Influence of Alpha-Chloralose on Muscle Relaxant Effect of Chlorzoxazone in Rats and Pharmacodynamic Analysis

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The Pharmacological response intensity of chlorzoxazone (CZX) was measured by the crossed extensor reflex (CER) method in anesthetized rats. The appropriate dose of alpha-chloralose (ACH) was examined to bring about a quantitative effect of CZX. The pharmacodynamics of CZX was also characterized in the anesthetized rats at three different doses of CZX.

When rats were anesthetized with ACH at three different doses (80, 110, and 150 mg/kg i.p.), the CER response caused by CZX at the doses of 25, 50, and 75 mg/kg *p.o.* was depressed dose-dependently. Among these three different doses of ACH, 80 and 110 mg/kg gave similar results in describing the pharmacological response induced by CZX with time, whereas a dose of 150 mg/kg of ACH elicited a stronger and longer CZX response than the other two doses. The area under the intensity-time curve also showed the same tendency. These findings suggested that the dose size ranging from 80 to 110 mg/kg of ACH for surgical anesthesia was appropriate to evaluate the quantitative pharmacological response of CZX.

Pharmacodynamic analysis offered the results that the dose-normalized biophase levels of CZX were coincident with each other when CZX was given at three different doses under ACH anesthesia at the dose of 80 mg/kg. This suggested that the disposition of CZX in rats obeyed linear kinetics and the theoretical values of CZX-induced response would be predictable. This implied that pharmacological response intensity of CZX was reasonably related to the relative biophase CZX level *via* Hill's equation.

Keywords — chlorzoxazone; crossed extensor reflex; pharmacodynamics; Hill equation; alpha-chloralose; muscle relaxant; rat; area under the intensity curve; pharmacological response intensity

Introduction

In animal experiment, anesthetic was primarily used when the pharmacological response of the drug was measured and analyzed its relation to the pharmacokinetic behavior. When the quantitative evaluation of the drug action was taken into consideration, the dose of anesthetic should be as small as in the amount enough not to interfere the evaluation of pharmacological responses intensity of the drug, and thus synergistic effects between the drug and the anesthetic must be given careful consideration.

Alpha-chloralose (ACH) is mainly used as a surgical anesthetic to measure the pharmacological response of muscle relaxants in laboratory animals.¹⁾ It was reported that ACH caused the elevation of benzodiazepine derivatives sensitivity and depressed the crossed extensor reflex (CER) in the hind limb.²⁾ In our previous paper the result also showed that ACH elicited the

increment in the potency of chlorzoxazone (CZX) using the CER method.³⁾ From these experimental observations, it may indicate that the influence of ACH on the CZX-induced pharmacological response could not be completely excluded in assessing the quantitative CER response which reflects the CZX-induced pharmacological response. However, it is possible to quantitate the drug pharmacological response with time if ACH gives the same degree of contribution to the drug sensitivity in the body during the time of the experiment.

In the present study the influence of various doses of ACH on the muscle relaxant response of CZX was examined at first, and an appropriate dose of ACH was proposed to evaluate the intensity of pharmacological response of CZX. Secondly, under the fixed condition of ACH dose the pharmacological data of CZX was analyzed to estimate the disposition kinetics of CZX.

Materials and Methods

Materials — CZX was kindly supplied by Eisai Co., Japan. All other chemicals and solvents were of analytical grade and were used without further purification. Male albino Wistar strain rats were purchased from Japan SLC Inc.

Measurement of CER Method in Rats — Male Wistar strain rats were used. Surgery was performed after fasting the rats overnight. Rats were anesthetized with ACH which was suspended in 0.5% carboxymethyl cellulose sodium (CMC) solution at concentrations of 8, 11, and 15 mg/ml, and injected intraperitoneally at a dose of 80, 110, and 150 mg/kg, respectively. The dose of 80 mg/kg was enough to achieve the required level of anesthesia within 20 min.

