J. Pharm. Pharmacol. 1988, 40: 592–594
Communicated November 9, 1987

Circadian phase-dependent pharmacokinetics and acute toxicity of mepivacaine

BERNARD BRUGUEROLLE, MARIN PRAT, *Medical Pharmacology Laboratory, Faculty of Medicine, 27 Bd J. Moulin, F-13385 Marseille cedex 5, France*

Abstract—The aim of this study was to investigate the possible influence of the time of administration on mepivacaine acute toxicity and kinetics in mice. Four different groups of adult male NMRI mice maintained under controlled environmental conditions (lights on: =0600–1800 h) were injected at one of the following times: 1000, 1600, 1900, 2200, 0100 and 0400 h with one of four doses of mepivacaine at each time point to establish the acute toxicity (LD50). To assess chronokinetics, a single 60 mg kg^{-1} i.p. dose of mepivacaine was given to adult male NMRI mice at four fixed times: 1000, 1600, 2200 and 0400 h. Mepivacaine plasma concentrations were determined by GLC. Our data showed significant 24 h variations in the following parameters: Highest tmax value = 0·366 ± 0·073 h at 1000 h ($P < 0·005$, amplitude, maximum-minimum/mean × 100, = 184%), highest C_{max}/t_{max} ratio = 177·17 ± 9·49 at 2200 h ($P < 0·005$, amplitude = 192%), highest $V_d = 0·842 \pm 0·23$ L kg^{-1} at 2200 h ($P < 0·005$, amplitude = 158%) and highest β phase elimination half-life = 5·408 ± 1·36 h at 2200 h ($P < 0·025$, amplitude = 145%). C_{max} (amplitude = 15%), AUC_0^∞ (amplitude = 24%) and clearance (amplitude = 23%) were not significantly time-dependent. These data demonstrate a temporal pattern of mepivacaine kinetics similar to those reported previously for bupivacaine. The temporal changes in mepivacaine-induced acute toxicity may result in part from its chronokinetic changes.

Temporal changes of drug kinetics (chronokinetics) have been reported in animals and man for more than a hundred drugs (Lemmer 1981; Reinberg & Smolensky 1982; Bruguerolle 1983, 1987). We have reported data on chronokinetics of two local anaesthetic agents, lignocaine (Bruguerolle et al 1982) and bupivacaine (Bruguerolle & Prat 1987) in rodents. To document a possible relationship between chemical structure and temporal pattern of kinetics of local anaesthetics, the present report examines the pharmacokinetic changes of mepivacaine, another amide type anaesthetic agent, related to the hour of its administration by assessing temporal changes in its pharmacokinetic parameters after a single i.p. dose in the mouse. The present work also documents possible temporal changes in chronotoxicity of mepivacaine and its relationship with its chronokinetics.

Materials and methods

Adult male NMRI mice (n = 640) (30 g) were housed, ten to a cage, for a minimum of three weeks before use, with free access to food and water. Environmental conditions were controlled at a relative humidity of 50–55%, and temperature of 25 ± 1°C during October. Animals were synchronized in standard conditions to a light/dark regimen (l/d: 12: 12, with l = 0600 to 1800 h).

Acute toxicity study. Injections of mepivacaine chlorhydrate in 0·9% saline NaCl were made i.p. over 24 h at six different times: thus four groups of 20 animals each (four different doses at each time point) were injected at one of the following times: 1000, 1600, 1900, 2200, 0100 and 0400 h. After the injection, animals were returned to their cages and regularly observed during the 24 h following the administration: dead animals were removed at each observation. Acute toxicity was evaluated by calculating LD50 by the method of Miller & Tainter (1944). Single cosinor analysis (Nelson et al 1979) of LD50 was used to detect a circadian rhythm and to estimate the rhythm characteristics.

Kinetic study. At 1000, 1600, 2200 and 0400 h a total of 160 animals, 40 per chosen time, were given mepivacaine chlorhydrate solution (10 mg mL^{-1}) intraperitonealy as a single 60 mg kg^{-1} dose; blood samples were collected after decapitation 5, 10, 30, 45, 60, 120, 180 and 360 mins after drug administration. Total mepivacaine serum concentrations were determined by GLC with a flame ionization detector according to Desch et al (1981) modified by Prat & Bruguerolle (1986). Mepivacaine serum concentrations were plotted against time and pharmacokinetic parameters were determined assuming a two compartment open model: maximum concentration (C_{max}), time to reach it (t_{max}), the ratio C_{max}/t_{max}, α and β phase elimination half-lives ($t_{\frac{1}{2}}\alpha$, $t_{\frac{1}{2}}\beta$), apparent volume of distribution (V_d), plasmatic clearance (Cl) and area under the serum concentration curve extrapolated to infinity (AUC_0^∞) were assessed according to conventional methods (Wagner 1975) by a computer program. The curve fitting of the data was done by the method of residuals according to Wagner (1975); for instance the α and β phases were assessed by linear regression (3 to 4 points for α and 4 to 5 for β phase). All data were quantified (mean ± s.e.m.) and compared by statistical analysis (analysis of variance, ANOVA). For each kinetic parameter, the amplitude of the temporal change was calculated as maximum-minimum/mean × 100 giving percentage of the 24 h mean.

