

## Research Article

# Cardiovascular Effects of Nicorandil and Nitroprusside in Furosemide Plus Digoxin Pretreated Dogs

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

**ABSTRACT** In support of human congestive heart failure (CHF) trials, the cardiovascular effects of the vasodilators nicorandil (NIC) and nitroprusside (NP) were examined in anesthetized and conscious dogs pretreated with the diuretic furosemide (FURO) and the cardiac glycoside digoxin (DIG). In anesthetized control dogs, iv NP (2–19  $\mu\text{g/kg/min}$ ) and NIC (24–105  $\mu\text{g/kg/min}$ ) maximally reduced mean arterial pressure (MAP) by 43 and 40 mmHg, respectively, with moderate increases in heart rate (HR). These hypotensive responses to NP and NIC were unmodified by iv FURO (2.65 mg/kg) + DIG (0.075 mg/kg) pretreatment (PT). FURO + DIG reduced central venous pressure (CVP) by 3 mmHg, masking the separate effects of NP and NIC. In a third group, FURO's fluid volume depletion and DIG's plasma concentrations were unaffected by adjunctive NIC infused for 2.5 h at a mean 17  $\mu\text{g/kg/min}$  iv. No untoward interactions were seen with any combination. In conscious dogs, the hypotension and tachycardia seen with iv NP (2–20  $\mu\text{g/kg/min}$ ) and NIC (20–160  $\mu\text{g/kg/min}$ ) were also unchanged after 5 days of oral FURO (5 mg/kg/day) and DIG (0.0125 mg/kg/day), with no intolerance. Repeated oral NIC (7.5 mg/kg/day  $\times$  3 days) in these chronic FURO + DIG dogs was consistently hypotensive but steadily more tachycardiac. This study offers a prototype of 3-way CHF drug interaction, demonstrates that NIC and NP can be safely combined with acute and chronic FURO and DIG, and shows that these CHF agents minimally affect the cardiovascular responses to NIC and NP in dogs. © 1994 Wiley-Liss, Inc.

**Key Words:** blood pressure, heart rate, interactions

## INTRODUCTION

Drug therapy for human CHF typically combines a diuretic to reduce circulating fluid volume, a cardiac glycoside to improve myocardial contractility, and a vasodilator to reduce cardiac preload and impedance. Inherent with such 3-way drug combinations is the risk for deleterious cardiovascular drug interactions, such as excessive hypotension resulting from too vigorous volume depletion and/or vasodilation, or ventricular arrhythmias associated with higher plasma levels of cardiac glycosides and diuretic-induced hypokalemia. The introduction of a new therapeutic agent for CHF must therefore be

closely evaluated for safety and efficacy in combination with standard CHF agents.

Nicorandil (NIC; N-[2-hydroxyethyl]nicotinamide nitrate) is a direct peripheral vasodilator [Sakai et al., 1981; Inoue et al., 1984] which has undergone extensive clinical development in Europe and the

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USA for its potential utility in angina and CHF. In vitro studies have firmly established that NIC exerts a dual vasodilation [Meisheri et al., 1991], stimulating cyclic guanosine monophosphate (cGMP) [Holzmann, 1983] and opening adenosine triphosphate (ATP)-sensitive  $K^+$  channels [Furukawa et al., 1981] in vascular smooth muscle. Human and dog studies have further shown that NIC exerts a mixed arterial and venous dilatation [Thormann et al., 1983], with some selectivity for the coronary vasculature [Preuss et al., 1985].

Early clinical trials have confirmed that NIC has acute benefits in angina [Doring, 1992] and CHF [Tice et al., 1990]. Prior to launching chronic CHF trials, however, it was necessary to characterize NIC's in vivo cardiovascular interactions with those agents with which it would most frequently be combined clinically, namely, the diuretic furosemide (FURO) and the cardiac glycoside digoxin (DIG), to determine if these agents would affect NIC's vasodilation, or if NIC would alter DIG's somewhat narrow margin of safety [Doherty, 1981]. Acute and chronic drug interaction studies were therefore conducted in anesthetized and conscious dogs evaluating NIC's cardiovascular effects following FURO fluid volume depletion and DIG loading. For comparison purposes, these interaction studies also included hypotensive infusions of the cGMP-dependent vasodilator sodium nitroprusside (NP) [Karaki et al., 1986], which historically has been safely combined acutely with FURO and DIG in CHF. These comparative drug interaction studies thus examined whether NIC's cardiovascular effects are altered by FURO and DIG, or whether their 3-way combination results in any deleterious drug interactions which might preclude their safe concomitant use in CHF.

## MATERIALS AND METHODS

### Anesthetized Dog Studies

#### General preparations

Fourteen fasted mongrel dogs of either sex averaging 12.2 kg were anesthetized with  $Na^+$  pentobarbital (30 mg/kg iv; Butler, Columbus, OH). A cuffed endotracheal tube was inserted to facilitate spontaneous respiration, and a bladder catheter was implanted to collect urine. Body temperatures were maintained at 38°C with a heating pad, as monitored with a rectal thermometer. PE-60 (Clay-Adams, Parsippany, NJ) catheters were inserted in a cephalic and femoral vein to administer drug or vehicle by iv injection and infusion (Holter 911 peristaltic pumps), respectively. A PE-190 catheter was threaded via a

jugular vein into the right atrium to record CVP with a Statham P23 transducer and Grass 7D polygraph. MAP was likewise recorded from a PE-60 femoral artery catheter, while HR was recorded with a Grass 7P4 tachograph triggered by the lead II electrocardiogram (ECG). After 1 h of post-surgical equilibration, timed urine volumes (UVs) were collected at 30 to 90-min intervals, and aliquots were retained for urinary  $Na^+$  analysis. Venous blood samples were taken at selected time points for plasma  $Na^+$ ,  $K^+$ , and DIG analyses, and the sample volumes were replaced with isotonic saline. Each dog's net fluid balance was calculated by subtracting total UV excretion from their iv fluid load.

