ICI 181,037: A Novel Eukalemic Diuretic with Antiarrhythmic Activity

Sen Kau, Christopher Yochim, My Linh Do, Krystyna Leszczynska, Chester Andruskiewicz¹, Jack Schwartz¹, Jack Li and Burton Howe

Departments of Pharmacology and ¹Chemistry, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, Delaware 19897, U.S.A.

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ABSTRACT—I CI 181,037, the most active compound from a series of 1,1-diarylcarbin-1-ol-2 amines, was evaluated for diuretic and cardiovascular activity. In saline-loaded rats, the magnitude of water diuresis and saluresis produced by ICI 181,037 (10 mg/kg, p.o.) was equal to that of hydrochlorothiazide. Water diuresis and saluresis produced by ICI 181,037 were enhanced with SKF 525A, ampicillin or neomycin plus lincomycin, suggesting that ICI 181,037 is an active diuretic. In conscious dogs, the saluretic activity of ICI d-181,037 (5 mg/kg, p.o.) was about 80% of the corresponding hydrochlorothiazide value, whereas the l-isomer demonstrated only minimum saluretic activity. In both rats and dogs, the concurrent kaliuresis after ICI 181,037 or its enantiomers was minimal as compared to hydrochlorothiazide. Following chronic dosing with diuretic doses, the basal levels of plasma potassium in dogs were not altered. In amphibian in vitro models for mimicking mammalian nephron, ICI 181,037 and its enantiomers demonstrated antinatriferic and anticliloriferic activities, suggesting multiple renal sites of action for this agent. Racemic ICI 181,037 and its isomers reversed ouabain-induced arrhythmia in dogs and/or reduced the ouabain-induced mortality in mice after intravenous administration. It is concluded that ICI 181,037, particularly its d-isomer, is a novel eukalemic diuretic and possesses antiarrhythmic activity.

Diuretics have been used as a major treatment of renal and cardiovascular disorders for more than three decades. The relative success of diuretic therapies has been based upon their relatively low cost, easy titration, good patient tolerability, and few subjective and immediate side effects. Electrolyte alterations and metabolic abnormalities are the primary adverse effects. There is an abundance of literature suggesting that diuretic-induced hypokalemia may lead to the occurrence of cardiac arrhythmia (1–7), although current opinions on this issue are not necessarily unanimous. Nevertheless, the available data concerning the arrhythmogenic potential of kaliuretic diuretics are substantial enough to warrant some precautions on the part of the clinician (8). It is evident that hypokalemia induced by diuretics is a cause for concern in clinical practice. Thus, a saluretic agent which induces less urinary potassium loss resulting in euakalemia will be an attractive therapeutic agent. We report here on the pharmacology of ICI 181,037 (R* S* -2-[2-dimethylamino-1-hydroxy-1-(2-methoxy-5-(1,1-dimethylethyl)-phenyl)propyl] phenoxyacetamide), a eukalemic diuretic which possesses an antiarrhythmic property (Fig. 1).
MATERIALS AND METHODS

Diuretic activity in rats

Male Wistar rats (160–300 g) were used for the evaluation of ICI 181,037, its enantiomers and the reference agent, hydrochlorothiazide. The animals were paired and fasted overnight with free access to water according to the method developed by Kau et al. (9). Each pair of rats was given an oral load of normal saline (4% body weight) containing the test compound and placed in a metabolism cage. The test substances were prepared in suspension with the aid of 0.5% methylcellulose. The control group received saline alone. Urine samples were collected over a period of 6 hr, and their Na⁺, K⁺ and Cl⁻ concentrations were measured.

Drug interaction studies

Pretreatment with SKF 525A: SKF 525A is a highly effective microsomal enzyme inhibitor that can prevent metabolism of drugs which would otherwise convert them to pharmacologically active or inactive metabolites in animals (10). In the present study, the pretreatment of SKF 525A in rats was used to evaluate if the noted diuretic activity of ICI 181,037 resulted from the effect of its active metabolites.

Male Wistar rats (200–260 g) were divided into four groups: saline vehicle, SKF 525A, ICI 181,037, and SKF 525A plus ICI 181,037. The rats were pretreated intraperitoneally with normal saline (0.1% body weight) or a dose of SKF 525A (35 mg/kg) in the same quantity of vehicle. The pretreatment of SKF 525A was followed 30 min later by an oral load of saline (4% body weight) or ICI 181,037 (30 mg/kg) in the same quantity of saline load. The urine samples were collected over a 6 hr period, and their osmolality and electrolyte concentrations were analyzed.