After the righting reflex was eliminated, the rat was fixed in the supine position and the surgical procedure for CER method was performed as previously reported.³⁾ A cannula was inserted into the trachea to enable spontaneous breathing. The right sciatic nerve was exposed and stimulated to induce the contralateral extensor reflex. Quantitative measurements were started after stable and reproducible responses to a repetitive stimulation of the sciatic nerve were obtained. CZX was suspended in 0.5% CMC solution at concentrations of 2.5, 5, and 7.5 mg/ml, and injected *via* the gullet cannula at a dose of 25, 50, and 75 mg/kg, respectively. Pharmacological response intensity, I (%), was calculated from:

$$I = (H_0 - H_t)/H_0 \quad (1)$$

where H_0 and H_t are the average twitch height before and after drug administration, respectively and the intensities were calculated every 5 min.

Pharmacological Response Intensity Analysis — To assess the significance of the degree of intensity of pharmacological response of CZX, the area under the intensity-time curve ($AUIC$), from zero to the experiment period of 120 min was calculated by the linear trapezoid rule. This variable has been used to compare the pharmacological response intensity.⁴⁾

$$AUIC_{120} = \int_0^{120} I dt \quad (2)$$

Pharmacodynamic Analysis — The procedure of pharmacodynamic analysis was reported in the previous paper.³⁾ A brief outline was described as below. The relationship between the dose of drug and intensity of drug response was quantitated using a logarithmic-logistic function (Hill equation):

$$\log [I/(1-I)] = H \log (D) - \log (K') \quad (3)$$

where H is Hill's coefficient, K' is a constant, and D denotes the dose of CZX. When Hill's equation is satisfied, a relative biophase level, $f(I)$, can be calculated:

$$f(I) = \sqrt[H]{K' \cdot I/(1-I)} \quad (4)$$

If two assumptions (1) that CZX disposition obeys linear kinetics and (2) that the intensity of CZX pharmacological response is a single-valued function of CZX concentration in biophase, *i.e.*, at the site of drug action, are satisfied, then Eq. 5 is expressed by the concept of Smolen.⁵⁾

$$f(I) = D \sum_{i=1}^n A_i \exp(-m_i t) \quad (5)$$

where A_i and m_i are equation parameters.

Statistical Analysis — Each value was expressed the mean with S.E. except for Table IV where results were reported as mean $\pm$ S.D. Statistical significance of difference was determined by Student's t -test and $p < 0.05$ was considered to be significant.

Results and Discussion

One hour after the intraperitoneal administration of ACH 80, 110, and 150 mg/kg, CZX was orally administered to rats at three different doses of 25, 50, and 75 mg/kg, and the inhibitory effect of CZX on CER was determined. The results were shown in Figs. 1 to 3. In the series of ACH 80 mg/kg i.p. with different doses of CZX, the onset of CZX response occurred at around 5 min after the administration at each dose of CZX. The intensity to depress the twitch

Fig. 1. Time Course of the Effect of Oral Dose (25 mg/kg) of CZX on the CER Method in Rats Anesthetized with Different Doses of ACH

The mean twitch height ($\pm$ S.E.M.) was measured every 5 to 120 min after CZX administration. Key: ●, ACH 80 mg/kg ($n=5$); ■, ACH 110 mg/kg ($n=5$); ▲, ACH 150 mg/kg ($n=5$).

height was increased with time and reached a peak at 10 min at each dose of CZX used. Thereafter, a progressive decline of the depressant effect was observed, and then the responses were completely recovered within the experi-

Fig. 2. Time Course of the Effect of Oral Dose (50 mg/kg) of CZX on the CER Method in Rats Anesthetized with Different Doses of ACH

The mean twitch height ($\pm$ S.E.M.) was measured every 5 to 120 min after CZX administration. Key: ●, ACH 80 mg/kg ($n=7$); ■, ACH 110 mg/kg ($n=10$); ▲, ACH 150 mg/kg ($n=6$).

Fig. 3. Time Course of the Effect of Oral Dose (75 mg/kg) of CZX on the CER Method in Rats Anesthetized with Different Doses of ACH

The mean twitch height ($\pm$ S.E.M.) was measured every 5 to 120 min after CZX administration. Key: ●, ACH 80 mg/kg ($n=5$); ■, ACH 110 mg/kg ($n=7$); ▲, ACH 150 mg/kg ($n=6$).

mental period. The higher the dose of CZX, the more the intensity of pharmacological response was increased, and it suggested that CZX produced a dose-dependent effect in reducing the CER response. The phenomena of the dose-dependent effect of CZX were also observed under the ACH anesthesia at other doses of 110 and 150 mg/kg.