### Protocol and drug treatments

Pilot experiments in 4 FURO-diuresed dogs established the iv DIG dosage, rate of administration, and post-infusion equilibration necessary to consistently achieve signs of DIG intolerance, i.e., ectopic ventricular beats (EVBs) and gastric spasms. Having established these guidelines, a 6.5 h protocol was implemented, the first 5 h of which consisted of sequential pretreatment (PT; 0.5 h), FURO administration (2 h), DIG loading (1.5 h), and post-DIG equilibration (EQUIL; 1 h) intervals, as outlined in Table 1. This protocol was intended to volume deplete the dogs and expose them to therapeutic plasma DIG concentrations ( $p[DIG]_s$ ) prior to iv infusions of NP and NIC, and employed a vehicle (Group A) and 2 FURO + DIG pretreated groups (Groups B and C). After PT, 3 Group A control dogs received an initial 0.5 ml/kg bolus injection of 2%  $NaHCO_3$  (J.T. Baker Inc., Phillipsburg, NJ), followed by 0.15 ml/min of sterile isotonic saline (Kendall McGaw, Irvine, CA) infused for 3.5 h. In parallel fashion, 4 Group B dogs were given a 1.5 mg/kg injection of FURO (Sigma Chemical Co., St. Louis, MO), followed by a 0.58 mg/kg/h  $\times$  2 h FURO infusion (in 0.15 ml/min saline), for a total dose of 2.66 mg/kg. This was followed by 0.05 mg/kg/h of DIG (Sigma), infused for 1.5 h in 0.15 ml/min d-glucose (Baker), thereby totalling 0.075 mg/kg. These same FURO and DIG doses were administered to 3 Group C dogs in combination with a 2.5 h iv infusion of NIC, given between 1.5 to 4 h and individually titrated to produce threshold arterial hypotension. This NIC pre-infusion averaged 17  $\mu$ g/kg/min (total 2.55 mg/kg) in  $\leq$ 0.15 ml/min of saline, and was intended to determine whether low-level NIC vasodilatation would modify FURO's diuresis or the targeted  $p[DIG]_s$ .

After the preloading and EQUIL periods, all 10 dogs were given a 30-min step-dose infusion of NP (Mallinckrodt), followed by 30 min of post-NP recov-

**TABLE 1. Baseline Cardiovascular Hemodynamics and Urinary Na<sup>+</sup> Excretion in Pentobarbital Anesthetized Dogs, and the Effects of Furosemide and Digoxin Pretreatment Prior to Hypotensive Infusions of Nitroprusside and Nicorandil<sup>†</sup>**

Experimental interval and iv drug treatment	Time (h)	Treatment group and parameter											
		A. Vehicle control (n = 3)				B. FURO + DIG (n = 4)				C. FURO + DIG plus NIC preinfusion (n = 3)			
		MAP	HR	CVP	UVNa <sup>+</sup>	MAP	HR	CVP	UVNa <sup>+</sup>	MAP	HR	CVP	UVNa <sup>+</sup>
Pretreatment (PT)	0.0–0.5	120 ± 3	144 ± 23	-0.3 ± 0.5	2.1 ± 0.9	123 ± 7	115 ± 9	-0.4 ± 0.4	3.1 ± 2.6	114 ± 9	109 ± 7	-0.5 ± 0.3	1.2 ± 0.6
Furosemide (FURO)	0.5–2.5	+9 ± 8	+12 ± 16	-1.1 ± 0.4	1.7 ± 0.9	+1 ± 7	-4 ± 14	-1.4 ± 0.4*	33.4 ± 3.8*,**	-9 ± 8	+47 ± 16*,**	-2.4 ± 0.4*	35.3 ± 4.0*,**
Digoxin (DIG)	2.5–4.0	+7 ± 9	+11 ± 18	-0.7 ± 0.5	2.1 ± 0.4	+2 ± 8	-10 ± 15	-1.0 ± 0.5	12.0 ± 0.3*,**	-14 ± 9	+44 ± 18*,**	-2.0 ± 0.5*	6.2 ± 1.4*,**
Equilibration (EQUIL)	4.0–5.0	+3 ± 8	-4 ± 16	+0.4 ± 0.3	3.2 ± 1.7	-2 ± 7	-5 ± 13	-1.7 ± 0.3*,**	9.0 ± 4.6	-11 ± 8	+40 ± 16*	-2.3 ± 0.3*,**	2.9 ± 0.4

<sup>†</sup>Mean arterial pressure (MAP) and central venous pressure (CVP) units = mmHg; heart rate (HR) units = beats/min. Hemodynamic values are the mean ± s.e.m. absolute readings at PT and their respective changes at the conclusion of the FURO, DIG, and EQUIL intervals. Urinary Na<sup>+</sup> excretion (UVNa<sup>+</sup>) units =  $\mu\text{Eq Na}^+/\text{min/kg}$  (mean ± s.e.m. absolute rate of excretion for all time intervals). Total FURO dose = 2.66 mg/kg; total DIG dose = 0.075 mg/kg. NIC preinfusion given to Group C only between 1.5 and 4.0 h = mean 17  $\mu\text{g/kg/min}$  (total dose of 2.55 mg/kg).

\* $P \leq 0.05$  from PT (paired t-test).

\*\* $P \leq 0.05$  from Group A (ANOVA).

\*\*\* $P \leq 0.05$  from Group B (ANOVA).

ery, and a final 30-min step-dose infusion of NIC. This drug infusion sequence was utilized since NP is much shorter acting than NIC, and MAP and HR rapidly normalized after stopping the NP infusion. The NP and NIC step dose infusions were given in  $\leq 0.15$  ml/min of isotonic saline at 3 stepped doses individually titrated to progressively achieve minimal, moderate, and marked reductions in MAP (10 min/level). These graded infusions simulated the pilot human trials in which NIC was gradually titrated to avoid abrupt hypotensive episodes. For all dogs, the average NP step-dose range was 3–19  $\mu\text{g/kg/min}$  (total 0.26 mg/kg), and the average NIC step-dose range was 17–82  $\mu\text{g/kg/min}$  (total 1.3 mg/kg).

### Conscious Dog Studies

#### General preparations

Four beagle dogs of either sex averaging 11.5 kg were anesthetized with Na<sup>+</sup> pentobarbital and chronically cannulated with a sterile PE-60 abdominal aortic catheter as previously described [Humphrey and Zins, 1984]. To facilitate drug infusion, the dogs were also cannulated with a Silastic® (0.040" ID × 0.085" OD; Dow Corning, Midland, MI) venous catheter advanced 12 cm down a jugular vein. Both cannulas were anchored at the shoulder, exteriorized, and filled with 1,000 U/ml Na<sup>+</sup> heparin (Upjohn, Kalamazoo, MI). Subcutaneous (Combiotic®, Pfizer, Parsippany, NJ; 1 ml/day) and topical (Mycitracin®, Upjohn) antibiotics were given for 3 days postoperatively to prevent infection, during which time the dogs were

conditioned to sling restraint and continuous MAP and HR recording. For all tests, the dogs were fasted overnight and equilibrated for at least 1 h prior to drug treatment.