Pretreatment with antibiotics: Ampicillin is known to cause reduction in the anaerobic element and partial suppression of aerobes, while the combination of neomycin plus lincomycin treatment can dramatically reduce both the aerobic and anaerobic flora (11, 12). Thus, ampicillin plus lincomycin were used to evaluate the involvement of gastrointestinal metabolism for the diuretic effect of ICI 181,037.

Male Wistar rats (200–260 g) were pretreated with ampicillin (200 mg/kg, p.o.) or lincomycin plus neomycin (100 mg/kg, p.o., each) in normal saline, in a total volume of no more than 0.25% body weight, for five consecutive days. The rats that received the same quantity of saline alone served as the control. On the 5th day, 1 hr after the pretreatment with antibiotics, the rats were dosed orally with ICI 181,037 suspended in normal saline (4% body weight) or saline alone. The urine samples were collected and analyzed according to the protocol described previously.

Diuretic activity in dogs

Well trained conscious Beagles (9–15 kg) that had free access to water, but fasted from the previous afternoon, were dosed orally with ICI 181,037, its enantiomers or hydrochlorothiazide in gelatin capsules. The doses investigated were 2.5 to 10 mg/kg, p.o. The bladder was catheterized and urine spontaneously voided into preweighed tubes as described previously in our laboratory (13). After an initial equilibration period, urine was collected every hour for two control and six post-drug samples. During the study, each animal was lightly restrained in a nylon net dog sling and had no access to water, and hourly urine loss was not replaced with fluid. Urinary osmolality, Na⁺, K⁺ and Cl⁻ concentrations were
determined in addition to measuring the hourly urine output.

**Antinatriferic and antichloriferic activity in the toad urinary bladder and cornea**

The methods of preparing the isolated toad cornea and urinary bladder for assessing the inhibitory efficacy of drugs of \( \text{Na}^+ \) and \( \text{Cl}^- \) transport (antinatriferic and antichloriferic activity) have been described in detail elsewhere (13–15). In the present study, ICI 181,037 and its enantiomers were dissolved in ethanol and the vehicle concentration was less than 1%; they were evaluated in comparison with standard diuretic agents according to those protocols.

The antichloriferic and antinatriferic activity of a drug were quantitatively evaluated by the magnitude of its inhibition on the chloride and sodium current, respectively; and the \( \text{IC}_{50} \) or \( \text{IC}_{30} \), the concentration of a drug needed to reduce the relevant transport by 50% or 30%, was determined graphically from the plot of the percentage of reduction in the current against the concentration of drug in the logarithmic scale.

**Effect on plasma potassium in dogs after dosing for 14 days**

Studies were performed on Beagles (8–14 kg), which were housed in metabolism cages. The animals were allowed free access to standard canine diet and water at all times. They were randomly divided into five groups: placebo, ICl d-181,037 (5 and 15 mg/kg), hydrochlorothiazide, (1 and 5 mg/kg). Each agent was administrated orally to the respective group at 11:30 a.m. daily for 14 consecutive days. ICI d-181,037 and hydrochlorothiazide were packed in gelatin capsules, and the placebo animals were given empty capsules. The animal had been conditioned to venipuncture prior to the initiation of the study. Brachial venous blood (4–5 ml) was collected in heparinized tubes at 2:30 p.m. daily for 14 control days and on day 7, 11 and 14 of the drug administered period.

Standard methods described previously were used for the assay of plasma \( \text{K}^+ \), \( \text{Na}^+ \) and \( \text{Cl}^- \). Hematocrit was measured using a microhematocrit centrifuge (Autocrit II, Clay Adams, NJ).

**Autonomic and cardiovascular evaluation**

Adult Beagle dogs (8–14 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Supplemental doses of sodium pentobarbital (30–60 mg, total dose) were administered during the experiment when required. The right femoral vein and artery were cannulated for injection of compounds and measurement of arterial blood pressure with a Beckman pressure transducer (P231D). Heart rate was monitored from a Beckman cardiotachometer (Type 9857) which was triggered by the arterial pulse pressure. Electrocardiograms were recorded using Lead II. All measurements were recorded on a Beckman Dynograph (Beckman Instruments, Inc., Fullerton, CA). Following the surgical procedures, a 30-min stabilization period was allowed before drug administration. ICI 181,037 or its d-isomer, dissolved in PEG 400, was administered i.v. at doses of 0.3, 1 and 2 mg/kg (total dose = 3.3 mg/kg). The 0.3 mg/kg dose of the test compound was injected over 30 sec, the 1 mg/kg dose over approximately 1 min and the 2 mg/kg dose over a 3–4 min-time period. Each compound was studied in 4 dogs. Appropriate vehicle and volume controls of PEG 400 was also evaluated in each animal. The blood pressure and heart rate response to i.v. injected submaximal bolus doses of the following autonomic drugs was determined before and after each dose of ICI 181,037 or the d-isomer (norepinephrine, 0.5 \( \mu \text{g/kg} \); isoproterenol, 0.3 \( \mu \text{g/kg} \); Tyramine, 50 \( \mu \text{g/kg} \); acetylcholine, 3.0 \( \mu \text{g/kg} \); histamine, 1.5 \( \mu \text{g/kg} \), and DMPP, 10 \( \mu \text{g/kg} \)).

