Combined phosphodiesterase inhibition and β -blockade in the GI104313, decreases ischemia-induced arrhythmias in the rat

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Purpose: The purpose of the present study was to evaluate the effects of GI104313, a chimeric molecule containing a phosphodiesterase inhibiting pyradazinone and a blocking phenoxopropanolamine, on ischemia-induced arrhythmias in anesthetized rats.

Method: The coronary artery was occluded 15 min after commencing drug administration and myocardial ischemia was maintained for 30 min during which the heart rate and mean blood pressure were recorded. Cyclic AMP and GMP were determined by radio-immunoassay.

Results: GI104313 ($0.1 \mu\text{mol}\cdot\text{kg}^{-1}$ plus $0.01 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or $1 \mu\text{mol}\cdot\text{kg}^{-1}$ plus $0.1 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) decreased the incidence of ventricular tachycardia (86% and 75%), ventricular fibrillation (28%, $P < 0.01$ and 12%, $P < 0.001$) and premature ventricular beats (164 ± 27.0 and 114 ± 28.5 , $P < 0.05$) following coronary artery ligation, resulting in a decrease in mortality (29% and 12%, $P < 0.05$).

Changes in cyclic nucleotide concentrations have been implicated in the genesis of ischemia-induced arrhythmias. However, in the present study GI104313 did not change the concentrations of adenosine 3': 5'-cyclic monophosphate (cyclic AMP) ($1.0 \pm 0.07 \text{ pmol}\cdot\text{mg}^{-1}$, $1.0 \pm 0.05 \text{ pmol}\cdot\text{mg}^{-1}$) or guanosine 3': 5'-cyclic monophosphate (cyclic GMP) ($0.025 \pm 0.008 \text{ pmol}\cdot\text{mg}^{-1}$ protein, $0.017 \pm 0.004 \text{ pmol}\cdot\text{mg}^{-1}$ protein) in the left ventricle during ischemia-induced arrhythmias in anesthetized rats compared to saline ($0.9 \pm 0.1 \text{ pmol}\cdot\text{mg}^{-1}$ and $0.013 \pm 0.002 \text{ pmol}\cdot\text{mg}^{-1}$, respectively).

Conclusion: Our results demonstrate that, in rats, GI104313 induced a decrease in both incidence of arrhythmias and mortality which was not associated with changes in ventricular cyclic nucleotide content.

Objectif : Évaluer les effets de la GI104313, une molécule chimérique contenant un pyradazinone inhibiteur de phosphodiesterase et une phénoxypropanolamine bloquante, sur des arythmies induites par ischémie chez des rats anesthésiés.

Méthode : L'artère coronaire a été bloquée, 15 min après le début de l'administration du médicament et l'ischémie myocardique a été maintenue pendant 30 min pendant lesquelles le débit cardiaque et la tension artérielle moyenne ont été enregistrés. L'adénosine monophosphate-3', 5' cyclique (AMP cyclique) et la guanosine monophosphate-3', 5' cyclique (GMP cyclique) ont été déterminés par radio-immunodosage.

Résultats : La GI104313 ($0,1 \text{ mmol}\cdot\text{kg}^{-1}$ plus $0,01 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ou $1 \text{ mmol}\cdot\text{kg}^{-1}$ plus $0,1 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) a réduit l'incidence de tachycardie ventriculaire (86 % et 75 %), de fibrillation ventriculaire (28 %, $P < 0,01$ et 12 %, $P < 0,001$) et d'extrasystole ventriculaire ($164 \pm 27,0$ et $114 \pm 28,5$, $P < 0,05$) après la ligature de l'artère coronaire, ce qui a réduit la mortalité (29 % et 12 %, $P < 0,05$).

Les changements de concentrations des nucléotides cycliques ont participé à la genèse des arythmies induites par l'ischémie. Cependant, dans la présente étude, la GI104313 n'a pas modifié les concentrations d'AMP cyclique ($1,0 \pm 0,07 \text{ pmol}\cdot\text{mg}^{-1}$, $1,0 \pm 0,05 \text{ pmol}\cdot\text{mg}^{-1}$) ou de GMP cyclique ($0,025 \pm 0,008 \text{ pmol}\cdot\text{mg}^{-1}$ de protéine, $0,017 \pm 0,004 \text{ pmol}\cdot\text{mg}^{-1}$ de protéine) dans le ventricule gauche pendant les arythmies induites par ischémie chez des rats anesthésiés, en comparaison avec une solution salée ($0,9 \pm 0,1 \text{ pmol}\cdot\text{mg}^{-1}$ et $0,013 \pm 0,002 \text{ pmol}\cdot\text{mg}^{-1}$, respectivement).

