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Influence of acetylator status on the haemodynamic effects and pharmacokinetics of cadralazine in healthy subjects

P. BRUNEL[1], J.-B. LECAILLON[2], T. T. GUYENE[3], P. IMHOF[1], & J. MÉNARD[1]
[1]Pharmaceutical Research and Development Department, CIBA-GEIGY Limited, Basle, Switzerland, [2]CIBA-GEIGY Biopharmaceutical Research Center, Rueil-Malmaison, and [3]Laboratoire de Radioimmunologie, Hôpital Broussais, Paris, France

1 Cadralazine is a new antihypertensive agent which causes peripheral vasodilation, probably mediated by a hydrazinopyridazine metabolite.

2 The possible influence of acetylator status on the pharmacokinetics and haemodynamics of the drug was studied in six fast and six slow acetylators over a period of 24 h after administration of a single 10 mg oral dose.

3 There were no differences between the two groups in AUC and C_{max} values of cadralazine and apparent metabolite, the latter defined as the sum of the free and conjugated hydrazinopyridazine. Peak plasma concentrations of these compounds were reached after 1 h. Thereafter, the concentration of the metabolite declined more slowly than that of cadralazine.

4 No effects on blood pressure were noted. Changes in heart rate and plasma renin were delayed by 3–5 h with respect to the time-course of drug and metabolite in plasma; maximum responses occurred at 4–6 h after drug administration. The extent of the increase in plasma renin activity was slightly greater in slow than in fast acetylators, but the difference was not significant statistically.

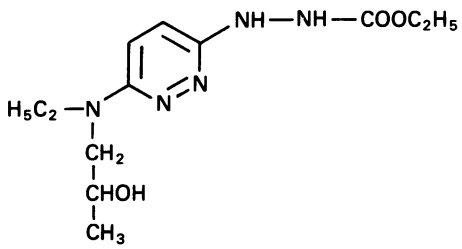
Keywords acetylation cadralazine hydrazino metabolite pharmacokinetics

Introduction

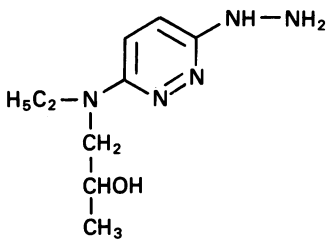
Cadralazine, 2-(3-[6-(2-hydroxypropyl) ethylamino] pyridazinyl) ethylcarbazate (Figure 1), is a peripheral vasodilator exerting a gradual and long-lasting antihypertensive effect (Amann & Bühler, 1979; Salvadeo *et al.*, 1985). Its mechanism of action is likely to be similar to that of other hydrazino-derivatives which are in therapeutic use. However cadralazine itself is a shielded hydrazino-derivative and it is likely that its effects are mediated by an active metabolite. Several metabolites of cadralazine have been identified (Schutz *et al.*, 1985). One of these, the hydrazinopyridazine product, CGP 22 639, has a much more potent effect that cadralazine on the blood pressure of hypertensive rats and is, therefore, believed to contribute towards the antihypertensive effect of the parent drug (Carpi *et al.*, 1981). A recent study in rats supports the contention that the slow onset and long duration of the pharmacological effect of cadralazine is related closely to the distribution of CGP 22 639 in vascular smooth muscle (Terauchi *et al.*, 1988).

In the present study, the influence of acetylator status on the pharmacokinetics of cadralazine and its metabolite was investigated in healthy subjects. Haemodynamic effects and plasma renin activity were measured in relation to the time-course of plasma cadralazine and CGP 22 639.

Correspondence: Dr P. Brunel, CIBA-GEIGY Limited, K-147.4.17, CH-4002 Basle, Switzerland.



Cadralazine



Hydrazinopyridazine metabolite
(CGP 22 639)

Figure 1 Chemical structures of cadralazine and its hydrazino-pyridazine metabolite (CGP 22 639).

