Infusion Rate-Dependent Positive Inotropic Action of Ouabain in Rabbits\textsuperscript{1)}

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The effect of the infusion rate on the relationship between plasma concentration (Cp) and positive inotropic action (PIA) of ouabain was studied in rabbits. The maximum of the first derivative of the left ventricular pressure (dP/dt\textsubscript{max}) was used as an index of PIA. The Cp of ouabain was measured by counting the radioactivity of \textsuperscript{3}H-ouabain, a nonmetabolizable cardiac glycoside. \textsuperscript{3}H-Ouabain was infused via the femoral vein at 2.7, 13 and 38 nmol/min/kg, and the time courses of Cp and dP/dt\textsubscript{max} were measured simultaneously. Non-linear initial volume of distribution was suggested from the different slopes of the Cp curves normalized by infusion rate during the initial 5 min. The relationship between Cp and PIA depended remarkably on the infusion rate. The values of Cp which produced the maximum PIA at the low, medium and high infusion rates were 136 ± 18, 700 ± 210 and 2300 ± 545 nm (mean ± S.E.M.), respectively. The total amounts of infused ouabain until the maximum PIA at the low, medium and high infusion rates were 81 ± 6.8, 130 ± 6.5 and 152 ± 7.2 nmol/kg (mean ± S.E.M.), respectively. It was clear that ouabain does not exhibit its action in the central compartment, although it is considered to be a fast-acting cardiac glycoside. It is suggested that a slow step exists in the appearance of PIA of ouabain in rabbits.

Keywords—ouabain; positive inotropic action; rabbit; infusion rate dependency; pharmacodynamics; pharmacokinetics; non-linear distribution volume

Introduction

Cardiac glycosides are used most frequently to increase the adequacy of the circulation in patients with congestive heart failure. This effect is owing to their direct action to increase the force of myocardial contraction, \textit{i.e.}, a positive inotropic action (PIA).\textsuperscript{2)} At present, it seems reasonable to assume that the following sequence of events occurs.\textsuperscript{3)} Cardiac glycosides bind to a specific site on the membrane Na\textsuperscript{+}, K\textsuperscript{+}-adenosine triphosphatase (ATPase) and decrease the active extrusion of intracellular Na\textsuperscript{+}. The consequent increase in the intracellular Na\textsuperscript{+} decreases the exchange of the extracellular Na\textsuperscript{+} for the intracellular Ca\textsuperscript{2+}. The elevated concentration of intracellular Ca\textsuperscript{2+} might increase contractility by several mechanisms.

Both in man and dog, a good correlation was found between the plasma concentration (Cp) and PIA in the elimination phase of digoxin and digitoxin.\textsuperscript{4–6)} Reuning et al.\textsuperscript{7)} and Kramer et al.\textsuperscript{8)} reported a pharmacokinetic analysis of the time course of PIA in man based on the amounts of digoxin in the deep compartment, which was calculated from Cp–time curve after i.v. administration.

In the present study, we examined the effect of the infusion rate on the relationship between PIA and Cp of ouabain, which is known to be a fast-acting, nonmetabolizable cardiac glycoside.\textsuperscript{9)}

Experimental

Materials—\textsuperscript{3}H-Ouabain was obtained from New England Nuclear Corp., Boston, MA. The specific activity
given by the manufacturer was 20 Ci/mmol. The radioactive compound was confirmed to be at least 98% pure by thin-layer chromatography (TLC) with chloroform–methanol–water (65:30:5). Unlabelled ouabain was purchased from Merck, Darmstadt, F.R.G. All other chemicals were commercial products of analytical grade.

Animal Experiments—Male, Japanese White rabbits (Nihon Igakou Dobutsu, Tokyo, Japan) weighing 2.5—3.5 kg were used. The rabbits were anesthetized with ethyl carbamate (urethan; 600 mg/kg) and α-chloralose (60 m/kg) intraperitoneally. Body temperature was kept at 37 °C using a heat lamp. The femoral vein and artery were cannulated with polyethylene tubing (PE-50) for drug administration and blood sampling, respectively. The throat was opened and a tracheal cannula was introduced for spontaneous respiration. A rigid catheter (i.d. 1.5 mm) was introduced into the left ventricle through the carotid artery. Systolic and diastolic ventricular pressures were recorded with a Gould Statham transducer (P23 i.d., Gould Inc., Oxnard, CA). The left ventricular pressure was damped by using a hemodynamic damping device (CorrecTORR; Norton, Akron, OH), to obtain an appropriate frequency response. From the ventricular pressure (P), the first derivative of P (dP/dt) was obtained by electronic differentiation. The maximum of dP/dt was used as an index of PIA (dP/dtmax). The lead II electrocardiogram (ECG) was also recorded continuously. Unlabelled ouabain was dissolved in saline and was mixed with radioactive glycoside in the range of 0.8—2.5 μCi/ml. Approximately 30 min after surgery, ouabain solution was infused intravenously through the femoral vein at 2.7, 13 and 38 nmol/min/kg (flow rates: 35—45 μl/min/kg) using an infusion pump (Harvard Apparatus, model 975E, South Natick, MA). Blood samples (approximately 1 ml) were taken into a heparinized syringe. For the control study, physiological saline without ouabain was infused.