Conclusion : Nos résultats démontrent que, chez les rats, la GI104313 a réduit l'incidence des arythmies et de la mortalité, conséquence non associée aux changements du contenu en nucléotides cycliques ventriculaires.

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TH E pharmacological treatment of congestive heart failure (CHF), a condition associated with a high incidence of ventricular arrhythmias and sudden cardiac death is challenging. Elevated concentrations of adenosine 3',5'-cyclic monophosphate (cyclic AMP) have been implicated in the genesis of ischemia-induced arrhythmias and the lowering of fibrillation threshold in the dog, pig and baboon heart¹ and in the isolated rat heart.² Direct evidence for the arrhythmogenicity of cyclic AMP was provided by the decrease in ventricular fibrillation threshold noted during perfusion of the isolated rat heart with dibutyryl cyclic AMP, the permeant form of cyclic AMP.³ An infusion of cyclic AMP analogs or agents increasing myocardial cyclic AMP at the visible border of the ischemic zone in the pig heart can provoke ventricular tachycardia (VT) or ventricular fibrillation (VF).⁴ Furthermore, elevation of ventricular guanosine 3',5'-cyclic monophosphate (cyclic GMP) as well as cyclic AMP increased ischemia-induced ventricular arrhythmias in anesthetized rats.⁵ If such changes in cyclic nucleotide were to occur in heart failure patients this could prove detrimental in the treatment of a condition where ventricular arrhythmias are already prevalent.⁶

Recent experience in CHF patients with phosphodiesterase (PDE) inhibitors that have both inotropic and vasodilator activity has been disappointing.⁷ A different approach that has been advocated is the use of β -blockers, particularly in certain subsets of CHF patients.⁸⁻¹¹ These studies show some promise, but the risk for decreased cardiac function is a major concern.¹²⁻¹³ Both of these approaches, if used in combination, might offer distinct advantages by compensating for each other's limitations in a complementary way.

GI104313(6-{4-[N-[2-[3-(2-cyanophenoxy)-2-hydroxypropylamino]-2-methylpropyl]carbamoylmethoxy-3-chlorophenyl]}-4,5-dihydro-3(2H)pyridazinone) is a potent and selective inhibitor of type III PDE and is a potent, nonselective β -adrenoceptor blocker.¹⁴ This molecule could be useful in the treatment of ventricular arrhythmias associated with CHF. Therefore, the aim of the present study was to determine the effects of GI104313 on ventricular arrhythmias and cyclic nucleotide content by the use of a model of ischemia-induced arrhythmias in the pentobarbital anesthetized rat. The effects of milrinone selective PDE III inhibitors and propranolol (β -adrenoceptor antagonist) were also evaluated in this model.

Methods and materials

Surgical and recording procedures

The method used to produce coronary artery occlusion in anesthetized rats has been described previous-

ly.¹⁵ Briefly, male rats (250-350 g, Sprague-Dawley) were anesthetized with sodium pentobarbital (60 mg·kg⁻¹ *ip*). The trachea was cannulated and the lungs ventilated using a miniature Ideal respiration pump (47 strokes per minute, 4 ml tidal volume). The femoral vein and carotid artery were cannulated for drug administration and to record blood pressure, using a pressure transducer, respectively. Mean blood pressure was calculated as diastolic blood pressure + 1/3 pulse pressure. A left thoracotomy was performed, and the fourth rib removed. After the pericardium was opened, the heart was exteriorized and a fine silk ligature placed around the left anterior descending coronary artery. The heart was returned to the chest cavity and allowed to stabilize for 15 min before starting the experiment.

Animals with a mean blood pressure < 70 mmHg after placement of the ligature were excluded from the experiment as were those that demonstrated arrhythmias prior to the ligature being tied. Following coronary ligation, animals that died within the first five minutes due to progressive but complete loss of blood pressure but without arrhythmias were also excluded.

