J. Pharm. Pharmacol. 1993, 45: 678–679
Communicated August 31, 1992

Flumazenil and lignocaine-induced toxicity: is the inverse agonist type activity circadian-time dependent?

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Abstract—We have investigated the possible influence of time of day on the activity of flumazenil on lignocaine-induced toxicity in mice. The circadian pattern of the period of latency for convulsions and the induced mortality for lignocaine alone and for lignocaine plus flumazenil was not statistically significant (cosinor or analysis of variance) but the influence of flumazenil on lignocaine-induced toxicity was circadian-time dependent ($F = 27{\cdot}8$, $P = 0{\cdot}0001$).

We have previously reported a partial inverse agonist activity of flumazenil, an imidazodiazepine initially characterized as a benzodiazepine-receptor antagonist, on bupivacaine- and lignocaine-induced mortality and toxicity in mice (Bruguerolle & Emperaire 1991, 1992). Since chronotoxicity and chronokinetics have been documented for these two local anaesthetics in mice and rats (Prat & Bruguerolle 1990), it was of interest to document the possible influence of time of day on the activity of flumazenil on lignocaine-induced toxicity in mice.

Materials and methods

Animals. Male NMRI adult mice, 30 g, housed in groups of ten in plastic cages were used; food and water were freely available. All animals were maintained at a temperature of 25 ± 1°C for a minimum period of 2 weeks in an environmental room equipped with a cool white fluorescence light; the light cycle was automatically timed to last from 0600 h to 1800 h daily. This study was conducted during the month of October.

Experimental design. At each of six different time points (0400, 0800, 1000, 1300, 1800 or 2300 h) two groups of 10 animals each were injected either with a single 1 mg kg^{-1} intramuscular dose of flumazenil (flumazenil hydrochloride, 0·1 mg mL^{-1}, Roche) or saline; this dose was chosen according to previously reported data on the partial inverse agonist activity of flumazenil on lignocaine-induced mortality and toxicity in mice (Bruguerolle & Emperaire 1992). Fifteen minutes later, these two groups were injected with a 115 mg kg^{-1} intraperitoneal single dose of lignocaine (lignocaine hydrochloride, 5 mg mL^{-1}, Roger Bellon Lab.); the dose of lignocaine (115 mg kg^{-1}) was chosen as a dose inducing a near to 50% mortality along the 24 h scale. The 15 min time difference was chosen, as previously reported (Bruguerolle & Emperaire 1992), on the basis of the relative plasma half-lives of these two drugs. All animals were immediately replaced in their respective cages and observed: the number of animals presenting convulsions was noted for each group and expressed as convulsant activity percentage; the time of latency from the time of injection to the time of the beginning of induced seizures was measured for each animal. Dead animals were noted and removed. Mean ± s.e.m. was assessed and compared by two way analysis of variance and cosinor (latent period to convulsions) or by chi square (% of mortality) tests.

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Results

As shown in Table 1, the circadian pattern of the induced mortality for lignocaine alone and for lignocaine plus flumazenil was not statistically significant by the chi square test (chi square = 9·37, $P < 0{\cdot}1$ and chi square = 9·44, $P < 0{\cdot}1$, respectively) even when the highest mortality was found at 2300 h.

Table 1. Lignocaine-induced percentage of mortality with hour of administration in lignocaine alone and in lignocaine + flumazenil-treated animals.

	Clock time (h)						
Groups	08	10	13	18	23	04	Chi square
Lignocaine alone	30	40	40	10	70	20	9·37, $P < 0{\cdot}1$
Lignocaine + flumazenil	40	20	10	40	70	30	9·44, $P < 0{\cdot}1$

The convulsant activity percentage (number of animals presenting convulsions) was 100% for lignocaine alone except at 0800 h (90%) and at 1000 h (80%) and 100% for lignocaine plus flumazenil whatever the hour of administration.

The circadian pattern of the period of latency for convulsions for lignocaine alone and for lignocaine plus flumazenil shown in Table 2 was not statistically detected by cosinor or by analysis of variance.

The influence of flumazenil on lignocaine-induced toxicity was circadian time-dependent ($F = 27{\cdot}8$, $P = 0{\cdot}0001$) since the difference was significant at 0800 h ($P = 0{\cdot}007$), 1000 h ($P = 0{\cdot}008$), 1300 h ($P = 0{\cdot}05$) and at 1800 h ($P = 0{\cdot}01$) but not at 0400 h ($P > 0{\cdot}1$) or at 2300 h ($P > 0{\cdot}1$). In other words, the partial inverse agonist activity of flumazenil was significantly different during day-time but not during night-time.

Discussion

We have previously reported on the partial inverse agonist activity of flumazenil on bupivacaine (Bruguerolle & Emperaire 1991) and lignocaine-induced mortality and toxicity in mice (Bruguerolle & Emperaire 1992): the anaesthetic-induced convulsant activity (period of latency for convulsions) was significantly decreased by decreasing dosages of flumazenil (2–0·125 mg kg^{-1}); these studies were conducted between 0800 and 1200 h to avoid possible circadian variations (Bruguerolle & Prat 1987). The present data show that the influence of flumazenil on lignocaine-induced toxicity was circadian time-dependent since a partial inverse agonist activity of flumazenil was only statistically significant during the day but not during the night.

Our data should be compared with the previously reported results of Lutch & Morris (1967) on circadian time-dependency of lignocaine convulsing activity (65 mg kg^{-1} dose) with maximal values occurring during the dark (activity) period of mice, when the drug was administered at 2100 h. It should be noted that at 2300 h and 0400 h (hour of the maximal convulsant activity), flumazenil does not significantly modify the time to convulse. In contrast, the influence of flumazenil on this

Table 2. Mean ± s.e.m. period of latency for convulsions (s) induced by lignocaine according to the hour of administration in lignocaine alone and in lignocaine + flumazenil-treated animals. Statistical comparison by analysis of variance and cosinor analysis.