Determination of Radioactivity—The purity of 3H-ouabain in the plasma sample at 35 min was examined by TLC as mentioned above; more than 93% was intact ouabain and no metabolite was found. Thus, we determined directly the Cp of ouabain by measuring the radioactivity in plasma. A 500 μl aliquot of plasma was mixed with 10 ml of Biofluor, high-efficiency emulsifier cocktail (New England Nuclear Corp., Boston, MA), and the radioactivity was determined in a liquid scintillation spectrometer (Packard Instruments Corp., Downers Grove, IL). Quenching was determined using automatic external standardization.

Data Analysis—The index of the PIA, I, was calculated as follows:

\[ I = \frac{E_t - E_0}{E_{max} - E_0} \]

where \( E_0 \) and \( E_t \) are the values of dP/dtmax at the base line and at time t, respectively, and \( E_{max} \) is the maximal value of dP/dtmax. \( E_t \) increased with time and reached a maximum then decreased due to toxicity in each experiment. Therefore, \( E_{max} \) represents the maximum dP/dtmax just before the appearance of toxicity.

Results

Time Course of Cp of Ouabain

Ouabain was administered intravenously by constant infusion at one of three rates, i.e., 2.7, 13 and 38 nmol/min/kg. Time courses of ouabain Cp from the initiation of the infusion
until the PIA reached the maximum value are shown in Fig. 1. The first rapid distribution phase finished within 5 min, and a rather slow phase followed. Each time course of Cp of ouabain was fitted to the two-compartment open model and the calculated pharmacokinetic parameters were obtained (not shown); however, it is not necessarily valid to compare them directly in order to evaluate the infusion rate dependency of the pharmacokinetic parameters, because of the different periods of data collection. Thus, each time course for the initial 5 min was normalized with respect to the infusion rate (Fig. 2). The higher the infusion rate, the higher the normalized Cp. Infusion rate dependency was clearly observed in Cp during the initial 5 min.

Fig. 2. Time Courses of Ouabain Cp Normalized with Respect to the Infusion Rate

Cp of ouabain was divided by its infusion rate. Symbols are the same as those in Fig. 1.

Fig. 3. Time Courses of PIA at Three Infusion Rates

The change of \(dP/dt\) was expressed as a percentage of the maximum value according to the equation. Each point and vertical bar represents the mean and S.E.M. Symbols are the same as those in Fig. 1.

Fig. 4. Relationship between Cp and PIA of Ouabain

Each point and vertical bar represents the mean and S.E.M. Symbols are the same as those in Fig. 1.

Fig. 5. Effect of the Infusion Rate of Ouabain on Cp Which Produced the Maximum PIA (\(EC_{max}\))

Each point and vertical bar represents the mean and S.E.M. Symbols are the same as those in Fig. 1. The solid line represents the linear regression line, \(EC_{max} = 61.8 \times IR - 60.0\), where \(IR\) is the infusion rate (nmol/min/kg). The regression coefficient \(r\) is 0.999 and is statistically significant \((p<0.05)\).
Time Courses of PIA of Ouabain

The time courses of PIA at the three infusion rates are shown in Fig. 3. The maximum effects were observed at 32.5 ± 2.5 min after initiation of the infusion for the low infusion rate, at 9.3 ± 0.5 min for the medium rate and at 4.0 ± 0.2 min for the high rate (mean ± S.E.M.; n = 4 or 5). The values of Cp of ouabain which produced the maximum PIA at the low, medium and high infusion rates were 135 ± 18, 700 ± 210 and 2300 ± 545 nm, respectively and the total amounts of infused ouabain were 81 ± 6.8, 130 ± 6.5 and 152 ± 7.2 nmol/kg, respectively. The values of Ec were 8695 ± 521, 7199 ± 956 and 7293 ± 1080 mmHg/s at the low, medium and high infusion rates, respectively. There is no significant difference among them. The maximum values of dP/dtmax for the low, medium and high infusion rates were 32.8 ± 5.0, 51.2 ± 22.8 and 44.4 ± 8.3%, respectively, and again there is no significant difference among them. In the control study, Et decreased by 0.5, 14.2 and 10.6% at 10, 20 and 40 min after the initiation of infusion, respectively. However, correction for this was not performed in this study.