Figs. 1, 2, and 3 were also depicted to reveal the influence of ACH on the CER response after oral administration of CZX at 25, 50 and 75 mg/kg, respectively. As shown in Fig. 1, the time courses of the effects of CZX under ACH anesthesia injected at both 80 and 110 mg/kg i.p. came together with time elapsed and no significant difference was observed on and after 10 min. However, CZX-induced pharmacological response under ACH anesthesia injected at 150 mg/kg i.p. was deviated from that obtained by both 80 and 110 mg/kg of anesthesia and lasted longer. The same tendency as shown in Fig. 1 was observed in Figs. 2 and 3 among the doses of ACH used.

To quantitate the influence of ACH on CER response, $AUIC_{120}$ was calculated. As shown in Figs. 1 to 3, the pharmacological response intensities of CZX under the treatment of ACH

TABLE I. Comparison of Pharmacological Response Intensity of CZX after Oral Administration (25 mg/kg) in Rats with Different Doses of Anesthetic

ACH dose (mg/kg)	$T_{\max}$ (min)	$I_{\max}^a$ (%)	$AUIC_{120}^a$ (min)
80 ($n=5$)	10	41.8 ± 7.85	14.3 ± 2.87
110 ($n=5$)	10	51.0 ± 4.93	20.3 ± 3.14
150 ($n=5$)	15	41.7 ± 12.8	32.8 ± 13.3

a) Each value represents the mean $\pm$ S.E.

TABLE II. Comparison of Pharmacological Response Intensity of CZX after Oral Administration (50 mg/kg) in Rats with Different Doses of Anesthetic

ACH dose (mg/kg)	$T_{\max}$ (min)	$I_{\max}^a$ (%)	$AUIC_{120}^a$ (min)
80 ($n=7$)	10	71.1 ± 3.88	22.5 ± 1.90^b
110 ($n=10$)	10	61.0 ± 3.68	24.2 ± 3.10^b
150 ($n=6$)	10	71.8 ± 6.27	58.8 ± 8.48

a) Each value represents the mean $\pm$ S.E. b) Significantly different from ACH 150 group, $p < 0.001$.

TABLE III. Comparison of Pharmacological Response Intensity of CZX after Oral Administration (75 mg/kg) in Rats with Different Doses of Anesthetic

ACH dose (mg/kg)	$T_{\max}$ (min)	$I_{\max}^a$ (%)	$AUIC_{120}^a$ (min)
80 ($n=5$)	10	78.0 ± 4.98	33.3 ± 1.71^b
110 ($n=7$)	15	63.6 ± 5.11	32.4 ± 4.53^b
150 ($n=6$)	10	85.1 ± 2.55	84.2 ± 4.53

a) Each value represents the mean $\pm$ S.E. b) Significantly different from ACH 150 group, $p < 0.001$.

80 and 110 mg/kg were eliminated by the time of 120 min, while the intensity under the treatment of ACH 150 mg/kg still lasted beyond 120 min. It was found that $AUIC_{120}$ under the treatment of ACH 150 mg/kg did not reflect the whole pharmacological response intensity of CZX in comparison with that under the treatment of ACH 80 and 110 mg/kg. Even under such conditions, however, this parameter, $AUIC_{120}$, was considered to be valuable enough to evaluate the degree of contribution of ACH on the CZX response. Based on this consideration, statistical analysis of $AUIC_{120}$ was carried out to observe the difference of the dose effect of ACH on the CZX response. Calculated $AUIC_{120}$ was listed in Tables I, II, and III and other variables ($I_{\max}$, maximum intensity, and $t_{\max}$, time at $I_{\max}$) were also calculated and listed in Tables I, II, and III. The results indicated that no significant differences in $AUIC_{120}$

