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Do nitrates differ?

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1 The organic nitrates all share a common biochemical and physiological mechanism of action.
2 The organic nitrates differ substantially in their pharmacologic potency and pharmacokinetics. *In vitro* potency differences appear larger than the corresponding *in vivo* activities.
3 The duration of action of organic nitrates, after a single immediate-release dose, is governed by the pharmacokinetics of the drug. However, the duration of action of available sustained-release preparations, whatever the nitrate or formulation, is limited to about 12 h, due to the development of pharmacologic tolerance.
4 Nitrates do not appear to differ in their production of undesirable effects.

Keywords organic nitrates mechanisms of action potency pharmacokinetics pharmacodynamics

Introduction

The organic nitrates are comprised of a number of compounds all of which contain one or more nitrate functional groups, ONO_2, in their chemical structure. These agents have long been known as active vasodilators and they have been employed therapeutically in the treatment of several cardiovascular diseases. The biochemical basis of pharmacologic action for these compounds is generally believed to involve metabolic activation of the nitrate group to the cellular mediator nitric oxide, which in turn activates the enzyme guanylate cyclase, leading to accumulation of cyclic GMP and subsequent vascular relaxation. Other compounds such as sodium nitroprusside, S-nitrosothiols, hydroxylamine, molsidomine, SIN-1, etc., can also cause vasorelaxation by the same biochemical pathway, but they should not be classified as organic nitrates because they lack the requisite nitrate functional group, and the mechanism of metabolic activation might be different. Another series of agents, the organic nitrites, (e.g. amyl nitrite and isobutyl nitrite), contain the nitrite functional group, ONO, but they are often classified as organic nitrates because of the similarities in pharmacologic effects. All these agents are known collectively as nitrovasodilators (Table 1).

Among the organic nitrates that have been used in cardiovascular therapy, the most important are nitroglycerin (GTN), isosorbide dinitrate (ISDN), and isosorbide-5-mononitrate (5-ISMN). Are there significant differences among these three nitrates? In answering this question, it may be convenient to examine their similarities and differences in terms of the following properties:

- mechanism of action
- pharmacologic potency
- pharmacokinetics
- pharmacodynamics
- undesirable effects

Table 1 Classification of nitrovasodilators

Nitrates	*Non-nitrates*
Erythrityl tetranitrate	Amyl nitrite
Isosorbide dinitrate	Hydroxylamine
Isosorbide-5-mononitrate	Molsidomine
Mannitol hexanitrate	S-nitrosothiols
Nitroglycerin	Sodium nitroprusside
Pentaerythrityl tetranitrate	

Mechanism of action

It is generally agreed that the organic nitrates exert a wide spectrum of physiological action on the cardiovascular system, which in turn lead to their beneficial effects in cardiovascular disease. In simplistic terms, nitrates dilate peripheral and regional arterial and venous smooth muscle, dilate coronary blood vessels,

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and increase coronary flow. These effects lead to a decrease in myocardial oxygen demand and preload, producing symptomatic relief in angina pectoris and congestive heart failure. Nitrates also reduce platelet aggregation in blood, and this action may contribute to their effectiveness in the treatment of unstable angina. All the known nitrates appear to exert the same scope of physiological action. There is no evidence that suggests otherwise, even in terms of the relative degrees of contribution of their multiple effects.

The biochemical mechanism of action is also not shown to be different among nitrates. These compounds are metabolised by the vascular smooth muscle cell, producing nitric oxide. Direct evidence for this reaction has now been obtained, and it appears that the enzyme system involved is bound to the cellular plasma membrane (Chung & Fung, 1990a). The nitric oxide produced may then react with endogenous thiols to form S-nitrosothiols in equilibrium, and both chemical species (nitric oxide and S-nitrosothiols) can activate guanylate cyclase to produce cyclic GMP, and then relaxation. Ignarro *et al.* (1981) have originally proposed that both inorganic nitrite ion and S-nitrosothiols are obligatory intermediates for the biochemical cascade leading to relaxation. However, recent evidence suggested that production of these biochemical intermediates may not be directly relevant in the biochemical mechanism of nitrate action (Craven & DeRubertis, 1983; Kowaluk & Fung, 1990; Romanin & Kukovetz, 1988).