Body weights (BW) were recorded daily and the dogs were maintained on 400 g/day of Purina Canine Chow and tap water, which provided an estimated daily intake of 69 mEq of Na<sup>+</sup> and 71 mEq of K<sup>+</sup>. Historic data from a similar group of 6 untreated beagle dogs maintained in this manner revealed little change in basal MAP, HR, or plasma electrolytes over 2 weeks. For this study, 3 ml venous blood samples were also taken daily for p[DIG] analysis and were replaced with sterile saline. IV drug infusions were given into the jugular catheters, while oral doses were given in gelatin capsules.

#### Protocol and drug treatment

An 8-day protocol examined the impact of chronic FURO and DIG on the cardiovascular effects of NP and NIC. On day 1, the dogs were first given a 30 min PT step-dose NP infusion at 2, 6, and 20  $\mu\text{g/kg/min}$  iv, delivered in 0.3 ml/min of saline (10 min/level; total 0.28 mg/kg). After 1 h of post-NP recovery, and with MAP and HR back to PT levels, the dogs were given a 35-min step-dose NIC infusion consisting of 20, 40, and 80  $\mu\text{g/kg/min}$  (10 min/level), and 160  $\mu\text{g/kg/min}$  (5 min), each infused in 0.3 ml/min of saline (total 2.2 mg/kg). After 1 h of post-NIC recovery, the first oral doses of FURO (5 mg/kg) and DIG (0.0125 mg/kg) were given, which were repeated once daily on days 2 through 8 following baseline cardio-

vascular recording. The FURO dosage was based on historic data showing it induces a 250 ml diuresis in fasted beagle dogs over 5 h, while the DIG dosage was based on the normal human loading dose. On day 5, the NP and NIC infusions were repeated 2 h after the oral FURO and DIG doses. Finally, on days 6 through 8, 7.5 mg/kg of NIC was given orally 2 h after the FURO and DIG doses, and MAP and HR were monitored for 3 h for comparison to historic data with these doses of NIC in conscious non-pretreated beagle dogs.

### Drug Formulations

DIG was prepared as a stock 0.5 mg/ml solution in a warm 20% ethanol/80% propylene glycol vehicle, and was diluted as needed with 5% d-glucose. NP and NIC were dissolved in sterile isotonic saline. FURO was dissolved in warm 2%  $\text{NaHCO}_3$  and was diluted as needed in saline.

### Plasma and Data Analyses

MAP and CVP (mmHg), HR (beat/min), and the lead II ECG were read manually. ECG endpoints included the PR and QTc ( $\text{QT} \div \sqrt{\text{R-R}}$ ) interval lengths (in sec), and ST segment height, expressed as mm deflection from the isoelectric baseline 40 msec after the J point. Urinary and plasma  $\text{Na}^+$  and  $\text{K}^+$  concentrations ( $\text{p}[\text{Na}^+]$ s and  $\text{p}[\text{K}^+]$ s) were measured with a NOVA-13 ion selective electrode analyzer.  $\text{P}[\text{DIG}]$ s were measured with a Nuclear Medical  $^{125}\text{I}$  radioimmuno assay kit. For the anesthetized dogs, the data was analyzed for significant differences from PT and from the vehicle control group using a paired t-test and an analysis of variance (ANOVA), respectively. For the conscious dogs, the data was analyzed for differences from PT using a paired t-test, and for differences from historic oral NIC data using an ANOVA. In all cases, the data was expressed as the mean  $\pm$  s.e.m., and a  $P$  value of  $\leq 0.05$  was regarded as statistically significant.

## RESULTS

### Anesthetized Dog Studies

#### Preparatory FURO and DIG loading

Table 1 summarizes the cardiovascular parameters and urinary  $\text{Na}^+$  excretion rates seen in the anesthetized dogs during the FURO + DIG loading and post-loading (EQUIL) intervals. The MAP, HR, and CVP values represent baseline readings at the end of PT, and their respective changes seen at the end of the listed experimental intervals. During this preparatory phase, the Group A controls experienced small increases in MAP, variable changes in HR and CVP,

and modest net fluid retention (mean  $+ 4 \pm 1$  ml/kg). In Group B, 2.66 mg/kg FURO and 0.075 mg/kg DIG resulted in stable MAPs and small declines in HR and CVP. In Group C, these same FURO and DIG doses, coupled with a threshold hypotensive pre-infusion of NIC (mean  $17 \mu\text{g/kg/min}$  for 2.5 h), resulted in small  $-9$  to  $-14$  mmHg reductions in MAP, which were accompanied by significant increases in HR and slightly greater reductions in CVP.

Table 1 also demonstrates the high natriuretic efficacy of 2.66 mg/kg FURO in these anesthetized dogs. In Groups B and C, urinary  $\text{Na}^+$  excretion during the 2 h FURO interval was about 20 times control. While not different during the FURO loading period, natriuresis was better sustained in Group B than in Group C during the 1.5 h DIG loading (0.075 mg/kg) and 1 h EQUIL periods, suggesting some attenuation of FURO's sustained effects with the hypotensive NIC pre-infusion. However, calculated over the 0.5 to 4.0 h preparatory sequence, Groups B and C showed significant fluid depletion relative to Group A, namely  $-38 \pm 2$  and  $-29 \pm 4$  ml/kg. Not tabulated for the subsequent vasodilator infusion sequences, urinary  $\text{Na}^+$  excretion declined to and remained stable at PT rates for all 3 groups during iv step-doses of NP (0.7 to  $1.7 \mu\text{Eq Na}^+/\text{min/kg}$ ), post-NP recovery (2.0 to  $3.9 \mu\text{Eq Na}^+/\text{min/kg}$ ), and iv step-doses of NIC (1.2 to  $2.3 \mu\text{Eq Na}^+/\text{min/kg}$ ). Consistent with these data, the fluid depletion originally achieved with FURO was fully maintained after these step-dose vasodilator infusions, with terminal fluid balances of  $-37 \pm 2$  and  $-27 \pm 5$  ml/kg for Groups B and C, respectively, as compared to  $+7 \pm 2$  ml/kg for Group A (both at  $P < 0.05$ ).

