References

- Alvn, G., Helleday, L., Lindholm, A., Sanz, E. & Villn, T. (1990). Diuretic effect and diuretic efficiency after intravenous dosage of frusemide. *Br. J. clin. Pharmac.*, **29**, 215–219.
- Hammarlund, M. M., Odland, B. & Paalzow, L. K. (1985). Acute tolerance to furosemide diuresis in humans. Pharmacokinetic-pharmacodynamic modelling. *J. Pharmac. exp. Ther.*, **233**, 447–453.
- Hammarlund-Udenaes, M. & Benet, L. Z. (1989). Furosemide pharmacokinetics and pharmacodynamics in health and disease — an update. *J. Pharmacokin. Biopharm.*, **17**, 1–46.
- Kaojarern, S., Day, B. & Brater, D. C. (1982). The time course of delivery of furosemide into urine: an independent determinant of overall response. *Kidney Int.*, **22**, 69–74.
- Noormohamed, F. H. (1990). Use of $E_{\max}$ model in diuretic studies. *Br. J. clin. Pharmac.*, **30**, 907–908.
- Sjstrm, P. A., Odland, B. G., Beermann, B. A. & Hammarlund-Udenaes, M. (1988). On the mechanism of acute tolerance to furosemide diuresis. *Scand. J. Urol. Nephrol.*, **22**, 133–140.
- Wagner, J. G. (1968). Kinetics of pharmacologic response. I. Proposed relationships between response and drug concentration in the intact animal and man. *J. theoret. Biol.*, **20**, 173–201.

Pharmacological activity of the dinitrate metabolites of nitroglycerin following their oral administration to healthy volunteers

The clinical efficacy of oral sustained release nitroglycerin (GTN) in the prophylactic management of stable angina is recognised (Davidov & Mroczek, 1977; Winsor & Berger, 1975), although there is little correlation between systemic GTN concentration and effect. Kohli *et al.* (1985) reported that in patients with stable angina, an oral dose of 6.5 mg sustained release GTN results in improved treadmill exercise times, despite no measurable GTN in plasma. The dinitrate metabolites of GTN i.e., 1,2 glyceryl dinitrate (1,2-GDN) and 1,3 glyceryl dinitrate (1,3-GDN) may play a significant role in the anti-anginal efficacy of GTN. This communication describes a preliminary study to determine if the GDNs possess pharmacological activity when administered to man.

The 1,2- and 1,3-GDN manufacturing, formulation and clinical study procedures were carried out under Investigational New Drug application 32,278. The clinical study was approved by the University of California, San Francisco Committee on Human Research and performed with volunteer 'informed consent'. The 1,2- and 1,3-GDNs were purified and characterised in our laboratory (Gumbleton *et al.*, 1989). Six healthy male volunteers between the ages 21 and 35 years, were recruited into the study. Following an overnight fast, three subjects received an oral dose of 4.2 mg 1,2-GDN, while a further three subjects received an oral dose of 2.4 mg 1,3-GDN. These first doses of GDNs administered to man were chosen to yield the approximate plasma dinitrate concentrations measured following oral 6.5 mg GTN solution doses (Nakashima *et al.*, 1990). The GDNs were administered as solutions in 50 ml of purified water. Serial blood samples were collected into chilled heparinized polyethylene syringes for 6 h after the dose. Processing of the blood samples, and the analysis

of 1,2- and 1,3-GDN in plasma were described previously (Nakashima *et al.*, 1990). Cardiovascular monitoring (Dinamap®; Critikon Inc, Tampa, FL) was performed for 20 min immediately prior to dosing, to record stable basal values, and throughout the 6 h study period. The subjects remained in a supine/semi-recumbent position throughout the study, receiving 100 ml of water to drink every hour, and a low fat lunch at 4 h post-dose.

Figure 1 shows the mean log plasma concentration-time curves together with mean systolic and diastolic blood pressure-time profiles for 1,2-GDN and 1,3-GDN. Both GDNs resulted in time-dependent decreases in systolic and diastolic blood pressures. Maximal decreases in systolic blood pressure ranged from 6–16 mm Hg for 1,2-GDN, and 14–17 mm Hg for 1,3-GDN; maximal decreases in diastolic pressure ranged from 11–27 mm Hg for 1,2-GDN, and 20–26 mm Hg for 1,3-GDN. The observed peak plasma 1,2-GDN concentrations ranged from 15.7 to 47.1 ng ml⁻¹, with the time to peak concentration ranging from 20 to 30 min. Peak concentrations following 1,3-GDN, ranged from 10.4 to 13.9 ng ml⁻¹, with a time to peak of 25 min. These preliminary results show an apparent correlation between the plasma 1,2- and 1,3-GDN concentration-time profiles and the blood pressure-time profiles, with effects upon diastolic blood pressure reflecting marked decreases in peripheral vascular resistance. No interconversion between the 1,2- and 1,3-GDN isomers was observed.