Experimental protocol

After stabilization, a trial drug or saline was administered intravenously as an initial bolus followed immediately by a continuous infusion. The drugs were given at two doses: GI104313 (0.1 μ mol·kg⁻¹ plus 0.01 μ mol·kg⁻¹·min⁻¹, n=10 or 1 μ mol·kg⁻¹ plus 0.1 μ mol·kg⁻¹·min⁻¹, n=10), milrinone (50 μ g·kg⁻¹ plus 0.5 μ g·kg⁻¹·min⁻¹, n=10 or 75 μ g·kg⁻¹ plus 0.7 μ g·kg⁻¹·min⁻¹, n=10) and propranolol (2 mg·kg⁻¹, n=10). Control animals (n=20) were treated with saline. The coronary artery was occluded 15 min after commencing drug administration and myocardial ischemia was maintained for 30 min during which the heart rate and mean blood pressure were recorded.

To study ventricular cyclic nucleotide content, the same protocol was followed, except that 30 min following coronary artery ligation, the heart was removed. Hearts were rapidly washed in ice-cold saline, the free wall of the left ventricle (ischemic tissue) and the right ventricle (comprising the right ventricle and the intraventricular septum, non-ischemic tissue) were frozen and stored at -80°C until the day of assay. The time taken to excise the heart, dissect and freeze was approximately 15 sec.

Determination of cyclic nucleotide content

Cyclic AMP and GMP were determined as mean of cyclic AMP ³H or cyclic GMP ³H by radioimmunoassay (Amersham International, Amersham, UK). Tissues were extracted in 0.3 M perchloric acid (in a ratio 1:10,

w v^{-1}), with a Polytron homogenizer. Extracts were centrifuged at 12000 rpm for 15 min, using a Biofuge A centrifuge (Heraeus, Germany). Supernatants were treated with potassium hydroxide solution until pH 7.5 was reached. The samples were centrifuged once and the supernatants were used for cyclic AMP and GMP analysis. The sensitivity of the assay for cyclic AMP and cyclic GMP was 2 pmol·ml⁻¹ and 10 pmol·ml⁻¹, respectively. Protein were determined according to a Lowry modified procedure.¹⁶

Determination of arrhythmias

The number of premature ventricular beats (PVBs) and the incidence and duration of VT (succession of seven or more PVBs) and VF (chaotic ventricular contraction associated with a complete collapse of blood pressure) were counted 20 min before ligature without drug (Control), 15 min before ligature with drug or vehicle (-15 min) and during the experiment.

Drugs

GI104313 (a gift from Glaxo Inc, EEUU), milrinone (Sanofi and Wintrop, Spain) and propranolol (Sigma Chemical Co. Spain) were dissolved in saline. The drugs were given as an *iv* bolus plus continuous infusion. Propranolol was not administered by continuous infusion. Control animals were treated with saline.

Statistical analysis

All data are presented as the mean $\pm$ SE. Data were analysed by one-way analysis of variance, followed by the Newman-Keuls post hoc test. The observed mortality in drug-treated groups was compared with the corresponding control group by Fisher's exact test.

Results

Ischemia-induced arrhythmias

Milrinone (50 $\mu\text{g}\cdot\text{kg}^{-1}$ plus 0.5 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) did not induce changes in the incidence of arrhythmias or in mortality compared with saline but, the higher dose of milrinone (75 $\mu\text{g}\cdot\text{kg}^{-1}$ plus 0.7 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) increased the incidence in VF (98 %, $P < 0.05$) and mortality (78%, $P < 0.01$). Propranolol did not modify the incidence of arrhythmias or mortality. In contrast, the incidence of VF was decreased in rats that received GI104313 (0.1 $\mu\text{mol}\cdot\text{kg}^{-1}$ plus 0.01 $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ or 1 $\mu\text{mol}\cdot\text{kg}^{-1}$ plus 0.1 $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ (28% $P < 0.01$ and 12% $P < .001$, respectively) resulting in a decrease in mortality with the higher dose of GI104313 (12%, $P < 0.05$) (Figures 1 A, 1B, 1C).