Table 2 summarizes the basal  $\text{p}[\text{Na}^+]$ s,  $\text{p}[\text{K}^+]$ s, and hematocrits for these dogs, and their respective changes at the end of the FURO, DIG, EQUIL, and sequential NP and NIC infusion periods. Starting  $\text{p}[\text{Na}^+]$ s (0 time) were similar in the 3 groups and remained stable over the entire 6.5 h test.  $\text{P}[\text{K}^+]$ s were somewhat variable in Group B, initially falling with acute FURO diuresis, and then rebounding with DIG loading. FURO's marked natriuresis in Group B also ultimately resulted in a significant  $+13\%$  increase in hematocrit. None of these parameters was affected by the 2.5 h NIC preinfusion superimposed in Group C, nor were any consistent changes noted subsequent to the NP and NIC step-dose infusions.

Not shown tabularly,  $\text{p}[\text{DIG}]$ s in Groups B and C were  $5.0 \pm 0.7$  and  $4.9 \pm 0.7$  ng/ml, respectively, at the end of the DIG infusion period, from which point they declined in parallel to  $1.4 \pm 0.2$  and  $1.2 \pm 0.3$  ng/ml, respectively, after the EQUIL interval, and to  $0.8 \pm 0.0$  and  $0.7 \pm 0.1$  ng/ml, respectively,

**TABLE 2. Plasma Na<sup>+</sup> and K<sup>+</sup> Concentrations and Venous Hematocrits in Pentobarbital Anesthetized Dogs Pretreated With Furosemide and Digoxin Prior to Hypotensive IV Infusions of Nitroprusside and Nicorandil\***

Experimental interval and iv drug treatment	Time (h)	Treatment group and parameter								
		A. Vehicle control (n = 3)			B. FURO + DIG (n = 4)			C. FURO + DIG plus NIC preinfusion (n = 3)		
		p[Na <sup>+</sup> ]	p[K <sup>+</sup> ]	Hematocrit	p[Na <sup>+</sup> ]	p[K <sup>+</sup> ]	Hematocrit	p[Na <sup>+</sup> ]	p[K <sup>+</sup> ]	Hematocrit
Pretreatment (PT)	0	152 ± 2	3.7 ± 0.0	49 ± 1	152 ± 3	3.6 ± 0.1	45 ± 1	148 ± 3	3.7 ± 0.1	41 ± 1**
Furosemide (FURO)	2.5	-1 ± 2	-0.1 ± 0.2	+3 ± 1*	+2 ± 2	-0.5 ± 0.1	+8 ± 1*,**	+5 ± 3	-0.8 ± 2*,**	+8 ± 1*,**
Digoxin (DIG)	4.0	-2 ± 2	-0.1 ± 0.2	+2 ± 1	+2 ± 2	+0.3 ± 0.2	+10 ± 1*,**	0 ± 2	-0.2 ± 0.2	+8 ± 1*,**
Equilibration (EQUIL)	5.0	-1 ± 2	0.0 ± 0.4	+3 ± 1	+1 ± 2	+0.8 ± 0.3	+11 ± 1*,**	+2 ± 2	+0.1 ± 0.4	+10 ± 1*,**
NP and NIC infusions	6.5	-1 ± 3	+0.1 ± 0.2	-3 ± 1	+1 ± 2	+0.4 ± 0.2	+13 ± 1*,**	+1 ± 3	-0.4 ± 0.1	+10 ± 1*,**

\*Plasma Na<sup>+</sup> and K<sup>+</sup> concentrations (p[Na<sup>+</sup>] and p[K<sup>+</sup>]) units = mEq/L; hematocrit = %. PT data are mean ± s.e.m. absolute values; all other values are changes from PT seen at the end of the listed treatment intervals. Dosages as described in the text and Table 1.

\*P ≤ 0.05 from PT (paired t-test).

\*\*P ≤ 0.05 from Group A (ANOVA).

after the NP and NIC step-dose infusions. Both sets of these p[*DIG*]s are regarded as pharmacologically active [Barr et al., 1972; Smith and Haber, 1974; Lewis, 1986]. Consistent with this view, this *DIG* loading sequence resulted in EVBs and gastric spasms in 2 of 4 Group B dogs and 1 of 3 Group C dogs. The incidence of these reactions did not increase during the step-dose vasodilator infusions (1 Group B dog with NP). These data demonstrate that a sustained threshold hypotensive infusion of NIC does not alter iv *DIG*'s initial peak plasma concentration, decay kinetics, or side effect frequency.

### Step-dose NP and NIC infusions

Having achieved significant FURO-mediated fluid volume depletion and pharmacologic p[*DIG*]s in Groups B and C, all dogs were subjected to individually titrated step-dose infusions of NP and NIC, as graphically displayed in Figure 1. The Group A control dogs (open circles) experienced dose-dependent reductions in MAP with titrated doses of NP (mean 2–19 µg/kg/min) and NIC (mean of 24 to 105 µg/kg/min). Both vasodilators prompted small increases in HR and modest decreases in CVP. NP was about 4 times more potent than NIC, but its vasodepressor effect rapidly dissipated upon stopping its infusion. Compared to these trends, the MAP responses to NP in Groups B (closed circles) and C (closed squares) were only modestly potentiated, while the responses to NIC were essentially unchanged. FURO + *DIG* pretreatment slightly attenuated NP's and NIC's tachycardia in Group B, while the low-level NIC preinfusion in Group C (closed squares) slightly accentuated

the HR responses to the step-dose sequence of both vasodilators. CVP changes with NP and NIC in Groups B and C were overshadowed by the marked depressor (i.e., volume depleting) effect achieved with FURO + *DIG*. Both vasodilators further reduced CVP marginally. Aside from the modest potentiation of NP's and NIC's tachycardia in Group C, these data show that the acute cardiovascular responses to NP and NIC are qualitatively unchanged in pentobarbital anesthetized dogs after acute FURO + *DIG*, and that these drug combinations do not precipitate any untoward interactions.