Methods

Subjects

The investigations were carried out in healthy male volunteers who had undergone a medical examination at the Out-patient Department of the Cantonal Hospital, Basle, not longer than 12 months previously and who had been passed as fit to participate in pharmacological studies. The protocol was approved by the Ethics Committee of the Cantonal Hospital. The acetylator status of each subject was determined using sulphadimidine (Bratton & Marshall, 1939) and six slow and six fast acetylators were identified. After being informed orally and in writing about the experiment the 12 subjects signed a declaration of consent. They were instructed to take no drugs for 2 weeks before and, with the exception of the test preparation, during the study. They were also told to drink no alcohol on the day before and during each day of drug or placebo administration and to refrain from sporting or other strenuous physical activities. The two groups were of similar ages (slow acetylators: 25–47 years, fast acetylators: 26–58 years), height (1.75 ± 0.04 m *vs* 1.79 ± 0.03 m), weight (74.6 ± 4.5 kg *vs* 77.7 ± 3.9 kg) and body surface area (1.90 ± 0.08 m^2 *vs* 1.97 ± 0.06 m^2), (means ± s.e. mean).

Protocol

The study was conducted according to a double-blind, cross-over design, each subject receiving single oral doses of cadralazine (10 mg tablet) and placebo in random sequence. An interval of at least 2 weeks was allowed between the two treatments. The volunteers took the tablet at 08.00h on an empty stomach together with about 100 ml of water. They received a light breakfast after 2 h and lunch after 4 h.

Analytical methods

Blood samples were collected in heparinized Vacutainer® tubes before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 24 h after drug administration. Plasma was separated after centrifugation and stored at −80° C until analysis. The concentrations of unchanged drug (Hauffe & Dubois, 1984) and of CGP 22 639 in plasma were measured by h.p.l.c. The concentration of CGP 22 639 was determined after hydrolysis of possible hydrazone derivatives and included free CGP 22 639 derived from the parent drug at acidic pH (3.9). Previous experiments investigated whether CGP 22 239 is transformed in plasma into compounds from which the parent drug could be recovered by hydrolysis. Thus, in plasma CGP 22 639 may behave like hydralazine (Reece *et al.*, 1980) and dihydralazine (Degen *et al.*, 1982) in forming hydrazones in equilibrium with CGP 22 639. It was shown that hydrolysis under strong acidic conditions must be performed to determine apparent CGP 22 639. In the present experiments 1 ml of plasma was diluted with 2 ml of 0.1 mol l^{-1} hydrochloric acid in a 15 ml glass tube. The tube was shaken and kept at room temperature for 60 min, owing to the lack of stability of cadralazine and CGP 22 639 in plasma, the compounds were added just before derivatization. The derivatization was performed with 10 μl of internal standard solution and 50 μl of aqueous sodium nitrite solution. The internal standard solution was prepared by dissolving 1 mg of hydralazine hydrochloride in 500 ml of 0.1 mol l^{-1} hydrochloric acid. The hydrazone derivatives were extracted with 2 ml of 2 mol l^{-1} NaOH and 5 ml of chloroform, then evaporated to dryness and the residue was dissolved in 100 μl of mobile phase containing 1.8×10^{-3} mol l^{-1} phosphoric acid-acetonitrile (86 : 14 v/v). Chromatography was performed on a Nucleosil C18 column and with u.v. detection at 240 nm. The limits of assay in plasma were

18 nmol of cadralazine l^{-1} (5 ng ml^{-1}) and 3.3 nmol of CGP 22 639 l^{-1} (1 ng ml^{-1}). The accuracy and precision of the method were tested by analysing biological samples spiked with known amounts of cadralazine (17.7 nmol l^{-1}–1765 nmol l^{-1}) and CGP 22 639 (1.66 nmol l^{-1}–66.2 nmol l^{-1}). Overall recoveries were 97.3 ± 7.9% (n = 69) for cadralazine and 97.3 ± 9.5% (n = 99) for CGP 22 639 (means ± s.d.).