	Clock time (h)					
Groups	08	10	13	18	23	04
Lignocaine alone	148 ± 9	177 ± 14	150 ± 9	145 ± 7	128 ± 11	154 ± 11
Lignocaine + flumazenil	112 ± 7	123 ± 11	123 ± 8	114 ± 7	115 ± 10	129 ± 13
Analysis of variance	H:		$F = 1{\cdot}90$, $P = 0{\cdot}10$			
	Treatment:		$F = 27{\cdot}8$, $P = 0{\cdot}0009$			
	Interaction:		$F = 0{\cdot}88$, $P = 0{\cdot}49$			
Cosinor	Lignocaine alone:		$P = 0{\cdot}28$ $\varnothing = 09{\cdot}59 \pm 1{\cdot}57$ $M = 147{\cdot}7 \pm 5{\cdot}6$			
	Lignocaine + flumazenil:		$P = 0{\cdot}79$ $\varnothing = 06{\cdot}22 \pm 5{\cdot}17$ $M = 119{\cdot}2 \pm 3{\cdot}2$			

convulsant activity is significantly increased when flumazenil is injected at 0800, 1000, 1300 and 1800 h; thus, the drug interaction appears to be circadian time-dependent.

Yokoyama et al (1992) recently reported the lack of effect of flumazenil (0·1 mg kg^{-1}) on lignocaine-induced convulsions in rats: our previously reported data (Bruguerolle & Emperaire 1992) on the effect of flumazenil on lignocaine-induced convulsions in mice do not agree with these results: differences in the doses used in the two respective studies may explain such a difference.

The circadian time-dependent drug interaction documented in the present study may be explained by pharmacokinetic changes related to the hour of injection of both drugs; previously reported data on the influence of the hour of injection of local anaesthetic agents on their plasma, heart and brain levels (Prat & Bruguerolle 1990) indicated a significant temporal change of the brain passage of these drugs with higher values observed at 1000 h for bupivacaine and mepivacaine. Other workers (Klotz et al 1985) have found no significant effect of flumazenil on benzodiazepine kinetics. The pharmacokinetic effect involved in the presently reported circadian time-dependent drug interaction still remains to be documented.

The authors wish to thank J. Mouchet for excellent technical assistance.

References

Bruguerolle, B., Emperaire, N. (1991) Flumazenil and bupivacaine-induced toxicity: inverse agonist type activity. Life Sci. 49: 185–188

Bruguerolle, B., Emperaire, N. (1992) Local anaesthetics-induced toxicity may be modified by low doses of flumazenil. Life Sci. 50: 185–188

Bruguerolle, B., Prat, M. (1987) Temporal changes in bupivacaine kinetics. J. Pharm. Pharmacol. 39: 148–149

Klotz, U., Duka, T., Dorow, R., Doenicke, A. (1985) Flunitrazepam and lormetazepam do not affect the pharmacokinetics of the benzodiazepine antagonist Ro 15-1788. Br. J. Clin. Pharmacol. 19: 95–98

Lutch, E., Morris, R. (1967) Circadian periodicity in susceptibility to lidocaine hydrochloride. Science 156: 100–102

Prat, M., Bruguerolle, B. (1990) Temporal variations of brain tissue levels of the three local anaesthetics in the mouse. Ann. Rev. Chronopharmacol. 7: 261–264

Yokoyama, M., Benson, K., Arakawa, K., Goto, H. (1992) Effects of flumazenil on intravenous lidocaine-induced convulsions and anticonvulsant property of diazepam in rats. Anesth. Analg. 75: 87–90

J. Pharm. Pharmacol. 1993, 45: 679–680

Book Review

Receptor-Ligand Interactions. A Practical Approach
Edited by E. C. Hulme
Published 1992 Oxford University Press, Oxford
XX + 458 pages
ISBN 0 19 963091 7 (pbk) £25.00

My first reaction to opening the package containing the complimentary copy of the book was despair—a couple of weeks earlier I had ordered it on recommendation from a colleague. My second thought was that the book was a lot thicker than I expected! The book is edited by Ed Hulme—a very well respected grinder-and-binder and most of the chapters are written by scientists with comparable standing. The book covers many of the areas associated with ligand binding to homogenate preparations in depth and detail but disappointingly only briefly describes protocols for the performance of quantitative receptor autoradiography. The chapters guide the reader from techniques concerning tissue preparation to more complex issues about mathematical interpretation of the generated data. In addition to the more common uses of the ligand binding technique, the book also describes the use of antibodies and neurotoxins to probe receptor characteristics.

The book starts with an interesting chapter on the methods employed to synthesize radioligands and, whilst the majority of binders are more likely to utilize commercial sources for their radioligands, this is an area of knowledge all too often neglected by graduate students. The subsequent chapters comprehensively cover the important issues concerning the ligand binding technique. Of particular note is the 4th chapter by Ed Hulme and Nigel Birdsall which forms about one-quarter of the book—it provides an extensive review of the strategies, procedures and potential artefacts associated with radioligand binding (essential reading for all novice binders).

I liked the depth of knowledge in most of the chapters in this book. For instance, many previous books skip over the various practical approaches to the different methods applied to separate the bound from free radioligand (filtration, centrifugation, charcoal adsorption, gel-filtration) but this book includes chapters on each individual technique. Whilst this may not be considered essential reading by all, it at least provides an excellent reference for these techniques.