Table 3 summarizes the ECG changes seen in these anesthetized dogs. Small changes were largely restricted to ST segment depression early during the FURO + *DIG* loading intervals, and to PR interval shortening during Group C's tachycardia during the NIC pre-infusion. The subsequent NP and NIC step-dose infusions resulted in few other changes.

### Conscious Dog Experiments

Table 4 summarizes the daily baseline values recorded in 4 conscious beagle dogs before (day 1) and during (days 2–8) chronic oral treatment with FURO (5 mg/kg/day) and *DIG* (0.0125 mg/kg/day). During this treatment regimen, MAP, HR, and BW declined slightly, plasma electrolytes were relatively stable, and daily 0 time p[*DIG*]s plateaued at 1.35 ng/ml on day 6. Not tabulated, 2 h post-*DIG* levels peaked at 2.73 ng/ml on day 8, devoid of untoward gastric or ECG rhythm effects. Consistent with a FURO-induced fluid volume depletion, hematocrits increased from a basal 39 to 44% on days 5 and 6. Historic data

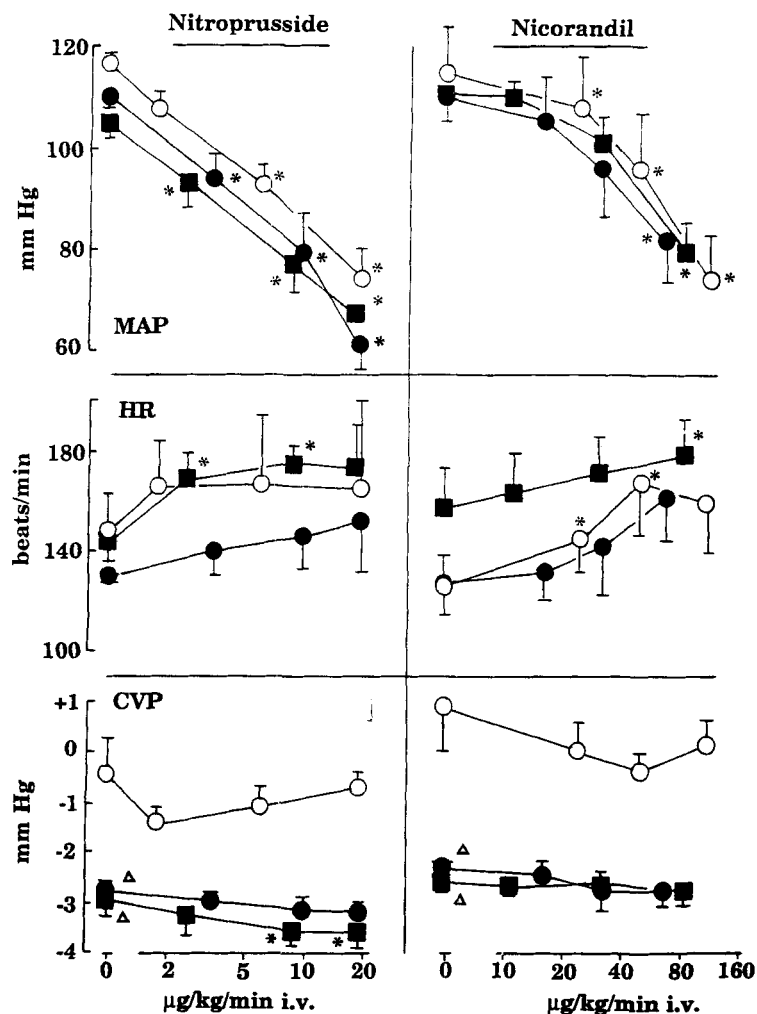


Figure 1. Cardiovascular changes in anesthetized FURO + DIG pretreated dogs subjected to iv infusions of NP and NIC. Values represent the mean  $\pm$  s.e.m. changes in MAP, HR, and CVP seen at 10 min with NP and NIC at infusion rates individually titrated to produce graded reductions in MAP. Dogs were pretreated with vehicle (Group A; open circles), FURO + DIG (Group B; closed

circles), or FURO + DIG with a 2.5 h sustained iv NIC pre-infusion (Group C; closed squares). Note the minimal shift in the hypotensive dose response to NP and NIC with pharmacologic doses of FURO and DIG in Groups B and C. \* =  $P \leq 0.05$  from PT (paired t-test);  $\Delta$  =  $P \leq 0.05$  from control Group A (ANOVA).

from an identically maintained group of 6 placebo-treated control beagle dogs showed directionally similar but smaller reductions in MAP (0 to  $-6$  mmHg), HR (0 to  $-12$  beat/min), and BW (0 to  $-0.15$  kg).

Figure 2 plots the acute MAP and HR effects of iv NP and NIC in these conscious dogs under control and FURO + DIG pretreated conditions. Under control conditions (open circles), acute iv NP at 2, 6, and 20  $\mu\text{g/kg/min}$  resulted in linear dose-dependent reductions in MAP, with only a modest acute tachycardia. While again 1/4 as potent as NP, acute iv NIC at 20, 40, 80, and 160  $\mu\text{g/kg/min}$  resulted in a steeper hypotensive dose response and a more pronounced tachycardia at higher doses. After 5 days of chronic

FURO + DIG treatment (closed circles), and starting from a lower basal MAP, NP's hypotensive dose response was largely unchanged. With its lower basal HR, NP's tachycardia was more apparent. Both the MAP and HR responses to NIC were virtually identical to the control circumstance. Neither vasodilator prompted any untoward ECG, gastric, or behavioral effects. Thus, in conscious dogs, the acute cardiovascular responses to NP and NIC are not affected by chronic oral FURO + DIG administration.