The autonomic drug challenge was started approximately 15 min after each dose of ICI 181,037 or the d-isomer. The blood pressure response to angiotensin I (0.3 and 1 \( \mu \text{g/kg} \)) was also evaluated before and after ICI 181,037.
Ouabain-induced arrhythmia in dogs

Adult Beagle dogs (7 - 15 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Arterial blood pressure, heart rate and lead II ECG were monitored continuously as described previously.

ICI 181,037, as well as the enantiomers were evaluated for their ability to reverse arrhythmias induced by intravenously administered ouabain. Each test compound including the vehicle (PEG 400) control was studied in four dogs. The protocol consisted of an initial administration of 20 μg/kg ouabain followed by 10 μg/kg every 10 min thereafter until a sustained arrhythmia developed. The arrhythmia usually persisted for an hour or more during which time several doses of the test compound could be administered. The total amount of ouabain required to produce a sustained arrhythmia ranged from 40 to 55 μg/kg. Once a sustained arrhythmia had developed the test compounds were administered i.v. at a dose of 1 mg/kg. The criterion for the antiarrhythmic activity was a return to normal sinus rhythm within a few minutes after drug administration and that the normal sinus rhythm persisted for at least 30 min. If the first 1 mg/kg dose did not reverse the arrhythmia, the same dose was then repeated at 5 to 10 min-intervals until a sustained sinus rhythm was achieved.

The effect of the ICI d-181,037 on ouabain induced arrhythmia was also investigated in vagotomized dogs (N = 3) and in animals pretreated with propranolol at the dose of 1 mg/kg, i.v. (N = 3). In the vagotomized dogs, a bilateral midcervical vagotomy was performed 1/2 hr before the administration of ouabain. In the animals receiving propranolol, the beta-blocker was given 20 min prior to the administration of ouabain. In the ouabain studies, ICI 181,037 and the enantiomers were injected over an approximate 1 min-time period.

Studies with ouabain-induced mortality in mice

Male albino mice (Swiss strain, HLA) weighing 25 - 27 g were used. The lethality of ICI d-181,037 and ouabain was assayed in groups of 5 - 10 mice by i.v. injection via the tail vein. A solution of ouabain (1 mg/ml) was prepared in normal saline, and ICI d-181,037 (5 mg/ml) was formulated in distilled water with the aid of 0.5% hydroxypropyl methylcellulose and 0.1% Tween 80. A total volume of less than 0.15 ml was given intravenously for the studies. The LD₅₀ and confidence limit was computed according to the method of Tallarida and Murray (16). The maximal non-lethal dose of ICI d-181,037 (12.5 mg/kg, i.v.) was selected to evaluate the effect of ICI d-181,037 on ouabain-induced mortality in the following experiments.

Groups of 5 - 10 mice were pretreated with the vehicle (normal saline 0.5% body weight) or ICI d-181,037 (12.5 mg/kg, i.v.) at selected time intervals (1, 15 or 30 min) prior to an i.v. injection of ouabain. Ouabain was given at 1, 2.5, 3.5 or 5.0 mg/kg to different groups of mice within 15 sec. The reduction of ouabain-induced mortality by ICI d-181,037 was computed and evaluated by comparing the response obtained with the vehicle.

Local anesthetic activity in mice

A modified method of Truant (17) was used to determine local anesthetic activity of the d- and 1-isomer of ICI 181,037 in comparison with procaine and propranolol as reference agents.

Male albino mice (Swiss Strain, HLA) weighing 25 - 27 g were used. All agents were dissolved in saline and tested in four different concentrations, and the volume for injection was restricted to 0.05 ml. The intramuscular administration of compounds using a 27-gauge needle was made into the popliteal space of the right hindlimb. The loss of motor activity on the right hindlimb was taken as a sign of producing local anesthesia. This can be seen decisively by having the mice walk on a wire screen held upside down. A positive local anesthetic activity was recorded when a mouse was walking using three limbs and the left hindlimb was hanging on the wire screen held upside down. An LD₅₀ and confidence limit was computed and expressed according to the
method of Tallarida and Murray (16).

