The cardiovascular continuum:  
from endothelial dysfunction to clinical events.  
The role of organic nitrates

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ABSTRACT: The endothelium plays a key role in the regulation of numerous physiological functions, including fibrinolysis, thrombosis, inflammation, cell growth and the maintenance of vascular tone. Several studies have shown that endothelial dysfunction is an early event in the course of the heart failure and changes in vascular reactivity have a series of hemodynamic consequences, including increases in total peripheral resistance and afterload, worsening of cardiac performance and a reduction in tolerance to exercise. Organic nitrates are the oldest drugs used in patients with coronary artery disease and heart failure and are able to deliver nitric oxide (NO) directly to the vascular wall inducing the well known vasodilator effects. Therefore, it is likely that the release of NO by organic nitrates is associated with antiaggregatory, antiadhesive, antiproliferative and antioxidative effects, all of which are considered to be vasoprotective. Recent data from animal models suggest that treatment with high doses of isosorbide mononitrate (ISMN) reduce the progression of atherosclerosis and this reduction of morphologic changes is associated with an improvement of the endothelial function. Organic nitrates and recent mononitrates (IS-5-MN and IS-2-MN) appear useful to protect arterial endothelium and inhibit the progression of the disease in heart failure and ischemic heart disease. (Heart International 2007; 3: 78-85)

KEY WORDS: Ischemic heart disease, Atherosclerosis, Heart failure, Mononitrates, Endothelial dysfunction, Vasodilation, Antioxidative effects, Vasoprotective effects

THE ROLE OF ENDOTHELium DYSFUNCTION IN THE PATHOGENESIS OF HEART FAILURE

The endothelium plays a fundamental role in the regulation of numerous physiological functions, including fibrinolysis, thrombosis, inflammation, cell growth and the maintenance of vascular tone (1). In particular, for its regulation of vascular tone, the endothelium releases a series of vasoactive substances, some of which are vasoconstrictive (for example, endothelin), while others are vasodilatory (for example, nitric oxide). Nitric oxide (NO) is synthesized from the amino acid L-arginine and, once produced in the endothelial cells through the effect of NO synthase, diffuses into the underlying smooth muscle cells of the arterial walls where it causes the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) which, in its turn, causes vasodilation through the reduction of the intracellular concentration of calcium ions (Fig. 1).

Clinical data have shown that heart failure can progress independently of the hemodynamic state of the patient, which has focused research on new, potential mechanisms underlying this progression. Patients with chronic heart failure (CHF) have altered vascular reactivity (2-4), as shown by reduced vasodilation in both the coronary arteries (5) and the brachial artery (6) in re-
The reduced response to acetylcholine is a manifestation of impaired endothelial-dependent vascular reactivity, i.e. the change in vascular tone that requires a healthy endothelium and the presence of NO. Endothelial-independent vascular reactivity, on the other hand, is largely preserved in patients with CHF, as demonstrated by the almost completely normal vasodilatory response to an infusion of nitroprusside, a direct NO donor, in the forearm (6).

The change in vascular reactivity observed in patients with heart failure has a series of hemodynamic consequences, including increases in total peripheral resistance and afterload, worsening of cardiac performance and a reduction in tolerance to exercise. Therefore, the endothelial dysfunction observed during the course of heart failure is one of the factors underlying the progression of the disease and it is extremely important to be able to measure it and monitor any changes that may occur (Fig. 2).

An increase in the generation of reactive oxygen species (ROS), produces not only a triggering apoptosis of muscle cells, but also loss of endogenous NO activity due to endothelial dysfunction (4, 5). The clinical consequences of persistent impaired NO activity and increased radical oxygen species activity lead mainly to endothelial dysfunction and myocardial dysfunction, reduction of myocardial pump function- or low-reflow phenomenon. Many actions are under the direction of NO: vasodilation, inhibition of platelet aggregation, reduction of proliferation and inflammation, antioxidant property (scavenging of O2), a direct or indirect increase in myocardial contractility, prevention of neutrophil adhesion.