To further determine the consistency of NIC's oral hypotensive response in combination with these chronic CHF agents, the FURO + DIG regimen was continued on days 6 to 8, with 7.5 mg/kg of NIC given

**TABLE 3. Electrocardiographic Changes in Pentobarbital Anesthetized Dogs Pretreated with Furosemide and Digoxin Prior to Hypotensive IV Infusions of Nitroprusside and Nicorandil<sup>a</sup>**

Experimental interval and iv drug treatment	Time (h)	Treatment group and electrocardiographic parameter								
		A. Vehicle control (n = 3)			B. FURO + DIG (n = 4)			C. FURO + DIG plus NIC preinfusion (n = 3)		
		PR	QT <sub>c</sub>	ST	PR	QT <sub>c</sub>	ST	PR	QT <sub>c</sub>	ST
Pretreatment (PT)	0	.09 ± .01	.45 ± .03	+0.9 ± 1.2	.10 ± .01	.45 ± .02	+0.5 ± 1.0	.11 ± .01	.46 ± .03	-0.9 ± 1.2
Change with furosemide	2.5	0 ± .00	+ .01 ± .01	-1.5 ± 0.6*	0 ± .00	-.01 ± .01	-2.2 ± 0.5*	-.02 ± .00*,**,***	-.01 ± .01	-1.9 ± 0.6*
Change with Digoxin	4.0	+ .01 ± .01	-.03 ± .03	-3.5 ± 1.2*	0 ± .00	0 ± .02	-1.5 ± 0.9	-.02 ± .01*,**,***	-.01 ± .01	-1.1 ± 1.0
Pre-nitroprusside	5.0	.10 ± .01	.40 ± .03	-3.4 ± 1.4	.09 ± .01	.47 ± .02	-1.6 ± 1.2	.10 ± .01	.38 ± .03	-0.9 ± 1.0
Change with nitroprusside	5.5	-.01 ± .01	-.01 ± .04	-0.8 ± 2.4	0 ± .01	+ .01 ± .04	+1.3 ± 2.0	0 ± .01	+ .01 ± .04	+4.8 ± 2.4
Pre-nicorandil	6.0	.11 ± .01	.43 ± .02	-3.2 ± 1.7	.10 ± .01	.42 ± .02	+0.5 ± 1.4	.10 ± .01	.41 ± .02	-0.1 ± 1.7
Change with nicorandil	6.5	-.01 ± .00*	+ .01 ± .04	-0.6 ± 2.4	0 ± .00	+ .05 ± .04	-0.7 ± 2.1	0 ± .00	-.01 ± .04	+0.5 ± 2.4

<sup>a</sup>PR and QT<sub>c</sub> interval length units = sec; ST segment heights = mm deflection from the isoelectric line. PT, pre-nitroprusside, and pre-nicorandil data represent mean ± s.e.m. absolute values; all other values represent the mean ± s.e.m. changes from those respective baselines. Drug dosages as described in the text and Figure 1.

\*P ≤ 0.05 from PT (paired t-test).

\*\*P ≤ 0.05 from Group A (ANOVA).

\*\*\*P ≤ 0.05 from Group B (ANOVA).

**TABLE 4. Daily Baseline Values in Conscious Beagle Dogs Treated Chronically With Oral Furosemide and Digoxin Prior to Acute IV Nitroprusside and Nicorandil, and With Repeated Oral Doses of Nicorandil**

Exp. day	Parameter and units						
	MAP (mmHg)	HR (b/min)	BW (kg)	p[Na <sup>+</sup> ] (mEq/L)	p[K <sup>+</sup> ] (mEq/L)	p[DIG] (ng/ml)	HCT <sup>a</sup> (%)
1	109 ± 3	91 ± 4	11.5 ± 0.2	150 ± 1	4.1 ± 0.1	0	39 ± 2
2	103 ± 3*	81 ± 4*	10.5 ± 0.2*	149 ± 2	3.9 ± 0.3	0.70 ± .41*	42 ± 2
3	101 ± 3*	90 ± 4	10.8 ± 0.2*	150 ± 2	4.0 ± 0.3	0.35 ± .13*	43 ± 2*
4	96 ± 3*	86 ± 4	11.0 ± 0.2*	149 ± 2	4.1 ± 0.2	0.70 ± .25*	42 ± 1
5	97 ± 3*	91 ± 4	10.5 ± 0.2*	150 ± 2	3.9 ± 0.2	0.95 ± .09*	43 ± 1*
6	100 ± 3*	85 ± 4	10.7 ± 0.2*	150 ± 2	4.0 ± 0.1	1.35 ± .42*	44 ± 2*
7	96 ± 3*	86 ± 4	10.3 ± 0.2*	148 ± 2	4.1 ± 0.2	1.35 ± .31*	41 ± 1
8	97 ± 3*	80 ± 4*	10.3 ± 0.2*	149 ± 3	4.2 ± 0.2	1.23 ± .18*	39 ± 1

\*P ≤ 0.05 from day 1 baseline value (paired t-test).

<sup>a</sup>HCT, hematocrit.

daily 2 h after these adjuncts. The time course of oral NIC's MAP and HR responses under these conditions are shown in Figure 3. Compared to NIC's profile in a parallel group of 6 control beagles (open circles; Wendling et al. unpublished data), the chronic FURO + DIG pretreated dogs had lower PT MAPs and HRs. Despite these minor differences, the first oral NIC dose (day 6) resulted in similar reductions in MAP and reflex increases in HR, with both effects peaking at about 1 h post-treatment. NIC's oral hypotensive response was replicated on days 7 and 8 with no untoward gastric or ECG effects. Addition-

ally, no major shifts in the dogs' 2 h post-treatment p[DIG]s were seen on days 6 through 8 (range of 1.93 to 2.73 ng/ml). However, despite stable baseline HRs and consistent hypotensive responses, NIC's tachycardia gradually increased with each daily dose, maximizing at 162 beat/min on day 8. These supplemental data further show that oral NIC's vasodilation is safely maintained chronically in the presence of FURO + DIG, and does not appear to alter DIG's steady-state pharmacokinetics. As per the iv test in anesthetized dogs, NIC's reflex tachycardia was slightly more pronounced with repeated oral administration.