GI104313 (1 $\mu\text{mol}\cdot\text{kg}^{-1}$ plus 0.1 $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$) decreased the PVBs count and the duration of VF ($P < 0.05$). Moreover, both doses of GI104313 reduced the

FIGURE 1 The incidence of ventricular tachycardia (VT, A), ventricular fibrillation (VF, B) and the mortality (C) on ischemia-induced arrhythmias in coronary ligated anaesthetized rats. Saline (1 ml·kg⁻¹·min⁻¹, n=20), Milrinone a (50 $\mu\text{g}\cdot\text{kg}^{-1}$ + 0.5 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, n=10), Milrinone b (75 $\mu\text{g}\cdot\text{kg}^{-1}$ + 0.7 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, n=10), Propranolol (2 mg·kg⁻¹, n=10), GI104313 a (0.1 $\mu\text{mol}\cdot\text{kg}^{-1}$ + 0.01 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, n=10) and GI104313 b (1 $\mu\text{g}\cdot\text{kg}^{-1}$ + 0.1 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, n=10). * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs saline.

duration of VT ($P < 0.01$ and $P < 0.05$, respectively). Milrinone (50 $\mu\text{g}\cdot\text{kg}^{-1}$ plus 0.5 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or 75 $\mu\text{g}\cdot\text{kg}^{-1}$ plus 0.7 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) did not affect either PVBs count or the duration of VT. However, with the higher dose of milrinone enhancement ($P < 0.05$) the duration of VF. Propranolol reduced the PVBs count without any changes in the VT and VF duration (Table I).

TABLE I Ischemia-induced arrhythmias in coronary ligated anesthetized rats.

Treatment	PVB count	VT (sec)	VF (sec)
Saline			
1.0 ml·kg ⁻¹ ·min ⁻¹	229 ± 44.53	101.1 ± 20.7	65.8 ± 20.6
Milrinone			
50 µg·kg ⁻¹ +			
0.5 µg·kg ⁻¹ ·min ⁻¹	195 ± 53.6	111.6 ± 38.7	75.3 ± 2.1
Milrinone			
75 µg·kg ⁻¹ +			
0.7 µg·kg ⁻¹ ·min ⁻¹	209 ± 45.0	91.4 ± 38.8	144.1 ± 10.7*
Propranolol			
2 mg·kg ⁻¹	103 ± 24.95*	63.0 ± 21.8	34.8 ± 17.8
GI104313			
0.1 µmol·kg ⁻¹ +			
0.01 µmol·kg ⁻¹ ·min ⁻¹	164 ± 27.0	20.8 ± 7.8†	19.4 ± 0.9
GI104313			
1 µmol·kg ⁻¹ +			
0.1 µmol·kg ⁻¹ ·min ⁻¹	114 ± 28.5*	22.2 ± 8.0*	12 ± 0.03*

Each value is the mean ± SEM. Saline group (n=20), Treatment groups (n=10).

* $P < 0.05$; † $P < 0.01$ vs saline

Hemodynamic effects

Coronary artery occlusion did not induce hemodynamic changes in the saline-treated rats. However, changes were observed with the different drugs used in this study. The lower dose of GI104313 decreased heart rate 15 min before and one minute after coronary ligature and remained significant descended after 30 min coronary ligature. However, only a transient increase in arterial blood pressure was observed 15 min before ligature which gradually reversed with the time. The same patterns of responses were observed with the higher dose of GI104313 and propranolol on the cardiac frequency although the magnitude of some changes was greater. Moreover, propranolol decreased mean blood pressure one minute after coronary ligature and remained low after 30 min coronary ligature whereas the higher dose of GI104313 only decreased mean blood pressure at 20 or 30 min postligature when compared with the saline group. With milrinone, heart rate decreased after 10 or 20 min postligature and remained decreased after 30 min drug infusion. Similar results were obtained in blood pressure. However at 30 min postligature, the decrease of mean blood pressure was not significant (Tables II, III).

Cyclic nucleotide concentrations

No differences in cyclic AMP content were observed between the ischemia left ventricle and non-ischemia right ventricle in the milrinone and propranolol groups

FIGURE 2 Effect of treatment on ventricular cyclic AMP (A) and GMP (B) content in coronary ligated anaesthetized rats. Saline (1 ml·kg⁻¹·min⁻¹, n=20), Milrinone a (50 µg·kg⁻¹ + 0.5 µg·kg⁻¹·min⁻¹, n=10), Milrinone b (75 µg·kg⁻¹ + 0.7 µg·kg⁻¹·min⁻¹, n=10), Propranolol (2 mg·kg⁻¹, n=10), GI104313 (0.1 µmol·kg⁻¹ + 0.01 µg·kg⁻¹·min⁻¹, n=10) and GI104313 (1 µg·kg⁻¹ + 0.1 µg·kg⁻¹·min⁻¹, n=10). * $P < 0.05$, † $P < 0.001$ vs left ventricle; * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ vs saline.