Nitrate tolerance that develops after chronic therapy is also thought to be biochemical in origin. Metabolic activation in the vasculature is reduced, perhaps due to the lack of critical sulph-hydryl cofactors necessary for the production of nitric oxide. The enzyme system that is responsible for this crucial metabolic activation step has not been clearly identified, but it has been generally accepted that it may be one of activities expressed by the widely distributed enzyme system known as glutathione-S-transferases (Habig *et al.*, 1975; Keen *et al.*, 1976). These enzymes are likely to be involved in the hepatic metabolism of organic nitrates after oral administration (Maier *et al.*, 1980), and a recent study suggested that they may also be involved in the expression of the pharmacologic effect of GTN in human aorta and heart (Tsuchida *et al.*, 1990). However, we have recently shown (Chung & Fung, 1990b), by a number of techniques, that microsomal glutathione-S-transferase activity cannot account for the nitric oxide-generating activity in the bovine coronary tissue. Therefore, the nature of the vascular enzyme involved in the metabolic activation of organic nitrates, in our view, still remains undefined.

Pharmacologic potency

Substantial differences in the *in vitro* vasodilator potency exist among the organic nitrates. In general, when the aqueous solubility is not limiting, the higher the number of nitrate groups in the molecule, the more potent the vasodilating activity. This may in part be due to the increasing lipophility of poly-nitrate compounds (Noack, 1984). Interestingly, the number of nitrate groups also appeared to correlate positively with the maximum velocity of organic nitrates as substrates for glutathione-organic nitrate reductase (Needleman & Hunter, 1965) which is primarily responsible for the metabolism of organic nitrates. This finding is therefore consistent with the current mechanistic view that nitrates need to be bioactivated to produce their pharmacologic activity.

Whatever the enzyme system involved, a puzzling aspect of nitrate potency is the apparent lack of correlation between the relative *in vitro* activity and their relative *in vivo* potency. For example, the *in vitro* potency of 5-ISMN in the Langendorff heart preparation was about 100-fold less than that of ISDN (Noack, 1984), and the potency difference was about 60-fold in a perfused dog hindleg preparation (Bogaert & Rosseel, 1972). In contrast, the relative *in vivo* potency ratio of these two nitrates appeared to be considerably smaller. For example, the ability of an 80 mg dose of 5-ISMN to reduce the pulmonary arterial pressure in man was found to be similar to that of a 30 mg dose of ISDN (Bussmann *et al.*, 1980), a potency difference of ISDN to 5-ISMN of only about 3. The reason for this apparent discrepancy between relative *in vivo* and *in vitro* potency is not known, but in the case involving oral ISDN, may be additionally complicated by the fact that the dinitrate is rapidly metabolised to two active mononitrate metabolites, each of which exerts its own activity.

In an animal study (Tzeng & Fung, 1990), we removed these complexities and examined the relative potency, by intravenous injection, of four isomeric mononitrates, all of which do not produce any active metabolites. These mononitrates were L-isoidide mononitrate, isomannide mononitrate, isosorbide-2-mononitrate, and 5-ISMN. We used pulse pressure as the *in vivo* haemodynamic index, and showed that the ED_{50}s (doses to reduce pulse pressure by 50%) were 10.2, 18.1, 43.2 and 48.6 mg kg^{-1}, for L-isoidide mononitrate, isosorbide-2-mononitrate, isomannide mononitrate and 5-ISMN, respectively (only a 5-fold range). In contrast, the relative *in vitro* potency, expressed as the EC_{50} for relaxation of isolated rat aorta, were 16.2, 57.5, 301 and 701 μM respectively (a 43-fold range). Thus, the relative *in vivo* potencies of organic mononitrates are considerably closer to each other than that predicted by *in vitro* results on vasorelaxation.

