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Kinetics of Bupivacaine After Nicorandil Treatment in Mice

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Abstract

Previous studies have reported interactions between potassium-channel agonists and 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 1 mg kg^{-1} intraperitoneal injection of nicorandil.

The kinetic variables of bupivacaine were determined after a single 20 mg kg^{-1} intraperitoneal dose of bupivacaine in controls (group 1) and in nicorandil-treated mice (group 2). The maximal concentration in the serum (C_{max} 0.618 ± 0.051 vs 0.408 ± 0.041 $\mu g\ mL^{-1}$ for group 1 vs 2, $P = 0.01$) and the area under the concentration curve (AUC 1.039 ± 0.051 vs 0.758 ± 0.072 $\mu g\ mL^{-1}$ h for group 1 vs 2, $P = 0.013$) of bupivacaine were significantly lower in nicorandil-treated mice, while CL (0.579 ± 0.025 vs 0.815 ± 0.079 for group 1 vs 2, $P = 0.022$) and V_d (0.506 ± 0.054 vs 0.981 ± 0.117 for group 1 vs 2, $P = 0.1006$) were increased in nicorandil-treated animals. The ratio of AUC for *N*-desbutylbupivacaine to AUC for bupivacaine, which may partially indicate the rate of metabolism, was higher in the presence of nicorandil (1.142 ± 0.017 compared with 0.877 ± 0.013, $P = 0.0001$).

Our data may indicate an increased metabolism of bupivacaine in nicorandil-treated mice. These results do not explain the previously reported enhanced anaesthetic activity of bupivacaine in the presence of nicorandil, but may participate, at least in part, in the relative protective effect of nicorandil against the previously reported bupivacaine-induced toxicity.

This study is concerned with a drug interaction of one of the most commonly used local anaesthetics, bupivacaine, with a potassium-channel agonist, nicorandil. The influence of potassium-channel agonists on bupivacaine toxicity (Gantenbein et al 1995) and local anaesthetic activity (Gantenbein et al 1996) have been recently documented. The authors reported that potassium-channel agonists, and in particular nicorandil, were shown to modify acute toxicity and local anaesthetic activity of bupivacaine. The mechanism of the described interaction was assumed to proceed, at least partially, from pharmacokinetic interactions, taking into account the possible influence of systemic cardiovascular effects of the studied potassium-channel agonists.

The present 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 injection of nicorandil.

Materials and Methods

Animals

Adult male NMRI mice, 30 g, 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 of relative humidity (50–55%), temperature $24 \pm 1°C$ and synchronization by a light–dark cycle (lights on 0600 h, lights off 1800 h). The experiment was conducted during the month of December.

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Procedure

Two groups of 35 animals each were used for this experiment. The first group received 20 mg kg^{-1} bupivacaine intraperitoneally, 30 min after a saline injection; the second group received the same dose of bupivacaine 30 min after 1 mg kg^{-1} nicorandil, intraperitoneally. For each group, bupivacaine was injected at 1000 h to avoid possible circadian influence, as we have already reported for bupivacaine kinetics (Bruguerolle & Prat 1987). Blood samples were collected by decapitation at 0·25, 0·5, 0·75, 1, 2, 4 and 6 h after drug administration.

In a second experiment, two groups of 15 mice each were used for the determination of bupivacaine protein binding according to the same protocol. Mice were decapitated 15 min after bupivacaine administration as previously reported data (Bruguerolle & Prat 1992; Bruguerolle & Lorec 1993) have shown that this lag time is sufficient to establish equilibrium between free and bound bupivacaine. The volume of serum obtained from one animal did not permit the separation of the free fraction and thus, for practical reasons, the sera were pooled (the same volumes of sera from three animals were mixed together). This pooled serum was used to determine the total and free levels. The free fraction was obtained by ultrafiltration and centrifugation of a portion (1 mL) of the pooled serum. The remainder was used for the total serum determination. Ultrafiltrations for free level determinations were performed with an apparatus equipped with a YMT (anisotropic and hydrophylic) ultrafiltration membrane (Centrifree, Amicon). Previous studies (Bruguerolle & Prat 1992) have demonstrated that in the range of levels studied (0·5–3 $\mu g\ mL^{-1}$) less than 3% adsorption of bupivacaine to the YMT membranes occured. For the *N*-desbutylbupivacaine, the lack of absorption to the YMT membrane has also been demonstrated (Bruguerolle & Lorec 1993). The percentage of

protein binding was calculated from the difference between total and free levels according to equation 1:

$$\% \text{ binding} = (\text{total} - \text{free}) \times 100/\text{total} \quad (1)$$