(Figure 2A). However, the cyclic AMP content in the GI 104313 (0.1 µmol·kg⁻¹ plus 0.01 µmol·mg⁻¹·min⁻¹ or 1 µmol·kg⁻¹ plus 0.1 µmol·mg⁻¹·min⁻¹) treated groups was higher in the right ventricle (1.3 ± 0.09 pmol·mg⁻¹ and 1.8 ± 0.1 pmol·mg⁻¹, respectively) than in the left ventricle (1.0 ± 0.07 pmol·mg⁻¹, $P < 0.05$ and 1.0 ± 0.05 pmol·mg⁻¹, $P < 0.001$, respectively). The higher dose of GI104313 induced changes in right ventricle cyclic AMP content vs saline group (1.0 ± 0.1 pmol·mg⁻¹, $P < 0.001$) (Figure 2A).

However, differences in cyclic GMP content were observed between the ischemic left ventricle and the non- ischemic right ventricle in the milrinone and propranolol groups (Figure 2B). The higher dose of milrinone increased the cyclic GMP content in left (0.028 ± 0.006 pmol·mg, $P < 0.05$) and right ventricles (0.099 ± 0.01, $P < 0.01$) vs saline groups (0.013 ± 0.002 pmol·mg⁻¹ and 0.015 ± 0.001 pmol·mg⁻¹, respectively) whereas propranolol decreased the levels of cyclic GMP in the right ventricle (0.019 ± 0.003 pmol·mg⁻¹ vs left

TABLE II Drugs administration and heart rate (HR) at -10 min, coronary artery occlusion at 1 min, 10 min, 20 min and 30 min postligature.

HR (beat·min ⁻¹)	Control	-15 min	1 min	10 min	20 min	30 min
Saline						
1 ml·kg ⁻¹ ·min ⁻¹	425 ± 8.8	425 ± 8.8	412 ± 12.6	412 ± 7.6	410 ± 13.2	400 ± 15.8
Milrinone						
50 µg·kg ⁻¹ +						
0.5 µg·kg ⁻¹ ·min ⁻¹	400 ± 17.5	378 ± 24.1	365 ± 20.0	274 ± 31.2†	298 ± 19.8‡	300 ± 25.8
Milrinone						
75 µg·kg ⁻¹ +						
0.7 µg·kg ⁻¹ ·min ⁻¹	400 ± 17.5	380 ± 23.7	372 ± 27.9	278 ± 46.1*	360 ± 33.9†	360 ± 13.3
Propranolol						
2 mg·kg ⁻¹	400 ± 32.9	230 ± 4.1‡	213 ± 10.8‡	191 ± 11.6‡	170 ± 7.07‡	170 ± 7.07‡
GI104313						
0.1 µmol·kg ⁻¹ +						
0.01 µmol·kg ⁻¹ ·min ⁻¹	407 ± 6.6	342 ± 12.2‡	327 ± 6.13‡	251 ± 32.7‡	262 ± 38.4†	259 ± 37.2†
GI104313						
1 µmol·kg ⁻¹ + 0.1						
µmol·kg ⁻¹ ·min ⁻¹	412 ± 14.6	293 ± 14.9‡	293 ± 14.8‡	257 ± 30.4†	247 ± 30.5‡	240 ± 29.4‡

Each value is the mean ± S.E.M. Saline group (n=20). Treatment-groups (n=10).

†*P* < 0.01; ‡*P* < 0.001 *vs* saline.

TABLE III Drug administration and mean blood pressure (BP) at 15 min coronary artery occlusion at 1 min, 15 min, 20 min and 30 min post ligature