Although a decrease in blood pressure, measured in healthy volunteers, does not necessarily predict anti-anginal efficacy in patients, these preliminary results suggest that the GDNs may contribute significantly to the therapeutic effects of GTN. Furthermore, although the data reported here are limited, it is intriguing to note

Figure 1 Mean plasma concentration (○) and blood pressure (●) vs time plots, (a) and (b) following a 4.2 mg oral 1,2-GDN dose in three healthy volunteers (c) and (d) following a 2.4 mg oral 1,3-GDN dose in a second set of three healthy volunteers; (a) and (c) systolic blood pressure measurements; (b) and (d) diastolic blood pressure measurements.

that the lower dose of 1,3-GDN appears to elicit an equal or an even greater decrease in blood pressure than the almost two-fold larger 1,2-GDN dose. These preliminary results should be considered in relation to previous studies with oral GTN dosing where GTN was shown to undergo almost complete first pass metabolism, resulting in considerable systemic concentrations of 1,2- and 1,3-GDN. Using a specific and sensitive assay, the finding by Noonan & Benet (1986) that high dose GTN, administered as an oral solution is less than 1% bio-available, negates the postulate that oral sustained release GTN can have any significant systemic availability as a result of saturable first-pass metabolism. Noonan & Benet (1986), Yu *et al.* (1988) and Nakashima *et al.* (1990), have all reported that substantial plasma concentrations of the dinitrate metabolites are achieved following oral GTN administration, with peak plasma concentrations of 1,2-GDN and 1,3-GDN that are at least 100 and 50 fold greater, respectively, than those of GTN (less than 0.3 ng ml^{-1}). Similarly, following transdermal GTN, significant amounts of the less rapidly eliminated GDNs will accumulate, resulting in combined (1,2- and 1,3-)

GDN steady-state plasma concentrations that can be 6–7 fold greater than the steady-state level of GTN (Noonan & Benet, 1987). The results of this study provide preliminary evidence that the 1,2- and 1,3-GDNs possess pharmacological activity in man, and provide corroborative evidence for the hypothesis that a significant proportion of the anti-anginal efficacy of GTN may be attributed to its dinitrate metabolites.

The authors would like to acknowledge ICI Americas Inc, Specialist Chemicals, Wilmington, DE for the supply of 'Dilute Nitrate Esters'. This work was supported by NIH Grant HL32243.

M. GUMBLETON & L. Z. BENET
Department of Pharmacy, University of California,
San Francisco, San Francisco, CA 94143-0446, USA

Received 9 July, 1990
accepted 14 September 1990

ADONIS 030652519100047W

References

- Davidov, M. E. & Mroczek, W. J. (1977). Effect of sustained release nitroglycerin capsules on anginal frequency and exercise capacity: A double-blind evaluation. *Angiology*, **28**, 181–189.
- Gumbleton, M., Cashman, J. R. & Benet, L. Z. (1989). Purification and characterization of the 1,2 and 1,3 glyceryl dinitrate esters. *Pharm. Res.*, **6** (suppl), S23.
- Kohli, R. S., Khurmi, N. S., Kardash, M. M., Hughes, L. O., Lahiri, A. & Raftery, E. B. (1985). Lack of correlation of blood nitrate levels with dose and exercise time in angina pectoris. In *Abstracts of the Cardiovascular Pharmacotherapy International Symposium*, Geneva (1985). Abstract 567.
- Nakashima, E., Rigod, J. R., Lin, E. T. & Benet, L. Z. (1990). Pharmacokinetics of nitroglycerin and its dinitrate metabolites over a thirtyfold range of oral doses. *Clin. Pharmac. Ther.*, **47**, 592–598.
- Noonan, P. K. & Benet, L. Z. (1986). The bioavailability of oral nitroglycerin. *J. pharm. Sci.*, **75**, 241–243.
- Noonan, P. K. & Benet, L. Z. (1987). Variable glyceryl dinitrate formation as a function of route of nitroglycerin administration. *Clin. Pharmac. Ther.*, **42**, 273–277.
- Winsor, T. & Berger, H. J. (1975). Oral nitroglycerin as a prophylactic antianginal drug: Clinical, physiologic, and statistical evidence of efficacy based on a three-phase experimental design. *Am. Heart J.*, **90**, 611–626.
- Yu, D. K., Williams, R. L., Benet, L. Z., Lin, E. T. & Giesing, D. H. (1988). Pharmacokinetics of nitroglycerin and metabolites in humans following oral dosing. *Biopharm. Drug Dispos.*, **9**, 557–565.