Haemodynamics

Haemodynamic measurements were made after 30 min rest in the recumbent position in an air-conditioned room at 20 ± 0.5° C, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 h after administration of 10 mg cadralazine or placebo. Arterial blood pressure in the left arm was recorded automatically at 5 min intervals over 15 min with an oscillometric recorder [Dinamap, Model 1846 SX, Critikon (Silas *et al.*, 1980)]. Heart rate was measured using the same apparatus. Mean arterial pressure was defined as the pressure corresponding to the maximum amplitude of oscillations. Non-invasive haemodynamic measurements (see below) were made within the 15 min following the resting period and blood samples were collected after the last haemodynamic measurement.

Impedance cardiography was performed with a Minnesota Impedance Cardiograph, Model 304B, and the tracings were recorded on a Hellige EK 36 Multiscriptor. Transthoracic impedance was measured according to Kubicek *et al.* (1966) using four strip electrodes positioned around the neck and chest. This allowed the estimation of stroke volume (Buell, 1988).

Simultaneous recordings of the carotid pulse, electrocardiogram and phonocardiogram allowed the measurement of systolic time-intervals (STI), including the pre-ejection period, left ventricular ejection time and total electromechanical systole. The regression equations used for adjustment of STI for changes in heart rate were those of Weissler *et al.* (1968).

Humeral artery haemodynamics were investigated using a bidimensional pulsed Doppler system (Echovar pulsed Doppler 8 MHz, Alvar Electronics, France). The probe was fixed with a stereotaxic device along the course of the right humeral artery, as described previously by Safar *et al.* (1981). Forearm blood flow was calculated as the product of mean blood velocity (electronic integration) and cross-sectional area of the artery (assuming a perfect cylinder).

Plasma renin activity was measured by an immunoradiometric assay (IRMA) using monoclonal antibodies (Ménard *et al.*, 1985) in blood collected in EDTA Vacutainers from an indwelling catheter in the right arm after 30 min rest in the supine position. The blood was stored at −20° C until analysis.

Statistical methods

Data are expressed as means ± s.e. mean. Based on the observation that the pharmacodynamic effect took place 2 to 8 h after intake of the drug the means during this interval were submitted to analysis of variance according to a cross-over design. The analysis of variance considered periods, treatments and interaction as sources of variation. A paired t-test was used to investigate differences between slow and fast acetylators with respect to the effects of cadralazine. The areas under the plasma drug concentration-time curves (AUC) were calculated using the linear trapezoidal rule. Correlation coefficients were calculated by the method of least squares. A 5% level of significance was adopted for all tests.

Results

Pharmacokinetics (Figures 2,3)

The mean plasma concentrations of cadralazine and of apparent GP 22 639 were slightly higher in slow compared with fast acetylators, but the differences were not significant statistically (Table 1). The concentrations of apparent CGP 22 639 were 0 to 6% of those of the unchanged drug. The elimination half-life of cadralazine was 2.38 ± 0.12 h (n = 12). Apparent CGP 22 639 disappeared from plasma slightly more slowly than the unchanged drug. A decrease in the plasma concentration of apparent CGP 22 639 was observed in some subjects but as the concentrations could be measured precisely only up to 6 h in most subjects, terminal $t_{½}$ values were not calculated.

Haemodynamics

Blood pressure and heart rate (Figure 2) Systolic blood pressure increased slightly after treatment, reaching a maximum after 3 h (+6.2 ± 6 mm Hg, $P < 0.05$); diastolic pressure tended to decrease. Heart rate increased significantly ($P < 0.01$), reaching a maximum after 4–6 h, and remained elevated up to 24 h (7.7 beats min^{-1} higher than after placebo; $P < 0.01$). Pulse rate was accelerated to a slightly greater extent in slow than in fast acetylators, but the difference was not significant statistically ($P = 0.1$).