BP (mmHg)	Control	-15 min	1 min	10 min	20 min	30 min
saline						
1 ml·kg ⁻¹ ·min ⁻¹	82.0 ± 4.8	89. ± 3.2	63. ± 8.2	62 ± 10.6	89.4 ± 2.9	92.8 ± 1.6
Milrinone						
50 µg·kg ⁻¹ +						
0.5 µg·kg ⁻¹ ·min ⁻¹	85.7 ± 3.9	89.7 ± 5.3	71.4 ± 6.9	65.1 ± 9.3	67.3 ± 6.8*	66.8 ± 8.2*
Milrinone						
75 µg·kg ⁻¹ +						
0.7 µg·kg ⁻¹ ·min ⁻¹	81. ± 7.0	79.5 ± 4.6	52.2 ± 6.4	52.2 ± 6.4	50.5 ± 8.2†	60.5 ± 9.1†
Propranolol						
2 mg·kg ⁻¹	77.6 ± 3.2	56.0 ± 0.6‡	45.5 ± 4.2	28.0 ± 11.9*	30.0 ± 4.9‡	32.0 ± 6.3‡
GI104313						
0.1 µmol·kg ⁻¹ +						
0.01 µmol·kg ⁻¹ ·min ⁻¹	82.1 ± 6.1	106.2 ± 4.4†	75.4 ± 6.7	58.8 ± 10.6	78.4 ± 11.2	76 ± 10.3
GI104313						
1 µmol·kg ⁻¹ + 0.1µmol·kg ⁻¹ ·min ⁻¹	74.8 ± 4.8	77.7 ± 6.8	59.2 ± 5.5	50.1 ± 3.8	52.5 ± 5.4‡	49.0 ± 4.8‡

Each value is the mean ± S.E.M. Saline group (n=20). Treatment groups (n=10).

**P* < 0.05; †*P* < 0.01; ‡*P* < 0.001 *vs* saline.

ventricle (0.033 ± 0.006 pmol·mg⁻¹, *P* < 0.05) and increased the cyclic GMP content in the left ventricle *vs* saline group (*P* < 0.01). Milrinone (50 µg·kg⁻¹ plus 0.5 µg·kg⁻¹·min⁻¹) and both doses of GI104313 did not induce changes in left or right ventricle cyclic GMP content *vs* saline. The higher dose of milrinone increased cyclic GMP content in right ventricle *vs* left ventricle (*P* < 0.05) (Figure 2B).

Discussion

In anesthetized rats undergoing coronary artery ligation, the PVBS count observed at the 30 min postischemic period was lower than that found by others authors although the overall mortality and incidence of arrhythmias was similar to that previously reported.^{5,15,17-20} The cyclic nucleotide levels obtained in this study were similar to that described previously in anesthetized rat.⁵

These results indicate that GI104313, a novel molecule, highly inhibitor of type III PDE and potent beta adrenoceptor blocker, reduced the incidence and duration of arrhythmias which is accompanied by a reduction in the mortality. Both doses of GI104313 increased the cyclic AMP content in the right ventricle *vs* left ventricle. Moreover GI104313 ($1 \mu\text{mol}\cdot\text{kg}^{-1}$ plus $0.1 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) increased cyclic AMP levels in right ventricle *vs* saline group without any changes in left ventricle. In contrast, this drug did not modify the content of cyclic GMP in the right or left ventricle. Milrinone and propranolol did not change the content of cyclic AMP in the left or right ventricle. The higher doses of milrinone ($75 \mu\text{g}\cdot\text{kg}^{-1}$ plus $0.7 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) exacerbated arrhythmias during acute myocardial ischemia. Furthermore, the mortality due to sustained VF was increased in the group that received the higher dose of milrinone. Moreover, milrinone enhanced the cyclic GMP levels in this model of ischemia-induced arrhythmias. Drug-induced elevation in cyclic nucleotides caused by either selective PDE type III inhibition, β -adrenoceptor activation or direct activation of adenylyl cyclase are associated with an increased incidence of arrhythmias.^{1,4,21} In addition, clinical studies have suggested that PDE III inhibitors such as amrinone and milrinone have arrhythmogenic effects during myocardial ischemia or reperfusion.^{22,23} Moreover, the higher dose of milrinone that exacerbated the arrhythmias increase the cyclic GMP content in left ventricle whereas, propranolol increased the cyclic GMP content but did not modify the incidence of arrhythmias or mortality. In contrast, GI104313 did not modify the content of the cyclic GMP but decreased the incidence of arrhythmias.

The decrease in incidence of arrhythmias induced by GI104313 was not accompanied by reduction in cyclic nucleotide. In agreement with the present data a previous study²⁰ showed that in anesthetized rats ischemia-induced arrhythmias did not modify the cyclic nucleotides content in ischemia ventricular tissue. In addition propranolol increase cyclic GMP content in left ventricle without changes in the incidence of arrhythmias suggesting that cyclic nucleotide are not involved in this model of ischemia.