Determination of bupivacaine concentrations and pharmacokinetic variables

Total and free bupivacaine and its main metabolite, *N*-desbutylbupivacaine, serum concentrations 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). The sensitivity of the method was 15 ng mL^{-1} and the reproducibility was good (coefficient of variation < 6%). Nicorandil did not interfere with the assay. Serum bupivacaine concentrations were plotted against time and pharmacokinetic variables were determined assuming a two-compartment open model. The C_{max} and T_{max} were directly assessed from individual data. The β-phase elimination half-life ($t½_\beta$), the area under the serum concentration curve ($AUC_{0-\infty}$), the total plasma clearance (CL) and the total volume of distribution (V_d) were assessed according to a nonlinear fitting method using PharmK (SoftRes Inc., Atlanta, USA), a pharmacokinetic software package for the Macintosh computer (Lu & Mao 1993). The $t½_\beta$ was calculated after nonlinear regression according to equation 2:

$$CL = F \times \text{dose}/AUC \quad (2)$$

where $F = 1$ and $V_d = CL/\beta$. The values for C_{max}, T_{max} and the ratio AUC for *N*-desbutylbupivacaine to the AUC for bupivacaine were calculated. All data is summarized as mean ± s.e.m. and comparisons were by Student's *t*-test.

Results

Serum bupivacaine and *N*-desbutylbupivacaine concentrations are shown in Figs 1 and 2.

Bupivacaine pharmacokinetic variables (C_{max}, T_{max}, AUC, V_d, CL, $t½_\beta$) are shown in Table 1. The maximal concentration in the serum (C_{max}: 0·618 ± 0·051 μg mL^{-1} vs 0·408 ± 0·041 μg mL^{-1} for group 1 vs 2, $P = 0·01$) and the area under the concentration curve of bupivacaine (AUC: 1·039 ± 0·051 vs 0·758 ± 0·072 μg mL^{-1} h for group 1 vs 2, $P = 0·013$) were significantly lower in nicorandil-treated mice, whilst CL (0·579 ± 0·025 vs 0·815 ± 0·079 for group 1 vs 2, $P = 0·022$) and V_d (0·506 ± 0·054 vs 0·981 ± 0·117 for group 1 vs 2, $P = 0·006$) were increased in nicorandil-treated animals. The ratio of AUC *N*-desbutylbupivacaine/AUC bupivacaine (Table 2), which may partially indicate the rate of metabolism, was higher in nicorandil-treated animals (1·142 ± 0·017 (group 2) compared with 0·877 ± 0·013 (group 1), $P = 0·0001$).

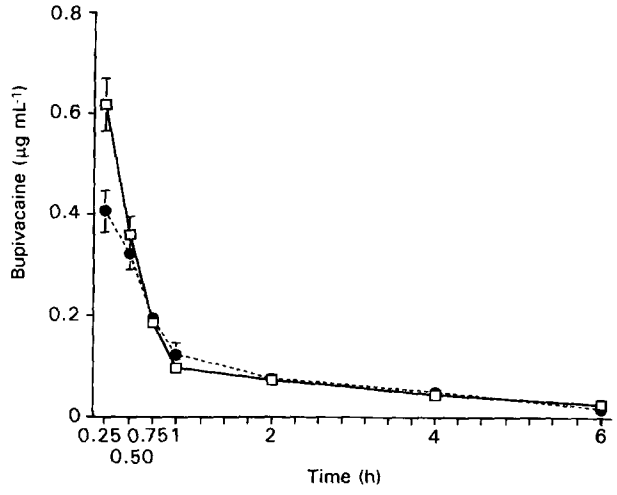

Fig. 1. Bupivacaine serum concentrations (mean ± s.e.m.) in mice receiving 20 mg kg^{-1} bupivacaine following either saline (□) or 1 mg kg^{-1} nicorandil (●).

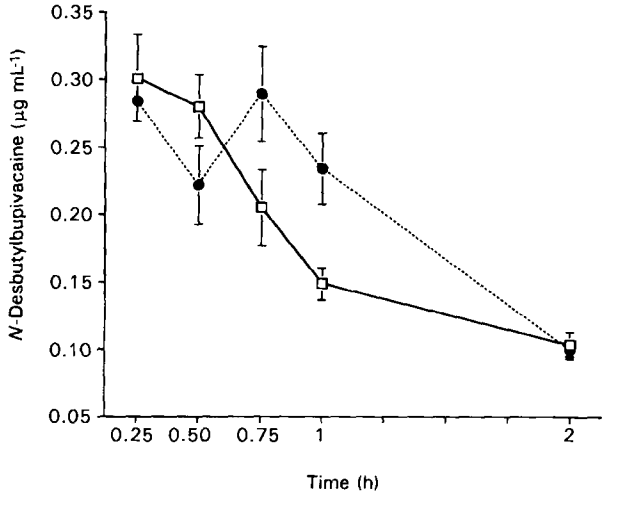

Fig. 2. *N*-Desbutylbupivacaine serum concentrations (mean ± s.e.m.) in mice receiving 20 mg kg^{-1} bupivacaine after either saline (□) or 1 mg kg^{-1} nicorandil (●).

As shown in Table 3, the protein binding percentages of bupivacaine and *N*-desbutylbupivacaine were significantly decreased with nicorandil treatment ($P = 0·03$ and 0·045, respectively). Bupivacaine total serum level and free *N*-desbutylbupivacaine serum levels were also significantly modified by nicorandil ($P = 0·0001$ and 0·009, respectively).