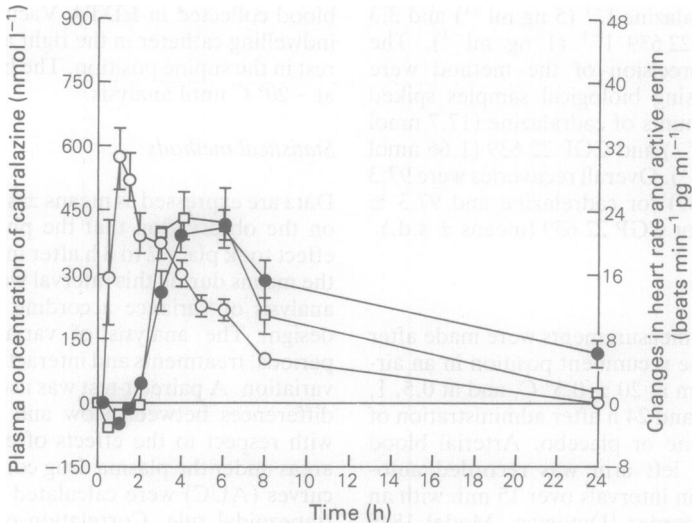


Figure 2 Plasma concentrations of cadralazine (○) and changes in heart rate (●) and active renin (□) after a single oral dose of 10 mg cadralazine to 12 healthy volunteers (means ± s.e. mean).

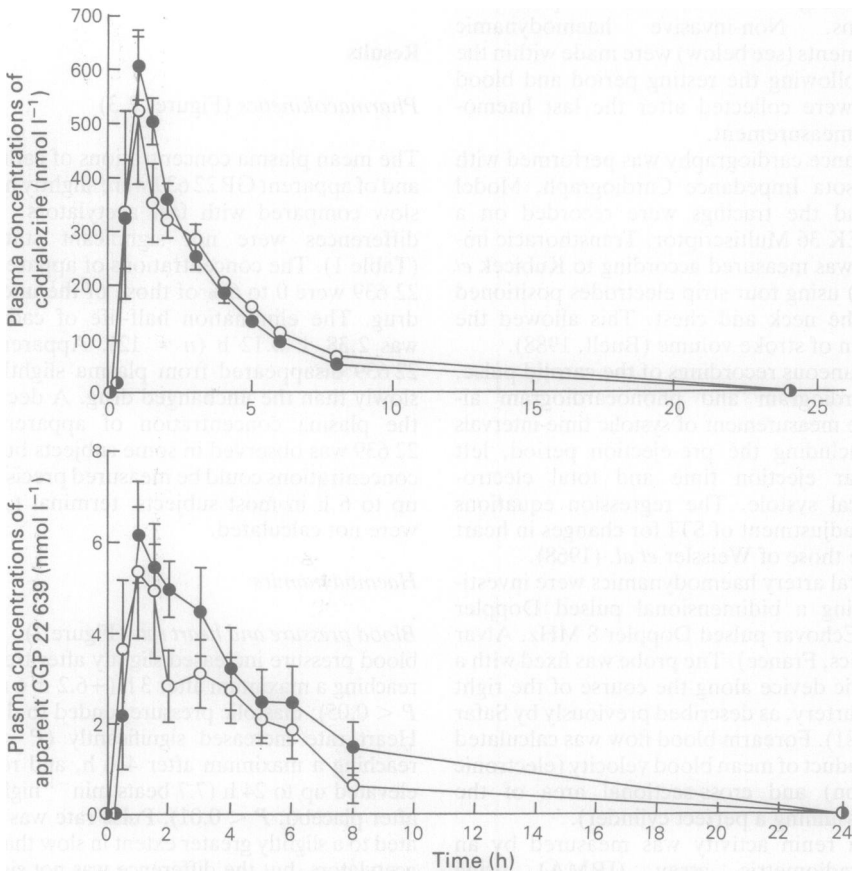


Figure 3 Mean (± s.e. mean) cadralazine and CGP 22 639 plasma concentrations in fast (○) and slow (●) acetylators ($n = 6$ in each group).

