J. Pharm. Pharmacol. 1997, 49: 293–295
Received April 30, 1996
Accepted August 21, 1996

© 1997 J. Pharm. Pharmacol.

Kinetics of Bupivacaine After Levcromakalim Treatment in Mice

M. GANTENBEIN, L. ATTOLINI AND B. BRUGUEROLLE

Laboratoire de Pharmacologie Médicale et Clinique, ER 311, Faculté de Médecine de Marseille, 27 Bld J. Moulin, F-13385 Marseille Cedex 5, France

Abstract

Previous workers have reported that 0·01 mg kg^{-1} of levcromakalim injected intraperitoneally did not modify bupivacaine-induced neurotoxicity but increased the duration of action of bupivacaine. This study was designed to document possible changes in the pharmacokinetic behaviour of bupivacaine and its main metabolite, *N*-desbutylbupivacaine in mice after a single 0·01 mg kg^{-1} intraperitoneal injection of levcromakalim.

The kinetic parameters of bupivacaine were determined after a single 20 mg kg^{-1} intraperitoneal injection of bupivacaine in controls and in levcromakalim-treated mice. It was found that levcromakalim did not change any kinetic parameters of bupivacaine or of its main metabolite, *N*-desbutylbupivacaine. The previously reported findings of the influence of the low dose (0·01 mg kg^{-1}) of levcromakalim on bupivacaine-induced toxicity agree well with the lack of influence of 0·01 mg kg^{-1} of levcromakalim on bupivacaine and *N*-desbutylbupivacaine pharmacokinetics, although the reported increase in the duration of action of bupivacaine after levcromakalim treatment can hardly be explained by the pharmacokinetics of bupivacaine when associated with levcromakalim.

We suggest that levcromakalim might interfere directly with ion-channel block caused by bupivacaine by altering conduction properties or indirectly by enhancing bupivacaine-induced voltage and time-dependent sodium-channel block.

This study is concerned with the interaction of one of the most commonly used local anaesthetic drugs, bupivacaine, with a potassium-channel agonist, levcromakalim. The influence of potassium-channel agonists on bupivacaine toxicity and local anaesthetic activity have recently been documented by Gantenbein et al (1995, 1996). They reported that levcromakalim, at the same concentration as that tested in this study, did not modify bupivacaine-induced neurotoxicity but increased the duration of action of bupivacaine. The characteristic pharmacological effects of levcromakalim are mediated, at least in part, via the opening of (voltage-independent) ATP-sensitive potassium channels (Edwards & Weston 1993). The benzopyran levcromakalim also has effects on intracellular calcium storage and release mechanisms (Hamilton et al 1993). Hamilton et al (1993) reported that the reduction in blood pressure produced by levcromakalim is a result of direct vasodilator action attributed to the activation of potassium channels in vascular smooth muscle. The specific cardiovascular properties of levcromakalim might provide an explanation of the observed interaction with bupivacaine, for instance by inducing variations in the blood flow and therefore influencing bupivacaine uptake. The mechanism of the described interaction was supposed to proceed, at least partially, as a result of pharmacokinetic interactions. This work aims to verify such a hypothesis by documenting possible changes in the kinetics of bupivacaine and its main metabolite *N*-desbutylbupivacaine in mice after a single intraperitoneal injection of 0·01 mg kg^{-1} levcromakalim.

Correspondence: B. Bruguerolle, Medical and Clinical Pharmacology Laboratory, Faculty of Medicine of Marseilles, 27 Bd J. Moulin, F-13385 Marseilles Cedex 5, France.

Materials and Methods

Animals

NMRI adult (30 g) male mice (from Iffa-Credo) were housed five to a cage with free access to food and water. For a minimum of two weeks before use all animals were kept under controlled conditions: relative humidity 50–55%, temperature $24 \pm 1°C$ and synchronized light-dark cycle (light 0600–1800 h, dark 1800–0600 h). The experiment was conducted during February. NMRI male mice were chosen because most of our studies of local anaesthetics were conducted on this strain (females were excluded from this work in order to avoid an additional factor of variation).

Protocol

Two groups of 35 animals each were used for this experiment. The first group received bupivacaine (20 mg kg^{-1} i.p.; bupivacaine hydrochloride, 5 mg mL, Astra France (Marcaïne, France; batch number 1017–1) 30 min after a saline injection; the second group received the same dose of bupivacaine 30 min after a 0·01 mg kg^{-1} intraperitoneal injection of levcromakalim (BRL 38227, batch number 5, 10 mg mL^{-1}, Smith Kline Beecham Laboratories). To avoid possible circadian influence bupivacaine was injected at 1000 h for each group (Bruguerolle & Prat (1987) have reported that bupivacaine kinetics vary according to the hour of administration). Blood samples were collected by decapitation 0·25, 0·5, 0·75, 1, 2, 4 and 6 h after drug administration.

