO by IS-2-MN could have important implications for the bioavailability of the sulphhydryl groups otherwise involved in the denitrication of the organic nitrates: in fact, a smaller proportion of sulphhydryl groups, particularly glutathione, would be subtracted, to biotransform the nitrates, from their role as scavengers to counteract oxidative stress in the vasculature.

CONCLUSIONS

The endothelium is an active biologic interface between the blood and all other tissues that forms a unique thromboresistant layer between blood and potentially thrombogenic subendothelial tissues. The endothelium also modulates tone, growth, hemostasis, and inflam-

mation throughout the circulatory system. Importantly, an excessive inflammatory and fibroproliferative response to the number of insults to the vascular endothelium is important in the development of atherosclerosis.

The role of endothelial dysfunction in atherosclerotic cardiovascular disease is well recognized, there is substantial evidence that endothelial dysfunction leading to impaired regulation of vascular tone may contribute significantly to the altered peripheral vascular tone observed in both animal and human models of HF. Irrespective of the setting, impairment in endothelial function is a marker for poorer cardiovascular prognosis.

Nitrovasodilators, such as organic nitrates (NTG, IS-5-MN, and ISDN), have been widely used as therapeutic agents. These compounds have been administered successfully in the treatment of symptomatic CAD, hypertension and heart failure and evidence suggests that they offer benefit in the management of vascular disor-

ders characterized by endothelial dysfunction and NO deficiency.

Appropriate treatment of vascular inflammation by direct or indirect NO donors, enhancement of the action of NO, and/or scavenging of ROS should be further explored for prevention of atherosclerosis. In this respect, new technologies such as controlled, slow, and regular release of NO from prolonged-release NO-donor capsules (IS-5-MN retard) or new evidences by IS-2-MN appear to be promising strategies.

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