Table 1 Plasma concentration-time parameters of cadralazine and its metabolite CGP 22 639 in fast and slow acetylators (means and ranges)

	Acetylator phenotype	C_{max} *(nmol l^{-1})*	t_{max} *(h)*	*AUC (nmol l^{-1} h)*
Cadralazine	Slow	749 (498–1030)	1	1884 (1373–2330)
	Fast	611 (433–1050)	1	1956 (1367–2226)
Apparent CGP 22 639	Slow	7.41 (3.95–10.0)	1	25.6 (7.0–40.7)
	Fast	6.40 (3.53–11.0)	1	20.0 (8.1–35.6)

Cardiac haemodynamics Stroke volume was increased slightly after cadralazine as compared with placebo and was lower for slow acetylators than for fast acetylators. Cardiac output increased in parallel with pulse rate ($P < 0.01$). Total peripheral resistance decreased after cadralazine and was lower than after placebo (950 ± 50 mm Hg min ml^{-1} *vs* 1126 ± 98 mm Hg min ml^{-1}; $P = 0.1$). No changes occurred in rate-corrected systolic time-intervals.

Forearm haemodynamics Compared with placebo, no changes were observed after cadralazine in humeral artery diameter and blood velocity; humeral local resistance was lower 24 h after cadralazine than placebo (130.2 ± 10.0 mm Hg min ml^{-1} *vs* 142.9 ± 11.9 mm Hg min ml^{-1}; $P = 0.3$) and was lower in slow compared to fast acetylators ($P = 0.02$). Humeral blood flow was higher in slow compared to fast acetylators ($P = 0.03$).

Plasma renin activity (Figure 2)

Cadralazine increased plasma renin activity significantly ($P < 0.05$) and to a slightly greater extent in the slow acetylators than in the fast acetylators (maximum 175 ± 44% after 4 h *vs* 132 ± 34% after 6 h; NS). The time-course of these changes was parallel to that of the change in heart rate. A correlation was found between change in active renin and change in heart rate after 4 h ($r = 0.600$, $P < 0.05$) and between the integrals of these measurements ($r = 0.580$, $P < 0.05$) (Figure 4).

Correlation between pharmacokinetics and haemodynamics

The time-courses of the effects on heart rate and plasma renin were not superimposable on the plasma concentration-time curves of either cadralazine or apparent CGP 22 639. Peak concentration of cadralazine and CGP 22 639 were reached after 1 h, whereas the maximum responses of heart rate and renin activity occurred between 4 and 6 h after administration.

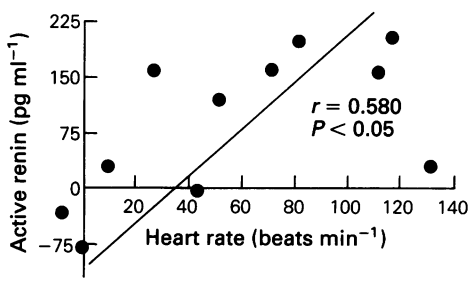


Figure 4 Relationships between renin activity and heart rate (placebo corrected areas under the curves for renin activity and heart rate).

Tolerability

Side-effects noted during the study comprised: (i) frontotemporal headache in one subject (fast acetylator) starting 9 h after drug administration and lasting until the 24th hour; (ii) moderate and transient frontal headache in another subject (fast acetylator); (iii) orthostatic hypotension in a third subject (slow acetylator).

Discussion

Since its introduction in 1952, hydralazine, an arteriolar vasodilator, has been widely used in the treatment of hypertension. Its chronic administration can provoke adverse effects, including a syndrome resembling lupus erythematosus which is ascribed to the hydrazine

group of the molecule and for which the frequency is influenced, amongst other factors, by the pharmacokinetics and metabolism of the drug (Reece *et al.*, 1980; Schmid *et al.*, 1981; Timbrell & Harland, 1979). After rapid and almost total absorption from the gastrointestinal tract, hydralazine is oxidized and acetylated in the liver. In the circulation hydralazine appears mainly as hydrazone derivatives such as pyruvic acid hydrazone. The metabolic pathway is dependent on acetylator phenotype: slow acetylators have 1.5 to 3 times higher plasma concentrations of apparent hydralazine (free plus hydrazone derivatives) than fast acetylators (Zacest & Koch-Weser, 1972). The acetylator phenotype also influences the urinary recovery of several metabolites of hydralazine (Reece *et al.*, 1980; Timbrell & Harland, 1979). This inter-individual variation in metabolism is believed to contribute to differences in the blood-pressure response to hydralazine.