'First dose' hypotension and venodilatation

Profound falls in blood pressure may occur in some patients when commencing captopril or enalapril (Cleland *et al.*, 1985; Hodsman *et al.*, 1983). Although these 'first dose' hypotensive reactions may be asymptomatic, in some patients myocardial ischaemia, transient hemiparesis, dysphasia or coma have developed (Cleland *et al.*, 1985; Hodsman *et al.*, 1983; Packer *et al.*, 1986; Todd & Heel, 1986; Webster, 1987); deaths have also occurred. Similar 'first dose' reactions have been described with phentolamine (Galloway *et al.*, 1975), prazosin (Graham *et al.*, 1976), ketanserin (Waller *et al.*, 1987), labetalol (Maronde, 1983), renin inhibitors (Semple *et al.*, 1988; Zusman *et al.*, 1983), β -adrenoceptor blockers and adrenergic neurone blockers (Kholeif & Isles, 1989; Webster, 1987). Although the underlying mechanism was originally assumed to be arterial vasodilatation, often enhanced by bradycardia (Cleland *et al.*, 1983; Semple *et al.*, 1988; Webster, 1987), recent evidence suggests that venous dilatation may also be important. Furthermore, classical 'first dose' hypotensive reactions do not occur with 'pure' arterial vasodilators such as nifedipine, felodipine or hydralazine, in spite of occasionally marked reductions in systemic vascular resistance and blood pressure (Elmfeldt *et al.*, 1987; Sorkin *et al.*, 1985).

Although Webster (1987) suggested that most drugs implicated in 'first dose' hypotensive reactions possess α -adrenoceptor blocking activity, this does not explain the hypotension following β -adrenoceptor blockers, renin inhibitors or angiotensin converting enzyme (ACE) inhibitors. However, all these agents inhibit the sympathetic nervous system, either directly or indirectly through reduction of angiotensin II levels (Webb & Cockcroft, 1989). Venous tone is principally controlled by the sympathetic nervous system (Rothe, 1983).

In susceptible patients, venodilatation may produce marked venous pooling with a consequent fall in cardiac

output and profound hypotension. Approximately 80% of the circulating blood volume is held within the venous system (Rothe, 1983) and changes in venous resistance affect venous return approximately eight times as much as similar resistance changes in the arterial circulation (Guyton, 1959). More than 30 years ago Guyton and colleagues demonstrated that small changes in venous tone may therefore have profound effects on venous return: cardiac output can fall by 40% following venodilatation due to sympathetic inhibition (Guyton, 1959; Guyton *et al.*, 1963). Even greater falls in cardiac output and blood pressure might be expected if the normal homeostatic reflex increases in peripheral vascular resistance and heart rate were also impaired or blocked. A failure of reflex tachycardia, even during profound hypotension, has been described when commencing ACE inhibitors in heart failure (Bayliss *et al.*, 1985; Chatterjee *et al.*, 1985; Cleland *et al.*, 1985) and occasionally in hypertension (Todd & Heel, 1986; Webster, 1987). Both decreased sympathetic activity and enhanced parasympathetic activity have been implicated (Ajayi *et al.*, 1985; Campbell *et al.*, 1985; Packer, 1985). The ensuing hypotension and bradycardia may then be enhanced by activation of the Bezold-Jarisch reflex (Semple *et al.*, 1988).

Although venodilatation has long been suspected in 'first dose' reactions (Cleland *et al.*, 1985; Hodsman *et al.*, 1983; Webster, 1987), direct evidence remains scant. Captopril (and by implication enalapril) reduces venous tone by up to 50% in patients with heart failure (Awan & Mason, 1982; Cowley *et al.*, 1982; Faxon *et al.*, 1981). Recently, using non-invasive radionuclide techniques (Clements *et al.*, 1981; Wathen *et al.*, 1983) captopril was shown to produce a 16% increase in peripheral venous volume, far more than the 11% increase following glyceryl trinitrate. Furthermore the fall in mean arterial blood pressure when commencing captopril correlated