Previous studies in the cat,²⁴ pig⁴ and baboon¹ suggest that cyclic AMP levels rose in ischemia tissue and that this rise preceded the onset of fibrillation. However, it has been observed that in rat isolated heart increases in cyclic AMP were observed 30-45 min after ischemia.⁴ Recently it has been reported that the mechanism by which cyclic AMP is elevated has a profound influence on the fibrillation threshold in isolated rat hearts. Large increases in cyclic AMP caused by forskolin did not cause such a large decrease in fib-

rillation threshold as that produced following the smaller increases in cyclic AMP caused by isoprenaline. Compartmentalisation of cyclic AMP may be an explanation for these effects.²⁵

On the other hand it is known that CHF is associated with a high incidence of ventricular arrhythmias and sudden cardiac death. Although numerous treatments are used, no single agent appears to provide adequate symptomatic relief and protection from sudden death in all patients. Inotropic stimulation using PDE inhibitors, without concomitant beta blockade, has been well studied. Although these agents elicit hemodynamic effects, which are maintained over a prolonged course of therapy, they do not improve survival.^{7,13} β -blockers, on the other hand, are of benefit in protecting patients from sudden death.^{26,27} At this time, it is not possible to predict which outcome is more likely in individual patients, but if beta blockers are most likely to exacerbate pump failure and PDE inhibitors are most likely to exacerbate arrhythmia sudden death, then it seems reasonable that both of these approaches (PDE-induced inotropy and β blockade), if used in combination, might offer distinct advantages by compensating for each other's limitations in a complementary way.

In conclusion, the present results demonstrated that GI104313 is associated with a decrease both in the incidence of arrhythmias and mortality without any changes in the cyclic nucleotides in the ischemia ventricle suggesting that cyclic nucleotides are not implicated in this model of arrhythmias. Recent studies suggest that other mechanisms, in addition to cyclic nucleotides, have been implicated in ischemia-induced arrhythmias.²⁸⁻³¹

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References

- 1 Podzuweit T. Early arrhythmias resulting from acute myocardial ischemia; possible role of cyclic AMP. In: Parratt JR (Ed.). Early Arrhythmias Resulting from Myocardial Ischemia, Mechanisms and Prevention by Drugs. London: Macmillan Press, 1982: 171-98.
- 2 Lubbe WF, Nguyen T, West EJ. Modulation of myocardial cyclic AMP and vulnerability to fibrillation in the rat heart. Federation Proceedings 1983; 42: 2460-4.
- 3 Lubbe WF, Podzuweit T, Davies PS, Opie LH. The role of cyclic adenosine monophosphate in adrenergic effects on ventricular vulnerability to fibrillation in the isolated perfused rat heart. J Clin Invest 1978; 61: 1260-9.
- 4 Podzuweit T, Els DJ, McCarthy J. Cyclic AMP mediated arrhythmias induced in the ischaemia pig heart. Basic Res Cardiol 1981; 76: 443-8.