What is the reason for this apparent lack of correlation between the *in vitro* potency and *in vivo* effects of organic nitrates? The precise explanation has not been identified, but it may be related, at least in part, to the different pharmacokinetic properties of the various compounds. Figure 1 shows a pharmacokinetic/pharmacodynamic scheme that has been proposed for organic nitrates (Fung, 1991). In this scheme, the drug has to partition into the vascular smooth muscle where it is converted to nitric oxide, via an enzymatic reaction that is possibly facilitated by a sulph-hydryl cofactor (X). In the systemic circulation, the organic nitrate is also cleared by metabolic processes to produce active metabolites (as in the case for GTN and ISDN), or inactive metabolites (as in the case for 5-ISMN). We

argued (Tzeng & Fung, 1990) that it is the competitive metabolism of the nitrate in the systemic circulation *vs* that in the vasculature that may partially account for the narrower relative *in vivo* potency differences among the organic nitrates. The more potent nitrates are those that are more susceptible to metabolism. When this takes place in the systemic circulation (which occurs *in vivo* but not *in vitro*), the net effect may then be a reduction in the availability of the organic nitrate for the vasculature, thus decreasing the *in vivo* potency of the more potent nitrates. Preliminary mathematical analysis suggests that this hypothesis is qualitatively correct, but further validation of this concept is needed.

Organic nitrates, therefore, do differ in their *in vivo* potency, but these differences appear much narrower *in vivo* than those observed *in vitro*.

Pharmacokinetics

Major differences exist in the pharmacokinetics of the various organic nitrates. Table 2 shows a summary of the systemic clearance and biological half-life of those organic nitrates that have been examined in man. The lead compound, GTN, possessed the most interesting pharmacokinetics of the group. Its vast clearance value (about 50 l min^{-1}) greatly exceeded that of cardiac output, which implicated extra-hepatic metabolism as an important pathway in its elimination. *In vitro* vascular metabolism of GTN and ISDN has been demonstrated (Fung *et al.*, 1984), and it was subsequently shown that the cardiac output accounts for about 75% systemic arterial clearance of intravenous GTN in rats (Fung *et al.*, 1986). This latter finding confirms that vascular metabolism of GTN might be important *in vivo*. The three factors of high systemic clearance, dependence of pharmacokinetics on haemodynamics, and extensive arterial-venous extraction (Armstrong *et al.*, 1980) provided a feasible explanation as to why venous GTN plasma concentrations after therapeutic doses were notoriously low and highly variable.

Table 2 also shows that the oral bioavailability of the three commonly used organic nitrates are vastly different. Oral GTN is extensively metabolised during absorption and any therapeutic activity that arises from oral nitroglycerin is most likely due to its dinitrate metabolites. Oral ISDN is completely absorbed, but only about 20% of the dose enters into the systemic circulation as intact drug, the rest being converted (at least initially) to the active mononitrate metabolites. 5-ISMN is not subjected to first-pass metabolism, and is therefore 100% orally bioavailable. Over the therapeutic dosing range, none of the three nitrates appeared to exhibit non-linear pharmacokinetics.

The apparent elimination half-life varied considerably among the nitrates, ranging from a few minutes for GTN, to about 4.5 h for 5-ISMN. This pharmacokinetic parameter, however, can be easily altered by prolonging the absorption rate of the drug (e.g. by the use of sustained-release preparations). Thus, in spite of its extremely fast elimination, steady-state concentrations of GTN can be readily maintained by the transdermal formulations. Other sustained-release dosage forms, primarily for the oral route, are also available for ISDN and 5-ISMN to maintain a longer residence time for plasma nitrate concentrations.