The alteration of vasodilatory capacity in the coronary, pulmonary and peripheral circulations by endothelial dysfunction is present in patients with CHF, due to ischemic or non-ischemic aetiology (6, 7). In CHF many signs and symptoms are present but none prevails, it is as in a theatrical performance where many actors alternate on the stand but no one is a main actor, all contribute to the representation. At the same way in CHF an increased endothelial dysfunction induces increasing of the peripheral vascular resistance, reducing flow to the skeletal muscles, particularly during exercise, and unbalance the regulation of pulmonary blood flow with a mismatch between the regulation of flow and perfusion. The alteration in the regulation of myocardial blood flow, leading to a mismatch demand and the metabolic supply with myocardial ischemia even in the presence of coronary vessels unjured by lesions detectable by coronarography. Neglia et al, using positron emission tomography, observed that the vasodilation of coronary vessels in patients with idiopathic dilated cardiomyopathy and myocardial dysfunction (mild-moderate) was compromised and hypothesized that etiology was
linked to functional alterations in the coronary microcirculation (8). The perfusion abnormalities were not correlated with clinical functional class or hemodynamic factors, suggesting a primary involvement of the coronary resistance vessels. These data, confirmed by other studies, lead to study the microcirculatory function in CHF, with important pathophysiological and prognostic implications (9). The principal mechanism of endothelial dysfunction in CHF is increased or abnormal production of reactive oxygen species (ROS), implying a reduction of the bioavailability of NO and increasing local oxidative stress, with production of peroxynitrites by direct reaction with NO, and inducing further oxidative damage to the endothelium (10, 11). Studies carried out on animal models demonstrated that increase of free radicals or a reduction of antioxidant defenses interfere with myocyte functions, reduce myocardial contractility, produce a damage of the cardiac tissue and induce apoptosis (12) (Fig. 3).

In ischemic and non-ischemic heart failure the presence of endothelial dysfunction and reduced NO bioavailability contribute to the process of left ventricular remodeling; the impaired NO production seems to be involved in the depressed contractility and apoptosis (16), and in the altered vascular response to circulating vasoactive substances (Fig. 4). Moreover, NO regulates the myocardial use of substrates and the mitochondrial respiratory chain (17). In CHF has been observed that there is a reduction in NO release in basal conditions, decreased sensitivity to NO synthase inhibition, a change in NO-mediated processes and an increase in oxidative stress. In patients with CHF the autoregulatory mechanism of peripheral vasodilatation activated by hypoxia during low output states is altered because there is a reduction of release of NO from S-nitrosohemoglobin (18).

Patients with CHF showed a reduction in the plasma thiol pool, evaluated as total sulphydryl groups; this has been suggested linked to an increased interaction between free radicals and membrane proteins. Other data indicated the presence of levels of red cell glutathione in patients with CHF. Therefore, it is not possible to suggest an interpretation of effect of this scavenger in the antioxidant defense system in heart failure (10, 19).

Ultrasound studies of the brachial artery are a relatively simple, non-invasive and well reproducible method for evaluating endothelial function in all patients.
with impaired cardiac contractility. Numerous studies exploiting this method have demonstrated the role of endothelial function in predicting the progression of heart failure and the risk of mortality (Figs. 5-8).