**Drugs**

ICI 181,037 hydrochloride and its enantiomers were synthesized at ICI Americas, Inc. Ampicillin sodium, furosemide, lidocaine hydrochloride, lincomycin hydrochloride, neomycin sulfate, ouabain, procaine hydrochloride and quinidine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Hydrochlorothiazide was obtained from Merck & Co. (Rahway, NJ), SKF 525A from Smith Kline & French Laboratories (Philadelphia, PA) and propranolol hydrochloride, from ICI (Alderley Park, England).

**Statistics**

Data are expressed as means ± S.E.M. unless otherwise indicated. Student's t-test or analysis of variance was used to determine the statistical significance between paired or unpaired data. P values of 0.05 or less were considered statistically significant.

**RESULTS**

**Diuretic activity in rats**

The increases in urine volume, Na⁺, K⁺ and Cl⁻ excretion after oral administration of ICI 181,037 and hydrochlorothiazide are shown in Fig. 2. ICI 181,037 dissimilar to hydrochlorothiazide produced diuretic responses in a distinct dose-related manner. The saluretic and water diuretic activity produced by ICI 181,037 at 10 mg/kg, p.o. was approximately 40–50% above the control. Hydrochlorothiazide produced comparable values at the dose equal to or greater than 10 mg/kg, p.o. ICI 181,037 appeared to be less potent than hydrochlorothiazide in terms of milligrams per kilogram required to produce the minimum water diuretic, natriuretic or chloruretic effect at a dose below 10 mg/kg, p.o. However, at doses above this dose, the effects of ICI 181,037 on water diuresis and natriuresis were significantly greater than hydrochlorothiazide, whereas the corresponding chloruretic activity of both compounds were similar. In contrast to the pronounced K⁺-wasting effect pro-

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**Fig. 2.** The dose-response relationship of the diuretic effect of ICI 181,037 (●) compared with that of hydrochlorothiazide (○) after oral administration in unanesthetized rats. Values are expressed as means ± S.E.M. for four to six experiments. UV, urine volume; UNaV, urine sodium excretion; UKV, urine potassium excretion; UClV, urine chloride excretion. Absolute values expressed per 100 g body weight for the vehicle control: UV = 2.78 ± 0.02 ml/100 g. UNaV = 0.45 ± 0.02 mEq/100 g. UKV = 0.07 ± 0.003 mEq/100 g. UClV = 0.47 ± 0.02 mEq/100 g.
duced by hydrochlorothiazide, as shown in Fig. 2, ICI 181,037 maintained kaliuresis near or slightly above the control level at all doses tested.

The d- and l-isomer of ICI 181,037 produced equal water diuresis and saluresis in rats; the potencies were similar to those of racemic ICI 181,037 (Fig. 3). In addition, both enantiomers like racemic ICI 181,037 only minimally altered urinary potassium excretion.

The pretreatment of SKF 525A, a microsomal enzyme inhibitor, did significantly modify urinary excretion of water and electrolytes. It reduced H2O, Na+ and Cl− excretion by about 40% and reduced K+ excretion and osmolality by 20% as compared to the vehicle control. Thus, as shown in Fig. 4A, the results from the groups treated with either ICI 181,037 or SKF 525A plus ICI 207,828 are summarized in terms of % vehicle control or % SKF 525A control, respectively. The water diuretic, natriuretic and chloruretic effects of ICI 181,037 in saline-loaded rats pretreated with SKF 525A (35 mg/kg, i.p.) were increased by 33%, 41%, and 52%, respectively. The effects of ICI 181,037 on urine osmolality and K+ excretion were also augmented after SKF 525A treatment. The pretreatment of ampicillin or lincomycin plus neomycin did not significantly alter urinary excretions of water and electrolytes. As illustrated in Fig. 4, B and C, co-administration of antibiotics and ICI 181,037 tended to enhance natriuresis, water diuresis and urine water and/or electrolyte output with the exception of potassium excretion.

**Diuretic activity in dogs**

By oral administration, ICI 181,037 was effective as a diuretic and saluretic agent. As illustrated in Fig. 5, ICI 181,037 produced water excretion approximately 90% of the hydrochlorothiazide value at the dose of 5 mg/kg, p.o., but the magnitude of natriuresis and chloruresis were less than the respective hydrochlorothiazide values. When the dose was increased to 10 mg/kg, p.o., ICI 181,037 increased water excretion equal to hydrochlorothiazide at 5 mg/kg, p.o., whereas the effects on Na+ and Cl− excretion were 76% and 70% of the hydrochlorothiazide values, respectively. In contrast to hydrochlorothiazide, ICI 181,037 at the doses tested, demonstrated no effect on K+ excretion. The diuretic activities elicited by ICI 181,037 in dogs appeared to predominantly reside in the d enantiomer since significant modification on urinary excretions of water and electrolytes was only noted after ICI d-181,037 but not with ICI l-181,037 (Fig. 6).