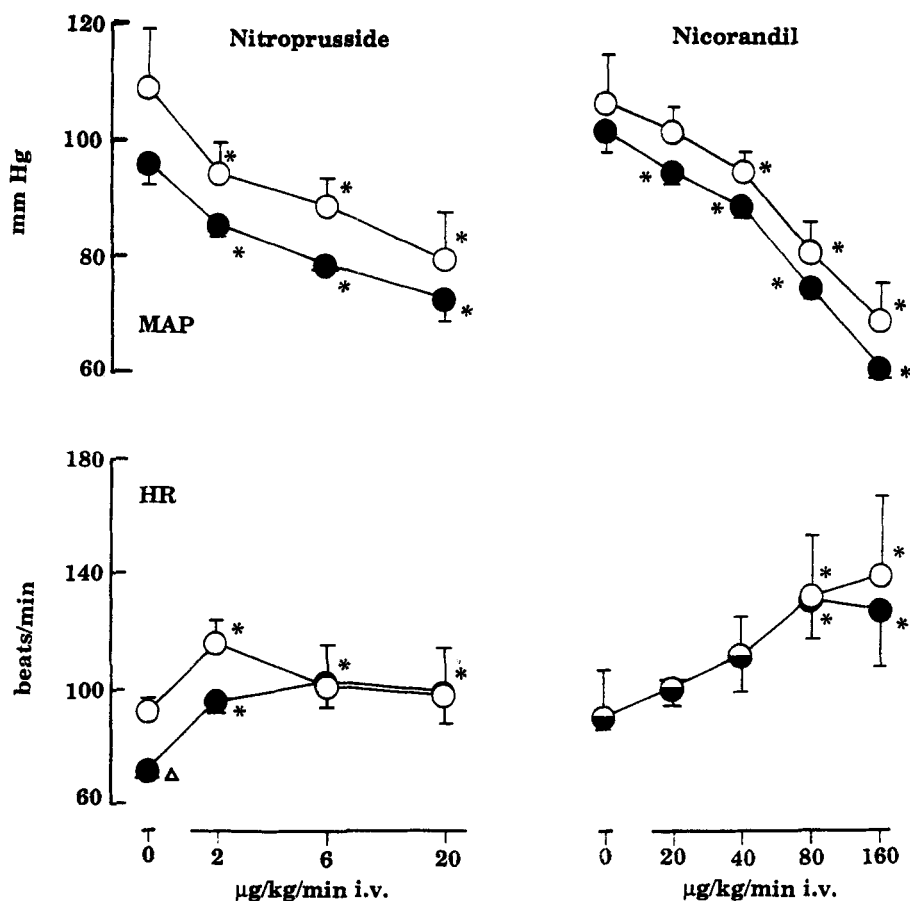


Figure 2. Cardiovascular changes in conscious beagle dogs treated with iv NP and NIC before and after chronic FURO + DIG. Values represent the mean  $\pm$  s.e.m. changes in MAP and HR seen at 10 min with stepped iv infusions of NP and NIC under control (open circles) and chronic FURO + DIG pretreated conditions (closed circles). Again note minimal shift in the MAP and HR dose responses under both circumstances. \* =  $P \leq 0.05$  from PT;  $\Delta$  =  $P \leq 0.05$  from non-pretreated condition (paired t-tests).

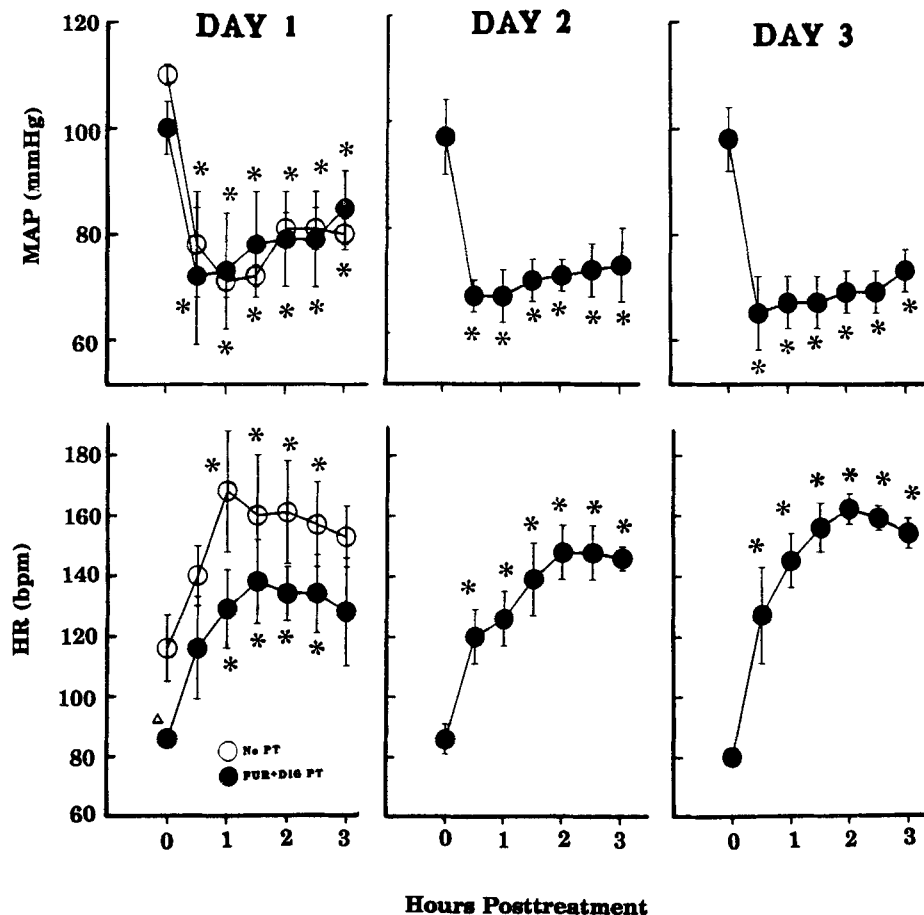
## DISCUSSION

In support of their concomitant clinical use, acute and chronic cardiovascular drug interaction studies were conducted in conscious and anesthetized dogs combining the vasodilator NIC with the diuretic FURO and the cardiac glycoside DIG. For comparison purposes, the acute phases of both experimental series included parallel interactions with the short-acting cGMP-dependent vasodilator NP [Tinker and Michenfelder, 1976], which historically has been safely used acutely with FURO and DIG in CHF patients. The primary goals of these investigations were to determine whether acute or chronic FURO + DIG would modify the cardiovascular responses to NIC or NP, whether NIC might affect FURO's acute diuresis or DIG's pharmacokinetics, and to confirm that these drug combinations are free of untoward ECG, gastric, and behavioral effects. Relative to these multiple end-

points, it should be noted that conventional toxicologic interaction tests were also conducted with NIC plus FURO and DIG in rats and dogs (Mesfin et al., unpublished data), focusing on acute and chronic pathological changes noted at high combined dosages. While also necessary as part of NIC's drug development, such studies typically do not generate pharmacologic information within the intended human dose range. This cardiovascular study was therefore implemented to quickly and efficiently establish whether these agents have any untoward or unpredicted drug interactions which might preclude their safe combined use in human CHF.