The possibility that abnormalities in vasodilatory capacity, 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 cardiac insufficiency. Contemporaneously, new, non-invasive techniques for evaluating both endothelial-dependent and endothelial-independent vascular reactivity are helping to clarify the pathophysiological mechanisms underlying microcirculatory abnormalities and can be used to evaluate the efficacy of treatments with vasodilators, such as NO donors, in slowing the progression of these abnormalities. Organic nitrates are the oldest drugs used in patients coronary artery disease and heart failure. For example, i.v. nitroglycerin is considered the first line therapy for unstable angina and oral nitrates might be of special use in patients with impaired left ventricular function, overt congestive heart failure, intermittent coronary vasoconstriction or
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Coronary artery spasm. Many studies with oral nitrate treatment in coronary artery disease and heart failure have clearly shown that these drugs are not only effective and safe but can also exert additional beneficial effects contributing to their therapeutic value (20). Among these, the impact of nitrates on the development of endothelial dysfunction seems to be of particular importance. Organic nitrates deliver NO directly to the vascular wall and are enzymatically de-nitrated to NO and the de-nitrated metabolites. During this enzymatic conversion which is most likely mediated by cytochrome P 450 type enzymes such as CYP3A4 three electrons are transferred to the nitrate nitrogen. Both vascular endothelial and smooth muscle express the enzymes necessary to generate NO from organic nitrates. The NO release from organic nitrates in the vascular wall induces the well-known vasodilator effects. As outlined above, NO exerts many other cardiovascular effects beside vasodilation. Therefore, it is likely that the release of NO by organic nitrates is associated with antiaggregatory, antiadhesive, antiproliferative and antioxidative effects, all of which are considered to be vasoprotective. This hypothesis has been tested in animal models. Initial studies with the NO-donor pentaerythritol tetranitrate have suggested that this is the case. A subsequent study has investigated whether high ISMN doses given in a commonly used eccentric dosing regimen impacts on the progression of atherosclerosis and endothelial dysfunction. The study was conducted in cholesterol-fed New Zealand White rabbits which received either vehicle, 1 mg ISMN/kg BW or 100 mg ISMN/kg BW in the morning and in the early afternoon. The study design is depicted in the Figure 9.

After 16 weeks of treatment, cross sections of the thoracic aorta were stained with sudan IV and intima-media thickness was measured. The 200 mg ISMN-group (C) had a significantly smaller intima-media thickness than the high fat vehicle group (B) and the 2 mg ISMN group (not shown). These data suggest that treatment with ISMN can reduce the progression of atherosclerosis in an animal model.

The authors also measured the function of the endothelium by applying increasing concentrations of acetylcholine. As expected, the high fat diet elicited a strong endothelial dysfunction. The maximally possible vasodilation was inhibited by >70%. It is obvious that treatment with 200 mg ISMN/kg BW/day resulted in a marked reduction in endothelial dysfunction. Therefore, the reduction of morphologic changes by ISMN is associated with an improvement in endothelial function. These data suggest that treatment of coronary artery disease patients with the organic nitrate can be useful.

The marked effects of ISMN on the development of atherosclerosis and endothelial dysfunction might be partially explained by the complete inhibition of vascular oxidative stress.

Figure 10 shows the cumulative generation of vascular superoxide production was greatly enhanced in atherosclerosis. Treatment with high ISMN doses completely inhibited this part of the pathomechanism of atherosclerosis. Nevertheless, atherosclerosis still occurred in the animals which demonstrates the multifactorial nature of the atherosclerosis pathogenesis and suggests that antioxidative effects are involved in the antiatherosclerotic effects of nitrates. In summary we conclude that high oral dose of ISMN could offer a vasoprotective therapeutic intervention in atherosclerosis.

Mononitrate

IS-2-MN, like IS-5-MN, is a metabolite of ISDN, from which it is formed by a denitrification reaction catalyzed by a glutathione-dependent hepatic nitrate-reductase. IS-2-MN, being a catabolite of ISDN, has the same pharmacodynamic characteristics of this latter drug, such that it has been hypothesized that it could be used as a treatment for angina pectoris, coronary heart
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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, peak concentration is reached after about 25 min and the drug has a half-life of 2.1 hr. 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.

Published pharmacokinetic studies (20, 21) have reported that IS-2-MN has a short elimination half-life ($T_{1/2} = 1.81 \pm 0.09$ h), which is about half that of IS-5-MN ($T_{1/2} = 4.58 \pm 0.76$ h). The clearance of IS-2-MN is higher (21.7 – 23.2 l/h) than that of IS-5-MN (6.5 – 8.5 l/h) whereas the volume of distribution is more or less the same.

IS-2-MN presents a high bioavailability (about 100%) with fast and complete intestinal absorption and does not undergo hepatic first-pass metabolism.