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**Fig. 3.** Comparative diuretic effects of ICI d-181,037 (○) and ICI l-181,037 (●) after oral administration in unanesthetized rats. Values are expressed as means ± S.E.M. for four to six experiments. UV, urine volume; UNaV, urine sodium excretion; UKV, urine potassium excretion; Uc,V, urine chloride excretion.
Fig. 4. The effects of (A) SKF 525A, (B) ampicillin and (C) lincomycin and neomycin pretreatment on diuretic activity of ICI 181,037 in unanesthetized rats. The results of ICI 181,037 alone (□) and drug pretreatment + ICI 181,037 (30 mg/kg) (■) are expressed in terms of % saline control or % drug pretreatment control, respectively. Each bar is expressed as means of ± S.E.M. of 6 rat pairs. *P < 0.05, **P < 0.01, ***P < 0.001, significantly different from the drug pretreated group. UV, urine volume; UNaV, urine sodium excretion; UKV, urine potassium excretion; UClV, urine chloride excretion; UOsmV, urine osmolality.

Fig. 5. Time course of the response to ICI 181,037 in comparison with hydrochlorothiazide (HCTZ) on water and electrolyte excretion in unanesthetized dogs after a dose of 5 mg/kg, p.o. Each point indicates the mean ± S.E.M. of four dogs. UV, urine volume; UNaV, urine sodium excretion; UKV, urine potassium excretion; UClV, urine chloride excretion; UOsmV, urine osmolality. □, placebo; ■, HCTZ; ○, ICI-181,037.
Fig. 6. Comparative effects of ICI d-181,037 and ICI l-181,037 on water and electrolyte excretion in unanesthetized dogs with hydrochlorothiazide (HCTZ) as the standard after a dose of 5 mg/kg, p.o. Experiments were performed in a crossover manner. Each point is the mean ± S.E.M. of three-four dogs. UV, urine volume; UN,V, urine sodium excretion; UKV, urine potassium; Uc-,V, urine chloride excretion. •, placebo (N = 4); ■, HCTZ (N = 4); ●, ICI l-181,037 (N = 4); ○, ICI d-181,037 (N = 3).

The dose-response curve for ICI d-181,037 was compared with that for hydrochlorothiazide and is displayed in Fig. 7. ICI d-181,037 seemed to be less effective in increasing salt excretion than hydrochlorothiazide based upon a mg/kg basis. At 5 mg/kg, p.o., ICI d-181,037 increased natriuresis and chloruresis, which were approximately 82% and 74% of the respective hydrochlorothiazide values. ICI d-181,037 appeared to be more effective in enhancing water diuresis than the corresponding saluresis as compared to hydrochlorothiazide. A striking difference in K⁺ excretion between these two agents was notable. ICI d-181,037 produced a negligible change in K⁺ excretion at the doses tested, whereas hydrochlorothiazide enhanced kaliuresis in a dose-related manner.

Effect on plasma potassium in dogs after dosing for 14 days

After 7 and 14 days of hydrochlorothiazide dosing, plasma K⁺ fell significantly (Fig. 8A). The magnitude of the reduction was 0.39 and 0.56 meq/l by 1 mg/kg, q.d. and 0.57 and 0.59 meq/l by 5 mg/kg, q.d., respectively. In contrast, ICI d-181,037 did not significantly change plasma K⁺ after 5 and 15 mg/kg, q.d. during a 14-day dosing regimen (Fig. 8B). The concurrent alterations of body weight, plasma electrolytes (Na⁺, K⁺ and Cl⁻) and hematocrit after both agents were negligible.

Antinatriferic and antichloriferic activity in the toad urinary bladder and cornea

Results of ICI 181,037 and its enantiomers on inhibition of Na⁺ transport in the toad bladder preparation compared to selected thiazides and amiloride are illustrated in Fig. 9, A and B. ICI 181,037 and the enantiomers all elicited a rapid and dose-dependent inhibition of Na⁺ transport when added to the mucosal side. In comparison to amiloride, a typical Na⁺ channel blocker (Fig. 9A), they were much weaker. However, in contrast to the lack of mucosal antinatriferic activity of the thiazide diuretics, their activities are noteworthy. The antinatriferic activity of racemic
ICI 181,037 is greater than the l-isomer but less than the d-isomer. These three ICI agents also demonstrated an antinatriergic response in a dose-dependent manner and equal potency when added to the serosal side. The reference agent trichlormethiazide in the serosal side inhibited sodium transport with a weaker potency than these ICI agents (Fig. 9B).