Relationship between Cp and PIA of Ouabain

From the time courses of Cp and PIA, the relationship between Cp and PIA was plotted as shown in Fig. 4. Remarkable infusion rate dependency is apparent in this relationship. The concentration-response curve shifted to the right with increase of the infusion rate. Then, the relationship between infusion rate and effective concentration (ECmax), the Cp which produced the maximum PIA, was plotted to examine the effect of the infusion rate on PIA; the result is shown in Fig. 5. There was a good linear relation between infusion rate and ECmax.

Discussion

The present study was focused on the distribution phase within 1 h after i.v. administration of ouabain, and the sampling times differed at the three infusion rates. It is not reasonable to discuss the non-linearity based on the pharmacokinetic parameters obtained from data sampled at different periods. Thus, Cp of ouabain was normalized with respect to the infusion rate, and a remarkable infusion rate dependency was observed (Fig. 2). Since the inverse of the initial slope represents the central volume of distribution, this infusion rate dependency may result from a difference in the central volume of distribution, depending on the infusion rate. Further study is needed to confirm the non-linear pharmacokinetics of ouabain.

In clinical studies, evidence of saturable binding of digoxin to skeletal muscle was reported. In addition, an increase in the volume of distribution of the peripheral compartment was reported for digoxin in hyperthyroidism. These studies suggested that the cause of the increased volume of distribution might be an increase in the amount of Na⁺, K⁺-ATPase, because Lindsay and Parker demonstrated that both digoxin and thyroxine treatment increased tissue Na⁺, K⁺-ATPase activity in rats. Lüllmann et al reported that the binding of ouabain in isolated papillary muscle of the guinea pig was saturable. Thus, it is suggested that the binding of ouabain to Na⁺, K⁺-ATPase may contribute to the distribution of ouabain in the body.

In this study, we used the maximum value of the first derivative of the left ventricular pressure (dP/dtmax) as a sensitive and direct index of PIA, although it is difficult to apply dP/dtmax to small animals. Kramer et al. used the electromechanical systole corrected for the heart rate (QS₂I) as an index of PIA in man. The merit of their method is the applicability to small animals, although it is an indirect method. There are some reports on the effect of infusion rate on the toxicity and inotropic action; it was shown that the lethal dose of ouabain changed depending on the infusion rate. As to toxicity, all guinea pigs producing arrhythmias died as a result of their ventricular arrhythmias, independently of the adminis-
tered dose of ouabain. In this study, the maximum effect was not altered significantly at the various infusion rates. Therefore, we expressed PIA in terms of \( I \), which is normalized with respect to \( E_{\text{max}} \).

A remarkable infusion rate dependency was demonstrated in the relationship between \( \text{Cp} \) and PIA (Fig. 4) and in the relationship between infusion rate and \( \text{Cp} \) at the maximum PIA (Fig. 5), although ouabain is known to be a fast-acting cardiac glycoside. This suggests that the effective compartment exists kinetically not in the plasma compartment but in the peripheral compartment. Kramer et al. reported that PIA of digoxin after i.v. administration to man could be explained in terms of the amount in the deep compartment. It is known that ouabain binds to \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase from the extracellular side and this step is considered to be the first step of PIA. Thus, this remarkable infusion rate-dependent PIA of ouabain (Fig. 4) may be explained by the slow binding process of ouabain with \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase or by a slow step after the occupation of \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase. In fact, extensive studies have been done on the binding of cardiac glycosides to \( \text{Na}^- \), \( \text{K}^- \)-ATPase and the slow binding process of ouabain to \( \text{Na}^+ \), \( \text{K}^+ \)-ATPase might well be one reason for the infusion rate dependency.

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References and Notes

1) Taken in part from a dissertation submitted by Hideyoshi Harashima to the Graduate School, Division of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.