Table 1. Bupivacaine pharmacokinetic parameters in serum without or with nicorandil treatment.

Group	C_{max} (μg mL^{-1})	T_{max} (h)	AUC (μg mL^{-1} h)	V_d (L)	CL (L h^{-1})	$t½_\beta$ (h)
Without	0·618 ± 0·051	0·250 ± 0·000	1·039 ± 0·051	0·506 ± 0·054	0·579 ± 0·025	7·530 ± 1·180
With	0·408 ± 0·041	0·250 ± 0·000	0·758 ± 0·072	0·981 ± 0·117	0·815 ± 0·079	4·510 ± 0·570
P value	0·01	> 0·05	0·013	0·006	0·022	0·051

Values are mean ± s.e.m.

Table 2. *N*-Desbutylbupivacaine pharmacokinctic parameters in serum without or with nicorandil treatment.

Group	C_{max} ($\mu g\ mL^{-1}$)	T_{max} (h)	AUC_{0-2} ($\mu g\ mL^{-1}$ h)	AUC_{0-2} (metabolite)/AUC_{0-2} (bupivacaine)
Without	0·308 ± 0·030	0·35 ± 0·06	0·341 ± 0·031	0·877 ± 0·013
With	0·316 ± 0·023	0·45 ± 0·12	0·395 ± 0·036	1·142 ± 0·017
P	> 0·05	> 0·05	> 0·05	0·0001

Values are mean ± s.e.m.

Table 3. Bupivacaine and *N*-desbutylbupivacaine total and free serum levels, and percent binding to plasma proteins without or with nicorandil treatment.

Group	Bupivacaine			*N*-Desbutylbupivacaine		
	Total ($\mu g\ mL^{-1}$)	Free ($\mu g\ mL^{-1}$)	Binding (%)	Total ($\mu g\ mL^{-1}$)	Free ($\mu g\ mL^{-1}$)	Binding (%)
Without	0·549 ± 0·013	0·061 ± 0·004	88·96 ± 0·65	0·210 ± 0·004	0·091 ± 0·001	56·63 ± 0·85
With	0·385 ± 0·021	0·051 ± 0·004	87·08 ± 0·50	0·243 ± 0·021	0·111 ± 0·007	53·06 ± 1·45
P	0·0001	0·09	0·03	0·129	0·009	0·045

Values are mean ± s.e.m.

Discussion

The present work indicates lower C_{max} and AUC values of bupivacaine in the presence of nicorandil. Elsewhere, the increased ratio of formation of *N*-desbutylbupivacaine and the increased clearance demonstrate an increase in the metabolism and elimination of bupivacaine after nicorandil treatment. Bupivacaine is known to be mainly metabolized to *N*-desbutylbupivacaine by *N*-dealkylation in the liver and excreted in urine as small amounts of pipecolylxylidine (10%), unchanged drug (5%) and other metabolises (Tucker & Mather 1979). Nicorandil is metabolized in the liver by oxidation to nicorandil *N*-oxide and hydroxy-2-nicorandil and by denitratation to *N*-2-hydroxyethylnicotinamide (Frydman 1989). Previous studies have established that bupivacaine rapidly penetrates through the peritoneal cavity, but the importance of a possible first-pass effect has not yet been described in detail.

Under the conditions of the present study (intraperitoneal route), we cannot evaluate the uptake of bupivacaine from the peritoneal route or the possibility that nicorandil may alter this uptake. Nevertheless, nicorandil does not seem to affect the amount of bupivacaine reaching the systemic circulation. Thus the described interaction seems to proceed from a metabolic interaction in the liver.

The present study aimed to verify a possible implication of bupivacaine kinetic changes induced by nicorandil, in previously reported variations of bupivacaine activity and toxicity. Gantenbein et al (1995) reported that the bupivacaine-induced convulsant activity was not significantly modified by nicorandil but that the time to convulse was enhanced in nicorandil-treated mice. Furthermore, the bupivacaine-induced mortality was decreased in nicorandil-treated mice, indicating a relative protective effect of nicorandil. This protection against bupivacaine-induced neurotoxicity agrees with the present findings, indicating a lower C_{max} and a lower AUC for bupivacaine in serum when nicorandil is present. On the contrary, the previously reported prolongation of the anaesthetic effect of bupivacaine after nicorandil treatment (Gantenbein et al 1996) cannot be explained by the present kinetic findings.

In conclusion, this study has documented a pharmacokinctic drug interaction between nicorandil and bupivacaine, suggesting an induction of the bupivacaine metabolism. Further in-vitro experimental studies are necessary to determine the precise mechanisms involved. As Oda et al (1989) reported that CYP 3A was involved in the metabolism of lidocaine, another amide type local anaesthetic, it is possible that bupivacaine is metabolized by this isozyme of CYP 450.

Although the findings of the present study do not explain the previously reported increase in the total bupivacaine anaesthetic effect, they may nevertheless explain, in part, the protection against bupivacaine-induced neurotoxicity in nicorandil-treated mice.

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