Fig. 8. Comparative effects of ICI d-181,037 and hydrochlorothiazide (HCTZ) on plasma potassium following a 14-day dosing in unanesthetized dogs. Each point represents the mean ± S.E.M. of three–four dogs. A. HCTZ: □, 1 mg/kg (N = 3); ■, 5 mg/kg (N = 4); ●, placebo (N = 3); B. ICI d-181,037: ▲, 5 mg/kg (N = 3); △, 15 mg/kg (N = 3); ●, placebo (N = 3).

ICI 181,037 is greater than the l-isomer but less than the d-isomer. These three ICI agents also demonstrated an antinatriergic response in a dose-dependent manner and equal potency when added to the serosal side. The reference agent trichlormethiazide in the serosal side inhibited sodium transport with a weaker potency than these ICI agents (Fig. 9B).

Results for the inhibition of these three ICI agents and the loop diuretic furosemide on Cl⁻ transport in the toad cornea preparation are shown in Fig. 9C. All three ICI agents demonstrated a definite antichlorferic response in a dose-dependent manner, although their potencies were weaker than those of furosemide. The difference of the antichlorferic responses among these three ICI agents was not significant.
Cardiovascular and autonomic evaluation in anesthetized, vagotomized dogs

The racemate and d-isomer were evaluated in the dose range of 0.3–2 mg/kg, i.v. At 0.3 and 1 mg/kg, both compounds had only minimal influence on the measured hemodynamic parameters. Following the 2 mg/kg dose, both compounds produced a slight (10–15%) transient (2–10 min) decline in mean arterial blood pressure with slight and variable effects on heart rate. A slight PR prolongation (95 ± 10 to 109 ± 9 msec, P < 0.05, after the racemate; 90 ± 5 to 112 ± 5 msec, P < 0.05, after d-isomer) and a widened P wave (35 ± 3 to 46 ± 4 msec, P < 0.05, after the racemate; 38 ± 2 to 52 ± 3 msec, P < 0.05, after the d-isomer) were observed on the electrocardiogram with both drugs following the 2 mg/kg dose (total dose = 3.3 mg/kg). Significant widening of the QRS and QT intervals was not observed with these doses of either the racemate or d-isomer.

The arterial blood pressure responses to i.v. doses of norepinephrine (0.5 μg/kg), tyramine (50 μg/kg), DMPP (10 μg/kg), isoproterenol (0.3 μg/kg), acetylcholine (3 μg/kg), and histamine (1.5 μg/kg) were not altered by either compound following a total dose of 3.3 mg/kg. A slight but statistically insignificant attenuation of the tachycardia response to a submaximal chronotropic dose of isoproterenol (0.3 μg/kg, i.v.) was noted after the cumulative dose of 3.3 mg/kg of either compound (heart rate response of isoproterenol before racemate: +86 ± 14 beats/min, after racemate: +76 ± 12 beats/min; heart rate response before d-isomer: +89 ± 7 beats/min, after d-isomer: +77 ± 8 beats/min). In addition, the pressor response to i.v. angiotensin I (0.3 and 1 mg/kg) was not altered by ICI 181.037.

Antagonism of arrhythmias induced by intravenous infusion of ouabain

The racemate and the d- and l-isomers were evaluated for their effectiveness in reversing ouabain-induced arrhythmias in anesthetized, non-vagotomized dogs. All three compounds converted ouabain-induced arrhythmias to
normal sinus rhythm. An example of the anti-arrhythmic effect of the d-isomer is shown in Fig. 10. The average effective cumulative i.v. dose for converting the arrhythmias was $1.5 \pm 0.3 \text{ mg/kg (range -1 to 2 mg/kg)}$ for the d-isomer, $2.5 \pm 0.6 \text{ mg/kg (range -1 to 4 mg/kg)}$ for the racemate, and $2.8 \pm 0.5 \text{ mg/kg (range -2 to 4 mg/kg)}$ for the l-isomer.

The antiarrhythmic effects of the d-isomer versus ouabain-induced arrhythmias was also evaluated in vagotomized dogs and in animals pretreated with propranolol (1 mg/kg, i.v.). The d-isomer effectively reversed the ouabain arrhythmias under both experimental conditions and at a dose level similar to that of the vagus intact dogs (Fig. 11).