Results

Acute toxicity. The results are presented in Fig. 1; these data

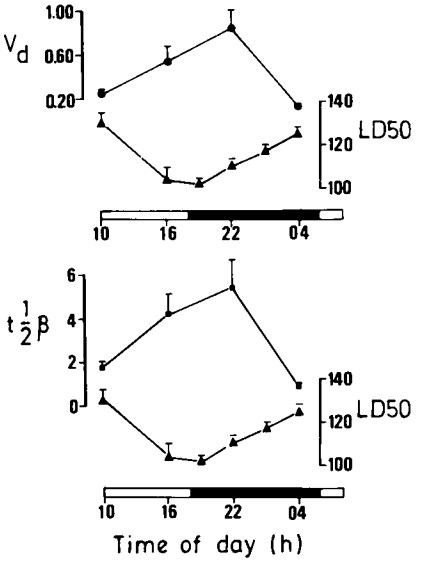

FIG. 1. Temporal variations of mepivacaine acute toxicity (LD 50) (▲——▲) of apparent volume of distribution of mepivacaine (●——●) and of its β elimination half-life (■——■).

Correspondence to: B. Bruguerolle, Medical Pharmacology Laboratory, Faculty of Medicine, 27 Bd J. Moulin, F-13385 Marseille cedex 5, France.

Table 1. Circadian variations of the pharmacokinetic parameters of mepivacaine after a single 60 mg kg^{-1} i.p. dose at four different times.

Hour	C_{max}	t_{max}	C_{max}/t_{max}	V_d	$t_{\frac{1}{2}}\alpha$	$t_{\frac{1}{2}}\beta$	Cl	AUC_0^∞
1000 h	12·63 ± 1·49	0·37 ± 0·07	49·74 ± 0·45	0·27 ± 0·01	0·49 ± 0·06	1·79 ± 0·29	0·096 ± 0·010	10·99 ± 1·1
1600 h	14·13 ± 1·93	0·08 ± 0·0	170·27 ± 23·23	0·54 ± 0·14	0·54 ± 0·03	4·23 ± 1·00	0·084 ± 0·006	12·16 ± 0·92
2200 h	14·70 ± 0·79	0·08 ± 0·0	177·17 ± 9·49	0·84 ± 0·23	0·42 ± 0·03	5·41 ± 1·36	0·106 ± 0·006	9·57 ± 0·57
0400 h	14·44 ± 0·24	0·08 ± 0·0	173·92 ± 5·90	0·14 ± 0·01	0·40 ± 0·01	0·93 ± 0·02	0·103 ± 0·008	10·05 ± 0·89
ANOVA	F = 0·41 ns	F = 11·99 $P < 0·005$	F = 13·51 $P < 0·005$	F = 4·24 $P < 0·025$	F = 2·31 ns	F = 5·20 $P < 0·025$	F = 1·04 ns	F = 0·40 ns
Amplitude %	14·8	184·0	192·2	158·0	31·0	145·0	23·0	24·0

C_{max} ($\mu g\ mL^{-1}$) = maximum concentration. t_{max} (h) = time to reach it. V_d (Lkg^{-1}) = apparent volume of distribution, $t_{\frac{1}{2}}\alpha$ (h) and $t_{\frac{1}{2}}\beta$ (h) = α or β phase elimination half-life. Cl($Lkg^{-1}\ h^{-1}$) = total plasma clearance. AUC_0^∞ ($\mu g\ mL^{-1}\ h^{-1}$) = area under the serum concentration curve.

indicate a circadian variation in the acute toxicity of mepivacaine: the maximum LD50 value occurred at 1000 h and the minimum value (i.e. maximal toxicity) occurred at 1900, i.e. at the beginning of the dark phase of the photoperiod. Single cosinor data validated a statistically significant circadian rhythm ($P < 0·01$, $\%R = 95·2$) with estimates for rhythm parameters ± s.e.m. as follows:

mesor (24 h-mean) = 117·08 ± 1·39 mg kg^{-1}

Amplitude (half the peak-through difference) = 14·69 ± 1·86 mg kg^{-1}

Acrophase (peak time of the variation) = 06·57 ± 0·30 (h/min)