Cadralazine is also an arteriolar vasodilator with a potent antihypertensive action (Carpi *et al.*, 1981), but appears to be free of the pharmacokinetic drawbacks of hydralazine. Its blood concentrations are independent of acetylator status and 60–80% of an oral dose is excreted unchanged in the urine (Schütz *et al.*, 1985). About 2% of the dose appears as CGP 22 639, a potent antihypertensive metabolite (Terauchi *et al.*, 1988), and its acetylhydrazino and methyltriazolo products. In the present study, the concentrations of apparent CGP 22 639 in plasma were found to be similar to those reported after administration of ^{14}C-labelled cadralazine, i.e. 5% of those of cadralazine (Schutz *et al.*, 1985). In contrast to findings with hydralazine, neither the plasma concentrations of cadralazine nor of CGP 22 639 were affected appreciably by acetylator status. The time-course of the mean plasma concentration of apparent CGP 22 639 was similar to that of the unchanged drug (t_{max} = 1 h), but the absolute concentrations of CGP 22 639 were about 1% those of the unchanged drug. The final plasma $t_{½}$ of CGP 22 639 could not be measured because concentrations declined to values below the limit of the assay by 6 h in most subjects. Thus, the appearance in plasma of the hydrazino metabolite formed from cadralazine is minimal in comparison with the plasma concentrations of hydralazine, and is virtually uninfluenced by acetylator status. No cases of the lupus syndrome have been reported in patients receiving cadralazine. Furthermore, patients with hydralazine-induced lupus have subsequently been treated sucessfully with cadralazine (Andersson, 1987).

The secondary objective of the study was to compare the pharmacokinetic profiles of cadralazine and its active metabolite with the haemodynamic response. Like other vasodilators (Lees & Reid, 1985; Thuilliez *et al.*, 1984), cadralazine does not exert a hypotensive effect in normotensive subjects. Thus, the arteriolar vasodilatation induced by the drug activates feed back regulation of blood pressure by the sympathetic nervous system and the renin-angiotensin system. The increase in heart rate despite any reduction in mean arterial pressure is probably due to sympathetic stimulation or parasympathetic withdrawal. In the present study there was no detectable decrease in the diameter of the humeral artery, which contrasts with the reduction observed in hypertensive patients after a single 20 mg dose of cadralazine (Laurent *et al.*, 1988). Moreover absence of a change in diameter at unaltered pressure despite stimulation of the sympathetic nervous system and the renin-angiotensin system might indicate a direct vasodilator effect of the drug on the humeral artery. The elevation of plasma renin activity paralleled the rise in heart rate. This stimulation of the renin-angiotensin system is at least partly due to sympathetic stimulation and may help to counteract the hypotensive effect of cadralazine. It has been shown that sympathetic stimulation induced by intravenously administered nicardipine was partly dependent on stimulation of the renin-angiotensin system, since converting-enzyme inhibition minimized the increase in both heart rate and plasma noradrenaline (Bellet *et al.*, 1987).

With respect to the power of the study 1-β = 70% (to detect a 20% difference in heart rate after cadralazine in fast compared to slow acetylators at the P = 0.05 level) no statistically significant differences could be detected in haemodynamic and endocrine effects as a function of acetylator status. Our results also show that the time-course of the effects of cadralazine and CGP 22 639 on heart rate and plasma renin do not follow the plasma concentration-time curves of either drug or metabolite. The time delay between concentrations and effect seems to reflect a slow penetration of drug and active metabolite to the site of action. It has been shown in rats treated with 3 mg kg^{-1} of cadralazine p.o. that CGP 22 639 accumulates in the aorta over 2 h, with a slow elimination (half-life = 11.6 h) (Terauchi *et al.*, 1988).

Our observations in healthy volunteers support the contention that cadralazine is without many of the disadvantages of hydralazine, i.e. non-specific chemical reactivity of the unshielded hydrazino-group and resultant pharmacokinetic problem, short duration of action, and signifi-

cant exposure of the organism to hydrazine-containing compounds responsible for the dose-related lupus syndrome.

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