**Effect on ouabain-induced mortality**

The dose-response relationship of the lethal activity of ICI 181,037 and ouabain after intravenous administration have been evaluated in mice. The LD$_{50}$ (confidence limit) for both agents were 31.6 (25.5–38.2) mg/kg and 3.35 (2.83–4.02) mg/kg, respectively. The dose of 12.5 mg/kg, i.v. for ICI 181,037 was the maximum non-lethal dose, whereas ouabain at 5 mg/kg, i.v. produced nearly 100% mortality in mice. These doses were selected to evaluate the effect of ICI d-181,037 on ouabain-induced mortality in mice. The pretreatment of ICI d-181,037 demonstrated distinct prevention of ouabain-induced mortality. The LD$_{50}$ of ouabain-induced mortality after 1, 15 and 30-min pretreatment of ICI d-181,037 were 7.14, 5.03 and 4.24 mg/kg, i.v., respectively; these values were significantly less than that obtained with ouabain alone (3.35 mg/kg, i.v.).

![Figure 10](image-url)

*Fig. 10.* An example of the effect of ICI d-181,037 (1 mg/kg, i.v.) on ouabain induced arrhythmia in the non-vagotomized, anesthetized Beagle dog. Top panel shows a control tracing and a tracing taken 30 min after a steady state arrhythmia induced by ouabain (55 μg/kg, i.v.). The interval between the two arrows indicates that ICI d-181,037 was given over 1 min. The bottom panel shows the tracings at 5 and 30 min after injection of ICI d-181,037.
Fig. 11. An example of the effect of ICI d-181,037 (1 mg/kg, i.v.) on ouabain induced arrhythmia in a non-vagotomized, anesthetized Beagle dog pretreated with propranolol (1 mg/kg, i.v.). Top panel shows a control tracing and a tracing taken 15 min after propranolol. The administration of ouabain was started approximately 20 min after propranolol. The bottom panel shows a tracing 30 min after a steady arrhythmia induced by ouabain (60,ug/kg, i.v.) and just before the injection of ICI d-181,037. The interval between the two arrows indicates that ICI d-181,037 was given over 1 min.

Local anesthetic activity in mice

ICI d-181,037 and ICI1-181,037 did not exhibit local anesthetic activity in mice up to a concentration of 20 mg/ml. Procaine and propranolol consistently demonstrated significant activity during a 20-min observation period after injection. Procaine had an IC50 of 7.9 (4.8–11.4) mg/ml, while propranolol exhibited an IC50 of 3.1 (1.4–0.44) mg/ml at 5 min after subcutaneous administration to the popliteal space.

DISCUSSION

ICI 181,037 is a diarylcarbinol derivative with a novel diuretic profile. Pharmacologically, ICI 181,037 is considered eukalemic and also possesses antiarrhythmic activity. With regard to aquaretic and saluretic effects, the oral potency of ICI 181,037 is less than that of hydrochlorothiazide in unanesthetized rats and dogs. Its diuretic action in both species resembles, in some aspects, that of thiazide diuretics and is characterized by a distal tubular action and modest ceiling effect. The diuretic response does not appear to depend primarily upon changes in renal hemodynamics inasmuch as there is no change in either glomerular filtration rate or renal blood flow after acute administration in rats and dogs (S. Kau et al., unpublished observation). In the rat, racemic ICI 181,037 produced a diuretic dose-response relationship similar to either the d- or l-isomer, and the respective oral potencies of the d- and l-isomers as well as the racemate on water diuresis and saluresis are equal. Racemic ICI 181,037 and its d- and l-isomer demonstrate an interesting profile of potassium excretion which is not shared with hydrochlorothiazide. At equal water diuresis and saluresis, the concurrent potassium excretion is minimally increased by the racemate and
enantiomers of ICI 181,037 in contrast to the profound kaliuresis produced by hydrochlorothiazide. The diuretic responses of ICI 181,037 in rats are enhanced with the pretreatment of SKF 525A and antibiotics by inhibiting microsomal enzyme and intestinal flora activity, respectively. The results clearly indicate that ICI 181,037 itself is an active diuretic agent in rats. In the dog, the d-isomer of 181,037 demonstrates the water diuretic and saluretic response in a dose-dependent manner, whereas the l-isomer produced insignificant activity. The exact mechanism whereby ICI 181,037 demonstrates a stereospecificity of diuretic activity in dogs but not in rats is not clear. As a diuretic, ICI 181,037 appears to be less potent in dogs than in rats, whereas hydrochlorothiazide is equally potent in rats and dogs. Following a dose of 5 mg/kg, p.o., ICI d-181,037 increases the aquaretic and saluretic response to approximately 100% and 80% of the hydrochlorothiazide value, respectively. In contrast to hydrochlorothiazide, ICI d-181,037 maintains the basal level of potassium excretion. In separate studies (S. Kau et al., unpublished observation), the diuretic activity of ICI d-181,037 in dogs is not reduced with the pretreatment of SKF 525A which is consistent with the finding in the rat. It seems less likely that any metabolite will be predominately responsible for the observed diuretic activity of ICI d-181,037. Plasma potassium values in dogs are not significantly altered after a 14-day regimen of ICI d-181,037 at the diuretic dose which is in contradistinction to the consistent decrease in plasma potassium produced by hydrochlorothiazide.