Kinetics. Table 1 shows the different pharmacokinetic parameters (mean ± s.e.m.) of mepivacaine after a single 60 mg kg^{-1} i.p. dose at 1000, 1600, 2200 and 0400 h and statistical analysis (ANOVA) of the comparison. This table also shows, for each parameter, the amplitude of the temporal changes. Thus, our results indicate a significant change in absorption processes, C_{max}/t_{max} being highest at 2200 h, a significant change in elimination half-life, $t_{\frac{1}{2}}\beta$ being highest at 2200 and lowest at 0400 h. Volume of distribution varied with a maximum value observed when the drug was given at 2200. C_{max}, $t_{\frac{1}{2}}\alpha$. Cl and AUC_0^∞ were not found to be significantly different according to the hour of administration.

Discussion

Chronokinetics (i.e. temporal variations of kinetics) of some local anaesthetics have been previously reported by our laboratory (Bruguerolle et al 1982, 1987): lignocaine and bupivacaine kinetics were found to depend on the time of administration. The present study aimed to document chronokinetics of another amide type local anaesthetic agent, mepivacaine, which is structurally related to bupivacaine and lignocaine, to demonstrate a relation between chemical structure and circadian chronokinetic pattern; this work also aims to try to correlate chronokinetics with chronotoxicity of mepivacaine; indeed numerous studies have assessed chronotoxicity of drugs, particularly anaesthetic agents, but very few studies have tried to correlate chronotoxicity with chronokinetics. Our data demonstrate that the acute toxicity and kinetics of mepivacaine depend on the hour of its administration.

Concerning chronotoxicity, our results clearly show (Fig. 1) that the temporal variations of mortality (LD50) mirror the temporal changes of some kinetic parameters such as V_d or $t_{\frac{1}{2}}\beta$: thus, the lowest mortality (highest LD50 values) correspond to the highest values of V_d or $t_{\frac{1}{2}}\beta$ (i.e. 1000 and 0400 h); at the opposite, the highest mortality occurred at 1900 h, the longest $t_{\frac{1}{2}}\beta$ and the highest V_d occurring at 2200 h. Thus chronotoxicity of mepivacaine may be, in part at least, explained by chronokinetics.

Concerning chronokinetic data, these temporal changes may result from several mechanisms:

(i) *Absorption.* It could be supposed that temporal variations in drug absorption are not of great importance when the i.p. route is used. Our data reveal a temporal variation of mepivacaine resorption since t_{max} and C_{max}/t_{max} changes indicate a highest resorption in the dark period and a lowest resorption of 1000 h. These results totally agree with previous data on bupivacaine (Bruguerolle & Prat 1987) and lignocaine (Bruguerolle et al 1982) and may be explained as previously supposed by temporal variations of membrane permeability or temporal changes of peritoneal blood flow. As reported by Dore et al (1984), peripheral blood flow is highest in rodents during the dark period, maximal values of hepatic, intestinal and muscular blood flow being observed between 2100 and 0300 h. When the present data are compared with bupivacaine chronokinetic values it appears that the faster rate of absorption of mepivacaine agree with its well-known shorter duration of action. If the amplitude of the temporal changes of mepivacaine resorption are twice as high than those observed for bupivacaine, nevertheless the two local anaesthetic agents seem to undergo the same temporal pattern.

(ii) *Distribution.* Since blood flow and blood volume are known to be greater in rodents during the dark period (Lew 1976) it is not surprising that mepivacaine volume of distribution varies with time and is highest when the drug is administered at 2200 h. Since mepivacaine has been reported to be only 77% bound to plasma proteins (Tucker & Mather 1975), temporal changes in the apparent volume of distribution of the drug may result from blood volume, tissue perfusion or protein binding temporal variations. Eating habits during the dark activity period are known to enhance the peritoneal blood flow (Vatner et al 1970) and thus may participate in the observed changes.

(iii) *Metabolism.* Mepivacaine, is mainly metabolized in the liver where it undergoes *N*-demethylation and aromatic hydroxylation (Tucker & Mather 1975; Reynolds 1984). Circadian variations of liver enzyme activity have been reported by many authors (reviewed in Bruguerolle 1983); for instance, more recently Belanger et al (1987) demonstrated circadian changes in nitroanisole demethylase and aniline hydroxylase with a maximum occurring in the dark period. We also demonstrated a circadian variation in the *N*-acetylation of procainamide in rats with a peak at 0400 h (Bruguerolle & Jadot 1985). Since elimination half-life of mepivacaine is shortest when the drug is given at 0400 h, our findings indicate a greater metabolism of the drug in the last hours of the dark period. Bupivacaine elimination half-life was previously reported to be longest at 2200 h and shortest at 1000 h with an amplitude of 35·5% (Bruguerolle & Prat 1987). Mepivacaine elimination half-life temporal variations have a very similar pattern but the amplitude of the variation is more than four times higher.