Determination of bupivacaine concentrations and pharmacokinetic parameters

Serum concentrations of total and free bupivacaine and its main metabolite, *N*-desbutylbupivacaine, were determined by a specific gas-liquid chromatographic method according to the

technique of Bjork et al (1990) modified as described by Lorec et al (1994). For analytical reasons (*N*-desbutylbupivacaine concentrations reached the limit of quantification) *N*-desbutylbupivacaine could only be determined in the blood samples collected until 1 h after bupivacaine administration. The limit of quantification of the method was 15 ng mL^{-1} and the reproducibility between days was good (coefficient of variation was less than 6%). Levcromakalim did not interfere with the assay. Serum bupivacaine concentrations were plotted against time and pharmacokinetic parameters were determined assuming a two-compartment open model. The maximum serum concentration, C_{max}, and the time of maximum serum concentration, T_{max}, were derived directly from individual data. The β-phase elimination half-life ($t_{\frac{1}{2}}\beta$) was calculated by non-linear regression from the equation $t_{\frac{1}{2}}\beta = \log 2/\beta$ and the area under the serum concentration-time curve (AUC_0^{∞}) was calculated by the trapezoidal rule according to a non-linear fitting method using PharmK (SoftRes Inc., Atlanta, USA), pharmacokinetic software for the Macintosh computer (Lu & Mao 1993). Total plasma clearance (Cl) and total volume of distribution (Vd) were calculated according to the equations:

$$Cl = F \cdot dose/AUC \quad (1)$$

where F is equal to 1
and

$$Vd = Cl/\beta \quad (2)$$

The C_{max}, T_{max} and the ratio AUC *N*-desbutylbupivacaine/AUC bupivacaine were calculated. All data were summarized (means ± s.e.m.) and compared by use of Student's *t*-test.

Results

Serum bupivacaine and *N*-desbutylbupivacaine concentrations (mean ± s.e.m.) are plotted against time for controls and levcromakalim-treated animals in Figs 1 and 2. Bupivacaine pharmacokinetic parameters (C_{max}, T_{max}, AUC, Vd, Cl, $t_{\frac{1}{2}}\beta$) are shown in Table 1. Levcromakalim treatment did not significantly change any of the bupivacaine kinetic parameters. The main pharmacokinetic parameters (C_{max}, T_{max}, AUC *N*-desbutylbupivacaine/AUC bupivacaine) of *N*-desbutylbupivacaine are shown in Table 2. T_{max} for *N*-desbutylbupivacaine was significantly increased after levcromakalim treatment.

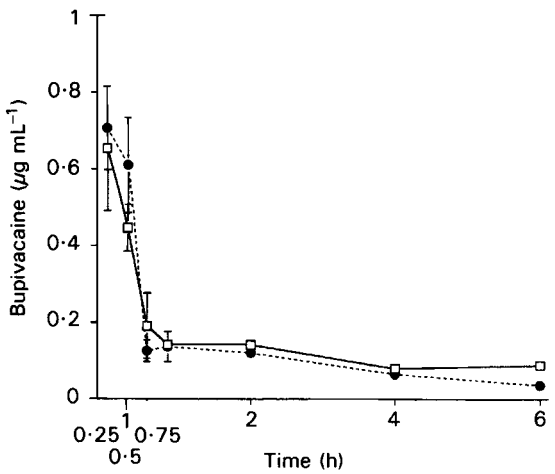

FIG. 1. Bupivacaine serum concentrations in mice receiving a single dose (20 mg kg^{-1}) of bupivacaine after either saline □, (group 1) or a single dose (0·01 mg kg^{-1}) of levcromakalim ●, (group 2).

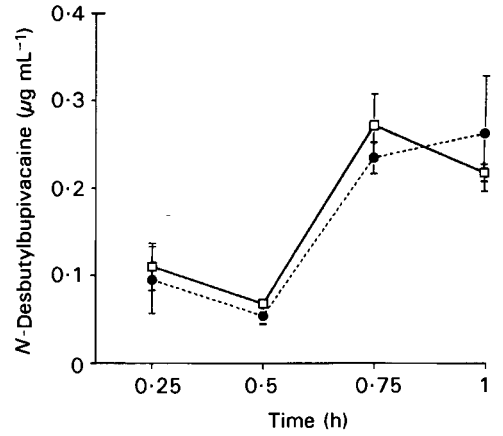

FIG. 2. *N*-desbutylbupivacaine serum concentrations in mice receiving a single dose (20 mg kg^{-1}) of bupivacaine after either saline □, (group 1) or a single dose (0·01 mg kg^{-1}) of levcromakalim ●, (group 2).