- 5 Kane KA, Morcillo-Sanchez EJ, Parratt JR, Rodger IW, Shahid M. The relationship between coronary artery occlusion-induced arrhythmias and myocardial cyclic nucleotide levels in the anaesthetized rat. *Br J Pharmacol* 1985; 84: 139-45.
- 6 Packer M. Effect of phosphodiesterase inhibitors on survival of patients with chronic congestive heart failure. *Am J Cardiol* 1989; 63: 41A-5.
- 7 DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC, Wright RA. A comparison of oral milrinone, digoxin and their combination in the treatment of patients with chronic heart failure. *N Engl J Med* 1989; 320: 677-83.
- 8 Waagstein F, Hjalmarson Å, Varnauskas E, Wallentin I. Effect of chronic beta-adrenergic receptor blockade in congestive cardiomyopathy. *Br Heart J* 1975; 37: 1022-36.
- 9 Weber KT, Likoff MJ, McCarthy D. Low-dose beta blockade in the treatment of chronic cardiac failure. *Am Heart J* 1982; 104: 877-9.
- 10 Engelmeier RS, O'Connell JB, Walsh R, Rad N, Scanlon PJ, Gunnar RM. Improvement in systems and exercise tolerance by metoprolol in patients with dilated cardiomyopathy: a double-blind, randomized, placebo-controlled trial. *Circulation* 1985; 72:536-46.
- 11 De Marco T, Chatterjee K. Phosphodiesterase inhibitors in refractory heart failure: bridge to beta-blockade. *JACC* 1998; 31: 1341-3.
- 12 Chatterjee K. Heart failure therapy in evolution (Editorial). *Circulation* 1996; 94: 2689-93.
- 13 Charlap S, Lichstein E, Frishman WM. -adrenergic blocking drugs in the treatment of congestive heart failure. *Med Clin N Am* 1989; 73: 373-85.
- 14 Shaffer JE, Grizzle MK, Anderson DK, Wheeler TN. The inotropic and beta blocking effects of a chimeric molecule that putatively inhibits both type III phosphodiesterase and β adrenoceptors in anesthetized dogs. *J Pharmacol Exp Ther* 1993; 265:1105-12.
- 15 Clark C, Foreman MI, Kane KA, McDonald FM, Parratt JR. Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. *J Pharmacol Methods* 1980; 3: 357-8.
- 16 Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* 1972; 48: 442-6.
- 17 Marshall RJ, Muir AW, Winslow E. Development of a severe model of early coronary artery ligation- induced dysrhythmias in the anaesthetized rat. *Br J Pharmacol* 1981; 73: 951-9.
- 18 Daugherty A, Frayn KN, Redfern WS, Woodward B. The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias in the rat *in vivo* and *in vitro*. *Br J Pharmacol* 1986; 87: 265-77.
- 19 Holdbrook M, Coker SJ. Effects of zaprinast and rolipram on platelet aggregation and arrhythmias following myocardial ischaemia and reperfusion in anaesthetized rabbits. *Br J Pharmacol* 1991; 103: 1973-9.
- 20 Jones RB, Frodsham G, Dickinson K, Foster GA. Effect of flosequinam on ischaemia-induced arrhythmias and on ventricular cyclic nucleotide content in the anaesthetized rat. *Br J Pharmacol* 1993; 108: 1111-6.
- 21 Sugiyama M, Ogawa K, Yamazaki N. Concentration of myocardial cyclic AMP and ventricular fibrillation induced by aminophylline. *Jap Heart J* 1979; 20: 177-82.
- 22 Colucci WS, Wright RF, Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. *N Engl J Med* 1986; 314: 349-58.
- 23 Lynch JJ, Kitzen JM, Hoff PT, Lucchesi BR. Effects of pimobendan (UD-CG 115 BS) a new positive inotropic agent, on ventricular tachycardia and ischemic ventricular fibrillation in a conscious canine model of recent myocardial infarction. *J Cardiovasc Pharmacol* 1988; 12: 547-54.
- 24 Corr PB, Witkowski FX, Sobel BE. Mechanisms contributing to malignant dysrhythmias induced by ischemia in the cat. *J Clin Invest* 1978; 61: 109-19.
- 25 Worthington MG, Opie LH. Contrasting effects of cyclic AMP increase caused by β -adrenergic stimulation or by adenylate cyclase activation on ventricular fibrillation threshold of isolated rat heart. *J Cardiovasc Pharmacol* 1992; 20: 595-600.
- 26 Chadda K, Goldstein S, Byington R, Curb JD. Effect of propranolol after acute myocardial infarction in patients with congestive heart failure. *Circulation* 1986; 73: 503-10.
- 27 Kendall MJ, Lynch KP, Hjalmarson Å, Kjekshus J. β -blockers and sudden cardiac death. *Ann Intern Med* 1995; 123: 358-67.
- 28 Hoag JB, Qian Y-Z, Nayeem MA, D'Angelo M, Kukreja RC. ATP-sensitive potassium channel mediates delayed ischemia protection by heat stress in rabbit heart. *Am J Physiol* 1997; 273: H2458-64.
- 29 Munch-Ellingsen J, Bugge E, Løkebo JE, Ytrehus K. Potassium channel blocker dofetilide does not abolish ischaemic preconditioning. *Scand J Clin Lab Invest* 1997; 57: 13-20.
- 30 Klein HH, Bohle RM, Pich S, Lindert-Heimberg S, Wollenweber J, Nebendahl K. Time delay of cell death by Na^+/H^+ -exchange inhibition in regionally ischemic, reperfused porcine hearts. *J Cardiovasc Pharmacol* 1997; 30: 235-40.
- 31 Fukuta M, Wakida Y, Iwa T, Uesugi M, Kobayashi T. Role of Na^+/H^+ exchange on reperfusion related myocardial injury and arrhythmias in an open-chest swine model. *Pacing Clin Electrophysiol* 1996; 19: 2027-33.