ICI 181,037 and its enantiomers inhibit sodium transport on both the mucosal and serosal sides of the isolated toad urinary bladder, a model widely used to investigate the effects of diuretics on sodium transport and to predict their potential renal sites of action (18–20). The ICI compounds have the characteristics of both amiloride and thiazide in this model, although they are not as active as amiloride. The balanced amiloride-like and thiazide-like diuretic activities of these compounds are compatible with and possibly contribute to their in vivo eukalemic profile.

A modest but definite inhibition of chloride transport was observed with ICI 181,037 and its enantiomers in the toad cornea model, in which furosemide demonstrates potent antichloriferic activity. Thus, ICI 181,037 and its enantiomers may also have some activity like loop diuretics. The in vitro results altogether suggest that these compounds could exert a direct renal effect with multiple sites of action in the mammalian nephron.

The d-isomer of ICI 181,037 has received extensive cardiovascular evaluation in anesthetized dogs. The effect of this compound of lead II ECG was not appreciable at doses below 2 mg/kg, i.v. A moderate prolongation of the PR interval and widening of the P wave become more evident as the dose of ICI d-181,037 was increased. In a separate study with unanesthetized dogs (S. Kau et al., unpublished observation), the d-isomer of ICI 181,037 was administered orally in acute single doses of 1, 5 and 15 mg/kg. No appreciable changes in lead II ECG was observed after the 1 and 5 mg/kg doses. At the 15 mg/kg dose level, there was moderate P wave widening and PR prolongation. Arterial blood pressure was not decreased in the conscious dog by ICI d-181,037 over the oral dose range of 1–15 mg/kg. Retest of the 15 mg/kg dose of ICI d-181,037 in the same dogs after pretreatment with SKF 525A (50 mg/kg, i.p. at 60 min prior to ICI d-181,037 administration) did not appreciably alter the magnitude of the ECG changes produced by ICI d-181,037 but prolonged the duration of the ECG changes. The results suggest that ICI d-181,037, rather than its metabolite, is responsible for the observed ECG effects. In addition, a group of three dogs receiving a 15 mg/kg, p.o. dose of ICI d-181,037 per day for 14 consecutive days demonstrated no clinical signs of toxicity; a moderate prolongation of the PR interval and widening of the P wave were noted. At 5 mg/kg, p.o. for 14 days, there were no signs of ECG changes. These ECG changes suggest a decrease in conduction velocity in the atria and
across the AV node. The racemate and l-isomer of ICI 181,037 appear to possess a similar profile of ECG changes, but they have been examined less extensively.

The results show that ICI 181,037 can effectively reverse the ouabain-induced ventricular arrhythmia after intravenous administration, it is slightly more effective than the l-isomer, but less potent than the d-isomer. The antiarrhythmic activity produced by the d-isomer is also well maintained under the conditions of beta adrenergic blockade and/or vagotomy. In addition, intravenous pretreatment with ICI d-181,037 was also found to reduce ouabain-induced mortality in mice. Seller et al. (21, 22) observed that the potassium-sparing diuretics amiloride and triamterene reduced the loss of myocardial potassium induced by digitalis in dogs. They proposed that these drugs may prevent digitalis induced arrhythmias not only by their antikaliuretic effects, but also by a direct action on the myocardium which antagonizes the loss of potassium from cardiac tissue. It is conceivable that these mechanisms proposed for amiloride and triamterene may also apply to the ICI compounds and explain their protective action against digitalis toxicity. Although ICI 181,037 and its d- and l-isomers lack significant local anesthetic activity, these compounds may possess potassium modulating properties. However, the exact mechanism(s) underlying the antiarrhythmic activity of the ICI compounds is presently unknown and studies are currently in progress in our laboratory investigating the mechanism(s) behind the antiarrhythmic activity of these ICI compounds.

Hypokalemia is a commonly encountered metabolic consequence of long-term diuretic therapy. A direct relationship between diuretic-induced hypokalemia and ventricular arrhythmia has been well-documented, although current opinions on this issue are not necessarily unanimous (8, 23, 24). Nevertheless, clinically, diuretic-induced hypokalemia is currently still considered as the primary adverse effect of the therapy in hypertension and heart failure, and it may increase the risk of digitalis-induced ventricular arrhythmia. Presently, we report ICI 181,037 and its enantiomers, especially the d-isomer, as novel eukalemic diuretics which are capable of protecting against ouabain-induced toxicity in animals. It is possible that ICI 181,037 and its enantiomers will be of great value as a research tool and potential therapy in the renal and cardiovascular area.

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