among ACH doses of 80, 110, and 150 mg/kg at CZX dose of 25 mg/kg *p.o.* were observed (Table I). When CZX was administered at each dose of 50 and 75 mg/kg, the following results were observed: (1) there were no significant differences in $AUIC_{120}$ at ACH doses between 80 and 110 mg/kg, (2) there were statistically significant differences as $p < 0.001$ between 150 and 80 mg/kg as well as between 150 and 110 mg/kg. From these results it was noted that ACH 150 mg/kg was quite different from either 80 or 110 mg/kg in the extent of influence on the CZX action on the muscle and elicited a longer pharmacological response of CZX. This long-lasting response could imply the existence of some contributing factors for the differentiation of the CZX response at the different dose of ACH. From the kinetic point of view one possible factor resulting in the long-lasting response may be due to a change of CZX's disposition in-

fluenced by ACH which might cause changes in the protein binding characteristics of CZX. In fact, it was reported that protein binding behavior of acidic drugs, such as warfarin and phenytoin, was changed under the treatments of anesthetics, such as enflurane, halothane, and trifluoroacetic acid,⁶⁾ and it was considered that such changes were possibly due to alteration of drug-interaction with proteins when such anesthetics were concomitantly administered. The long-lasting response might also depend on the pharmacodynamic interaction, since it was reported that the elevation of drug sensitivity might have resulted from an anesthetic state caused by ACH.²⁾ Based on these observations, it may be that the degree of interaction of CZX with protein is possibly changed by ACH in the body and this can lead to eliciting the high sensitivity of CZX response at the higher dose of ACH such as observed with 150 mg/kg of ACH. During the course of our *in situ* study on the measurement of muscle relaxant action of CZX, the pharmacological response has to be measured quantitatively under the influence of ACH, so that the pharmacodynamics of CZX could be elucidated in anesthetized rats. Besides, the dose size of ACH should be small enough not to interfere with the evaluation of pharmacological response intensity of CZX. Hence in the animal experiment which required anesthetic to measure the drug response, a feasible criterion to determine the dose size of ACH was set up to minimize the amount of the dose which could give the identical or, if possible, no degree of contribution to drug sensitivity independent of the dose of ACH. As it was indicated in Figs. 1, 2, and 3 CZX produced the CER response to the same extent when the rats were anesthetized with ACH of the two dose sizes of 80 and 110 mg/kg given. Thus, the dose size of ACH ranging from 80 to 110 mg/kg could be the reasonable amount in giving a quantitative pharmacological response of CZX in rats. Kawasaki *et al.* used ACH of 80 mg/kg i.p. to measure CER responses of diazepam⁷⁾ and Kontani *et al.* used the dose of ACH 100 mg/kg i.p. to determine the pharmacological response of 2-methyl-3-aminopropiophenone.⁸⁾ These results supported our dose size of ACH to anesthetize

Fig. 4. Hill Plot of CZX under Anesthesia with ACH 80 mg/kg i.p.

Each point represented the maximum intensity ($t_{\max} = 10$ min). Hill coefficient = 1.49, $K' = 158.7$, and $r = 0.988$.

rats in determining the CER response of CZX. In the previous data³⁾ the pharmacodynamic behavior of CZX under the treatment of ACH 150 mg/kg was well described using the concept of Smolen. However, it is desirable to minimize the dose of ACH in determining the pharmacological response of the muscle relaxant, CZX. Under such a condition, we would evaluate the drug response of CZX independent of the dose of ACH. In this context, the dose size of ACH ranging 80 to 110 mg/kg seems to be desirable as compared with the dose of 150 mg/kg.

The time course of CZX-induced CER response under anesthesia with ACH at the dose of 80 mg/kg were put on pharmacodynamic analysis and the quantitative evaluation was made with the data shown in Figs. 1 to 3. As the dose-response curve obtained from this data showed a straight line ($r = 0.988$, Fig. 4), relative bi-

TABLE IV. Pharmacodynamic Parameter Values Estimated by Fitting of Pharmacological Data to Eqs. 3 and 5

Parameter	Estimate $\pm$ S.D
Hill equation's parameters	
H	1.49 ± 0.225
K'	159 ± 131
Pharmacodynamic parameters	
A_1 ($\mu\text{g/ml}$)	1.72 ± 0.149
A_2 ($\mu\text{g/ml}$)	-1.72 ± 0.149
m_1 (/min)	0.0407 ± 0.00255
m_2 (/min)	0.198 ± 0.0216