Effective doses of NIC, NP, and FURO were based on prior experience with these agents in conscious beagle dogs. The DIG dosages were more difficult to select since it has a poorly defined dose response in normal animals, a delayed onset of action,





**Figure 3.** Cardiovascular changes in conscious beagle dogs treated with oral NIC during chronic FURO + DIG therapy (closed circles). Open circles represent historic acute oral NIC time course data in non-pretreated beagle dogs. Three repeated oral doses of NIC (7.5 mg/kg/day) were given on days 6, 7, and 8,

denoted as combined oral treatment days 1, 2, and 3, respectively. Note comparable acute reductions in MAP with the first oral NIC dosage, and its consistent MAP response with repeated doses. \* =  $P \leq 0.05$  from 0 time reading (paired t-test);  $\Delta = P \leq 0.05$  from historic non-pretreated dogs (ANOVA).

and a somewhat narrow therapeutic index [Smith and Haber, 1974]. Indeed, while DIG increases contractility in failed dog hearts at p[ DIG ]s of 0.5–2.0 ng/ml, in normal dogs often the only indications of activity are gastric and ECG side effects. DIG's pharmacokinetics also complicate combination studies since delayed separate responses to this cardiac glycoside could be misinterpreted as untoward drug interactions. Based on pilot tests, 0.075 mg/kg of DIG (7.5 times the normal human iv dose) was given to the anesthetized dogs, which acutely resulted in therapeutic plasma concentrations [Barr et al., 1972; Lewis, 1986] and threshold gastric and ECG side effects. Chronic DIG was given at 0.0125 mg/kg/day, the normal human dosage, which also gave stable therapeutic 0 time p[ DIG ]s by day 5. In both preparations, the p[ DIG ]s and indices of fluid balance (i.e., volume depletion, hematocrit, and BW) showed that

effective doses of FURO and DIG had been given prior to superimposing acute iv NIC and NP.

This study has demonstrated that acute NP and acute and chronic NIC are well tolerated in combination with FURO and DIG, and that their vasodilations, as judged by changes in MAP and HR, are largely unmodified by these conventional CHF adjuncts. The 2.5 h NIC preinfusion in anesthetized dogs did not appreciably affect FURO's volume depletion or DIG's acute pharmacokinetics, nor did repeated oral doses of NIC affect the steady-state p[ DIG ]s. It thus appears that, like NP, pharmacologic doses of NIC can be safely combined with FURO and DIG without untoward cardiovascular or pharmacokinetic interactions. These findings support an earlier test with NIC and ouabain in anesthetized dogs [Nakamura et al. 1984], but extend this conclusion to include NIC's acute and chronic interaction with

FURO + DIG as compared to NP in conscious and anesthetized dogs.

A fair criticism of this study may be the relatively small group sizes which were used. While slightly smaller than most conventional pharmacologic studies, it must be appreciated that 3-way drug interaction studies are difficult to design and implement, particularly when they include a fourth standard comparator (NP), a general anesthetic (pentobarbital) with separate tachycardiac effects, and 2 compounds with markedly different onsets of peak action (i.e., the immediacy of FURO and the delayed effects of DIG). Indeed, using a classic 3-way interaction design, at least 8 treatment groups (and 40 dogs at  $n = 5/\text{group}$ ) would have been necessary to examine the separate and combined effects of vehicle, NIC, FURO, and DIG under just one experimental circumstance. Balanced against this idealized interactive grid is the acknowledged need for efficient experimental drug evaluations and for limiting animal usage, particularly when it is apparent that the key drug combination, in this case, NIC's cardiovascular response in the presence of FURO and DIG, results in negligible drug interactions. These concerns were addressed by concentrating on the most relevant drug combinations, using serial drug administration, relying on parallel historic data, and conducting an interim data analysis to determine whether the targeted group size ( $n = 5/\text{group}$ ) were in fact justified. Specifically, when a preliminary analysis of the anesthetized dog data revealed that NIC could be safely and predictably combined with FURO + DIG, and, based on their individual variance, 1 to 2 more dogs/group would most likely not produce a significant MAP interaction, emphasis was shifted to conscious dogs free of anesthetic interference and amenable to chronic drug exposure. Likewise, the initial statistical analysis of the conscious dog data demonstrated that sustained FURO + DIG had only minor effects on NIC's hypotensive profile, which, based on the variation in the dogs' responses, would likely not be modified with an additional dog. It must also be emphasized that while the individual group sizes were small, collectively 7 anesthetized dogs (in Groups B and C) and 4 conscious dogs showed virtually the same result, namely, little change in NIC's hypotensive dose response and no untoward interactive effects, a finding subsequently supported by the clinical experience. Thus, the consistency of these findings in a total of 11 dogs under both acute anesthetized and acute and chronic conscious conditions would seem to compensate for their separate group sizes and solidify the conclusions of this study.

Beyond NIC's minimal interactions with FURO

+ DIG, this study has further defined the acute and chronic cardiovascular effects of this bimodal vasodilator in dogs. In both preparations, NIC was about 1/4 as potent as NP, evoked more tachycardia per level of hypotension, but was longer acting. Small shifts in NIC's HR response were also seen, depending on the duration of drug exposure. In the anesthetized dogs, the 2.5 h NIC preinfusion during the FURO + DIG loading tended to accentuate the tachycardia to both NIC and NP, suggesting greater sensitivity to arterial hypotension. In the conscious dogs, acute iv NIC prompted a moderate tachycardia intermediate to NP and known PCOs such as minoxidil [Humphrey and Zins, 1984], pinacidil [Kawashima and Liang, 1985], and Ro 31-6930 [Paciorek et al., 1990], as might be expected with its combined cGMP and PCO-dependent vasodilation [Meisheri et al., 1991]. With repeated oral therapy, however, NIC's reflex tachycardia appeared steadily more pronounced, eventually equalling those typically seen with pure  $K^+$  channel openers, suggesting that this component of NIC's vasodilation may become more prominent with repeated exposure. Relevant to this speculation, Giles et al. [1989] reported that chronic NIC increases HR and BW in CHF patients, effects commonly associated with PCO-like vasodilators. Thus, the relative balance and consistency of NIC's cGMP and PCO-dependent vasodilator components during extended or repeated exposure would seem a question worthy of further investigation.

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