These differences may also be explained by different metabolic patterns of the two drugs (oxidation by *N*-dealkylation for bupivacaine and *N*-demethylation or hydroxylation for mepivacaine).

(iv) *Elimination.* Mepivacaine is excreted in the urine as its metabolites resulting from aromatic hydroxylation and small amounts (5–10%) of unchanged drug. Mepivacaine chronokinetics may proceed partially from temporal changes in urinary excretion of the drug: for instance, variations of the urinary pH may lead to an acidification and thus enhance urinary excretion of mepivacaine.

In conclusion, chronokinetics of mepivacaine may be explained by several mechanisms involving temporal variations of absorption, distribution, metabolism and elimination: The present work assess temporal changes in mepivacaine induced-acute toxicity so that temporal variations in chronokinetics of this drug: chronotoxicity may be at least in part explained by chronokinetic data.

References

Belanger, P. M., Bruguerolle, B., Desgagne, M. (1987) Temporal variations in the inductive effect 3,4-benzpyrène in rat. in: Reinberg, Smolensky & Labrecque. (eds), Annual review of Chronopharmacology, 3, Pergamon Press, Oxford, pp 361–364

Bruguerolle, B. (1983) Influence de l'heure d'administration d'un médicament sur sa pharmacocinétique. Thérapie 38: 223–235

Bruguerolle, B. (1987) Chronopharmacokinetics. Pathol. Biol. 35: 925–934

Bruguerolle, B., Jadot, G. (1985) Circadian changes in procainamide and *N*-acetylprocainamide kinetics in the rat. J. Pharm. Pharmacol. 37: 654–656

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

Bruguerolle, B., Jadot, G., Valli, M., Bouyard, L., Bouyard, P. (1982) Etude chronocinétique de la lidocaine chez le rat. J. Pharmacol. (Paris) 13: 65–76

Desch, G., Cavadore, D., Jullien, Y., Mercier, L., Descomps, B., De Rodez, M. (1981) Dosage rapide et simultané des principaux anesthésiques locaux de type amide par chromatographie gaz-liquide. Ann. Anesth. Franc. 2: 158–168

Dore, F., Belanger, P. M., Labrecque, G. (1984) Distribution tissulaire de microsphères radioactives en fonction de l'heure du jour et de l'état nutritionnel chez le rat. L'union Med. Can. 38: 694

Lemmer, B. (1981) Chronopharmacokinetics. In: Breimer, D., Speiser, P. (eds) Topics in pharmaceutical sciences. Elsevier North Holland, Biomedical Press, pp 49–68

Lew, G. M. (1976) Circadian rhythms in blood pressure and norepinephine in genetically hypertensive and normotensive rats. Gen. Pharmacol. 7: 35–40

Miller, L., Tainter, M. L. (1944) Estimation of ED50 and its error by means of logarithmic-probit paper. Proc. Soc. Exp. Biol. Med. 57: 262–264

Nelson, W., Liang-Tong, Y., Lee, J. K., Halberg, F. (1979) Methods for cosinor rhythmometry. Chronobiologia 6: 305–323

Prat, M., Bruguerolle, B. (1986) Rapid simultaneous gas-liquid chromatographic determination of five local anaesthetic drugs in serum. Clin. Chem. 32: 2098

Reinberg, A., Smolensky, M. (1982) Circadian changes of drug disposition in man. Clin. Pharmacokinet. 7: 401–420

Reynolds, F. (1984) The pharmacology of local anaesthetic drugs. In: Churchill-Davidson, M. C. (ed.), Willie and Churchill-Davidson's A practice of anaesthesia. Lloyd-Luke, London, pp 830–843

Tucker, G. T., Mather, L. E. (1975) Pharmacokinetics of local anaesthetic agents. Br. J. Anaesth. 47: 213–224

Vatner, S. F., Franklin, D., Van Citters, R. L. (1970) Coronary and visceral vasoactivity associated with eating and digestion. Am. J. Physiol. 219: 1380–1385

Wagner, J. G. (1975) Fundamentals of clinical Pharmacokinetics. In: Drug Intelligence Publications (eds), Hamilton, pp 241–278