Fig. 5. Time Course of Dose-Normalized $f(I)$ after Oral Administration of CZX in Rats

The line represents the simulated values using the equation: $f(I)/D = 1.72 \times (\exp(-0.0407 \times t) - \exp(-0.198 \times t))$. Key: ●, CZX 25 mg/kg ($n = 5$); ■, CZX 50 mg/kg ($n = 7$); ▲, CZX 75 mg/kg ($n = 5$).

ophase concentration of CZX could be calculated from the intensity of pharmacological response according to the method described in experimental section and the estimated parameters, H and K' , were listed in Table IV.

Figure 5 was depicted by calculating dose-normalized $f(I)$. The time-course of dose-normalized biophase levels after oral administration of CZX was well distributed and coincident with each dose and corresponded well to the data reported in the previous papers.^{3,9} Thus, the intensity of CZX-induced response with time under ACH anesthesia was found to fit for pharmacodynamic analysis based on the dose independent kinetics. The coincident of $f(I)/\text{dose}$ was subsequently found to be best fitted to the equation of sum of exponential, which was estimated using MULTI program¹⁰ and the rest of the estimated parameters, A_1 , A_2 , m_1 , and m_2 , were listed in Table IV. The elimination phase of the time profile of $f(I)/\text{dose}$ showed a steeper slope as compared with the elimination phase described in the previous study under the treatment of ACH 150 mg/kg anesthesia.³ This difference of the elimination phase might depend on the protein binding as mentioned above

Fig. 6. Mean Intensity of Pharmacological Response vs. Time after Oral Administration of CZX in Rats

The lines represent the simulated values based on Eq. 4 with known parameter values. Key: ●, CZX 25 mg/kg ($n = 5$); ■, CZX 50 mg/kg ($n = 7$); ▲, CZX 75 mg/kg ($n = 5$).

and/or pharmacodynamic interaction. Kawasaki and Matsushita reported that ACH caused the elevation of benzodiazepine derivatives sensitivity and depressed the crossed extensor reflex in rat's hind limb.²⁾ The elevation of drug sensitivity was also reported on another study where a dose of 0.01 mg/kg i.v. of diazepam, which did not cause any behavioral changes in other reported test, caused about a 40% reduction in the long-latency supraspinal reflex of the chloralose-anesthetized cats.¹¹⁾ These results suggested that a chloralose-induced anesthetic state can give rise to a high sensitivity of CZX response and the elevation of sensitivity resulted from pharmacodynamic interaction of ACH and CZX might elicit the different slope in the elimination phase of $f(I)/\text{dose}$. The theoretical values of the time course of the intensity of CZX could be then calculated with all known parameter values and D of each dose *via* Eq. 4 (Fig. 6).³⁾ As it can be seen, the calculated line for each dose of CZX was fairly coincident with the observed values. From these results, it was explained that CZX disposition obeyed linear kinetics at least in the oral dose range ≤ 75 mg/kg and that the relative biophase CZX level, $f(I)$, could be reasonably related to its pharmacological response *via* Hill's equation. Sakiya *et*

*al.*¹²⁾ reported that CZX with an intravenous dose between 5 and 10 mg/250 g body weight in rat followed a linear kinetics, which was coincident with our linear kinetics, that is, CZX disposition obeyed linear kinetics in the oral dosing ≤ 75 mg/kg. Yasuhara and Levy reported that the cerebrospinal fluid (CSF) concentrations of CZX at the onset of loss of righting reflex were independent of the relatively slow infusion rate (0.173–0.257 mg/min) and essentially identical with the CSF concentrations at offset of the loss of righting reflex.¹³⁾ This appeared to mention that the CSF concentration of CZX reasonably represented the amount at around the site of action. It thus possibly described that the relative biophase levels of CZX reflected the CSF concentrations.

In conclusion, CZX-induced response was proven to be quantitated with time under ACH treatment and the pharmacodynamics of CZX could relate to the disposition of CZX.

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