Table 2 Pharmacokinetic parameters of GTN, ISDN and 5-ISMN in man after intravenous administration (values are approximate means at usual therapeutic doses, modified from Fung, 1987)

	GTN	*ISDN*	*5-ISMN*
Half-life (min) λ_1	3	10	280
λ_z		65	
Venous plasma clearance (l min^{-1})	50	4	0.1
Apparent volume of distribution (l kg^{-1})	3	4	0.6
Oral bioavailability (%)	$\simeq$ 0	20	100

Pharmacodynamics

In an earlier presentation (Fung, 1991), the influence of nitrate structure and formulation on the various pharmacodynamic aspects of nitrate action were discussed in relation to the scheme presented in Figure 1. In particular, the effects of the various rate processes on the onset of nitrate action, the duration of action and tolerance properties were examined. Here, only a summary of these discussions is provided.

In spite of a large difference among the nitrates in the systemic metabolic rate (and therefore possibly a similar difference in the metabolic activation rate in the vasculature), the onset of action of all the available compounds appears rapid. The duration of action of a single immediate-release dose of organic nitrate, however, appears to be directly related to the half-life of the drug, and the active metabolites it produces (corrected for potency, of course). However, upon steady-state input of the drug (e.g. either by transdermal patches or oral sustained-release preparations), the duration of therapeutic action will be limited by the development of pharmacologic tolerance. Using objective effects (e.g. exercise tolerance, lowering of left ventricular end-diastolic pressure) as therapeutic indices, the maximum

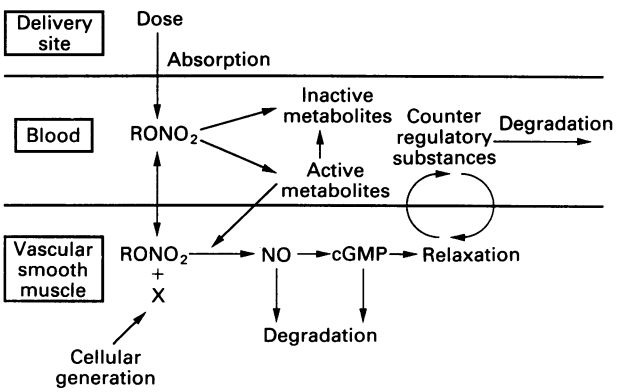

Figure 1 Pharmacokinetic/pharmacodynamic action scheme for organic nitrates (taken from Fung, 1991, with permission).

duration of effectiveness for any available nitrate preparation is presently limited to about 12 h. The exact *in vivo* reasons of tolerance are not known, but may involve vascular and/or systemic mechanisms (Figure 1). At the vascular level, depletion of critical cofactors after sub-chronic or chronic administration may reduce the extent of metabolic activation to nitric oxide. At the systemic level, neurohormonal factors may be produced to counteract the vasodilating effects of nitrates (Figure 1). Whatever the mechanism, it appears that a nitrate-poor or nitrate-free interval, usually of 10–12 h in duration, needs to be instituted to regenerate sensitivity toward the next nitrate dose.

Undesirable effects

The most common side effects of nitrates are headache and hypotension. There is a potential for methaemoglobinaemia, but this side effect is rare after clinical doses. In some patients, abrupt withdrawal of nitrates may bring about rebound responses in several haemodynamic parameters (Olivari *et al.*, 1983). In principle, the rate of drug input into, and disappearance out of, the systemic circulation could affect the intensity of these undesirable effects, but no thorough study has been undertaken to examine this aspect of nitrate therapy. The general qualitative observation is that occurrence of undesirable effects is perhaps more dependent on individual sensitivities and the doses used, rather than the nitrate or formulation chosen.

Conclusion

The question 'Do nitrates differ?' has a mixed answer. The available nitrates in clinical use do differ in their pharmacokinetics and pharmacologic potency, but they do not differ in their biochemical and physiological mechanisms of action or the profile of undesirable effects. The pharmacodynamics of nitrates, including the onset and duration of action, and the development of tolerance, are affected by the pharmacokinetic rate processes of the nitrate and formulation chosen. No available nitrate/formulation combination has yet been found to be able to provide round-the-clock protection in exercise tolerance in angina pectoris, or sustained haemodynamic effects in congestive heart failure.

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