The pharmacokinetic profile of IS-2-MN does not appear to be significantly different in patients with renal insufficiency. For the most part, this product of the biotransformation of ISDN is metabolized in the liver, through redox reactions similar to those used for IS-5-M. The drug is excreted in the urine unmodified and also in the form of various different compounds, all of which are pharmacologically active (23).

Table I summarizes the most important pharmacokinetic and pharmacodynamic differences between ISDN and the two mononitrates.

The induction of nitrate tolerance leads to a loss of activity of all nitroderivates. As can be seen in Figure 11, NTG induces tolerance in strips of rabbit aorta pre-incubated with this compound for 2 hrs.

However the desensitization degree was significantly less with IS-2-MN.

Figure 12 shows the percentage decrease in the inhibitory activity for NTG, ISDN, IS-5-MN and IS-2-MN.

The short plasma half-life of IS-2-MN could be an advantage in preventing the development of tolerance, a phenomenon that occurs frequently with the use of ISDN and IS-5-MN and that is due predominantly to the prolonged occupation of the receptors for the nitrates, with consequent depletion of the reduced sulphhydryl groups necessary for the therapeutic effect.

The different types of formulations of IS-2-MN (Tab. II), compared to those of IS-5-MN and ISDN, are also of substantial interest (L. Dei Cas: data on file):

**FUTURE PROSPECTS FOR IS-2-MN**

The pharmacodynamic and pharmacokinetic characteristics of this mononitrate and, in particular, its very high bioavailability, its lack of first-pass hepatic metabolism and almost equally balanced venous and arterial

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ISDN</th>
<th>IS-2-MN</th>
<th>IS-5-MN</th>
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<tbody>
<tr>
<td>Hepatic metabolism</td>
<td>X</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Short half-life</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Long half-life</td>
<td>–</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Beyond arteriolar vasodilation</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Less induction of tolerance</td>
<td>–</td>
<td>X</td>
<td>–</td>
</tr>
</tbody>
</table>
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vasodilating effects, suggest that it would be better tolerated by patients with liver disease or congestive heart failure with hepatic damage; in addition, in the light of the new evidence on the role of endothelial dysfunction in the progression of heart failure. Furthermore, the short half-life of IS-2-MN with its faster elimination compared to that of IS-5-MN and lesser depletion of sulphydryls lay the basis for less tolerance and maintained activity over time. The wide range of possible pharmaceutical formulations of IS-2-MN makes up for those lacking for IS-5-MN and renders it similar to ISDN but with better pharmacokinetic bioavailability.

Furthermore, the oxidative stress plays a main role in the progression of heart failure, the reduced occupation of the receptors for NO by mononitrates with less potency respect to ISDN or TNG could have important effects on the bioavailability of the sulphydryl groups, particularly glutathione counteract oxidative stress by a scavenger effect on the vascular system.

In conclusion, mononitrates play an important role in the cardiovascular continuum because they are able to interfere with the progression of the disease by the substitution of endogenous NO in the endothelial vessels and seem to inhibit atherosclerosis by high mononitrates doses offering vasoprotective therapeutic intervention in atherosclerosis.

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Fig. 11 - The figure shows the vasoconstriction of a strip of rabbit aorta following the addition of noradrenaline 10⁻⁶ M. The subsequent addition of NTG 10⁻⁷ M produced dilatation, restoring baseline values. Incubation with NTG 10⁻⁷ M for 2 hrs and the addition of NTG 10⁻⁷ M caused an approximately 50% reduction in the vasodilation (L. Dei Cas: data on file).

Fig. 12 - The degree of desensitization with IS-2-MN compared to with NTG, ISDN and IS-5-MN was less and with a significant inhibitory activity expressed as a % (L. Dei Cas: data on file).

TABLE II - DIFFERENT FORMULATIONS OF ISDN, IS-2-MN AND IS-5-MN

<table>
<thead>
<tr>
<th>Type of formulation</th>
<th>ISDN</th>
<th>IS-2-MN</th>
<th>IS-5-MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Delayed release</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sublingual</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Intravenous</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Spray</td>
<td>–</td>
<td>X</td>
<td>–</td>
</tr>
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</table>

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REFERENCES