Discussion

It has previously been reported that potassium-channel agonists, and in particular levcromakalim, modify the acute toxicity and the local anaesthetic activity of bupivacaine (Gantenbein et al 1995, 1996). The increase in bupivacaine-induced mortality caused by high doses of levcromakalim can be explained by delayed mortality but was not modified by the 0·01 mg kg^{-1} dosage of levcromakalim. The mean latency time for convulsion was, furthermore, increased in a dose-dependent manner by levcromakalim, except for the 0·01 mg kg^{-1} concentration. Finally bupivacaine-induced convulsant activity characterizing bupivacaine neurotoxicity was not significantly modified by the 0·01 mg kg^{-1} dose of levcromakalim. These discoveries of the influence of the low dose (0·01 mg kg^{-1}) of levcromakalim on bupivacaine-induced toxicity agree well with the lack of influence of 0·01 mg kg^{-1} of levcromakalim on bupivacaine and *N*-desbutylbupivacaine pharmacokinetics, although the reported increase in the duration of action of bupivacaine after levcromakalim treatment can hardly be explained by the pharmacokinetics of bupivacaine when administered with levcromakalim. We suggest that levcromakalim might interfere with bupivacaine-induced ion-channel block either directly by altering conduction properties or indirectly by enhancing bupivacaine-induced voltage and time-dependent sodium-channel block. Some complementary studies are necessary to explain the mechanisms involved in the levcromakalim-induced prolongation of local anaesthetic activity of bupivacaine.

Acknowledgements

This work was partly supported by a grant from DGRT, Aix-Marseilles II University Faculty of Medicine Marseilles, France. The authors wish to thank Dr Sue Patak from SmithKline Beecham Pharmaceutical Laboratories for the

Table 1. Pharmacokinetic parameters of bupivacaine in serum after administration of bupivacaine, either alone or with levcromakalim.

Parameter	Bupivacaine	Bupivacaine + levcromakalim	Student's *t*-test *P* value
Maximum serum concentration ($\mu g\ mL^{-1}$)	0·654 ± 0·161	0·718 ± 0·093	0·740
Time of maximum serum concentration (h)	0·250 ± 0·000	0·300 ± 0·050	0·347
Area under the serum concentration-time curve ($\mu g\ mL^{-1}\ h$)	0·876 ± 0·125	0·796 ± 0·119	0·653
Total volume of distribution (L)	0·749 ± 0·211	0·478 ± 0·123	0·300
Total plasma clearance ($L\ h^{-1}$)	0·349 ± 0·110	0·558 ± 0·069	0·147
β Phase elimination half-life (h)	9·24 ± 4·44	5·33 ± 2·38	0·437

Values are means ± s.e.m.

Table 2. Pharmacokinetic parameters of *N*-desbutylbupivacaine in serum after administration of bupivacaine, either alone or with levcromakalim.

Parameter	Bupivacaine	Bupivacaine + levcromakalim	Student's *t*-test *P* value
Maximum serum concentration ($\mu g\ mL^{-1}$)	0·272 ± 0·036	0·296 ± 0·038	0·660
Time of maximum serum concentration (h)	0·75 ± 0·00	0·95 ± 0·05	0·004
Area under the serum concentration–time curve ($\mu g\ mL^{-1}\ h$)	0·341 ± 0·031	0·395 ± 0·036	0·725
Ratio (area under the *N*-desbutylbupivacaine serum concentration–time curve)/(area under the bupivacaine serum concentration–time curve)	0·431 ± 0·031	0·349 ± 0·023	0·065

gracious gift of levcromakalim. Special thanks to J. Mouchet for technical assistance.

References

Bjork, M., Petterson, K. J., Osterlof, G. (1990) Capillary gas chromatographic method for the simultaneous determination of local anesthetics in plasma samples. J. Chromatogr. 533: 229–234

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

Edwards, G., Weston, A. H. (1993) The pharmacology of ATP-sensitive potassium channels. Annu. Rev. Pharmacol. Toxicol. 33: 597–637

Gantenbein, M., Attolini, L., Bruguerolle, B. (1995) Effects of four potassium-channel agonists on bupivacaine-induced toxicity in mice. Life Sci. 57: 113–116

Gantenbein, M., Attolini, L., Bruguerolle, B. (1996) Potassium-channel agonists modify the local anesthetic activity of bupivacaine in mice. Can. J. Anaesth. 43: 871–876

Hamilton, T. C., Beerahee, A., Moen, J. S., Price, R. K., Ramji, J. V., Clapham, J. C. (1993) Levcromakalim. Cardiovasc. Drug Rev. 11: 199–222

Lorec, A. M., Bruguerolle, B., Attolini, L., Roucoules, X. (1994) Rapid simultaneous determination of lidocaine, bupivacaine and their two main metabolites using capillary gas liquid chromatography with nitrogen phosphorus detector. Ther. Drug Monit. 16: 592–595

Lu, D. R., Mao, F. (1993) An interactive program for pharmacokinetic modeling. J. Pharm. Sci. 82: 537–542