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Investigation of Bioequivalence and Tolerability of Intramuscular Ceftriaxone Injections by Using 1% Lidocaine, Buffered Lidocaine, and Sterile Water Diluents

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The pharmacokinetics and tolerability of 1-g doses of ceftriaxone diluted in sterile water, 1% lidocaine, or buffered lidocaine were investigated. No difference in bioequivalence was noted between the three treatments. No difference in peak creatine kinase values was seen. By use of a quantitative pain scale, injection of ceftriaxone with the water diluent was significantly more painful than that with either of the other two diluents. No difference in injection pain was noted for lidocaine or buffered lidocaine.

Intramuscular (i.m.) administration of some members of the cephalosporin group causes pain at the injection site. This pain may be due to direct chemical irritation or the volume of solution administered (6). Pain on injection can be partially overcome by the use of a local anesthetic as a diluent (9, 15).

Ceftriaxone is a broad-spectrum cephalosporin which is indicated for once-daily dosing for a number of infections. When admixed with sterile water and given i.m., ceftriaxone causes significant pain. The use of 1% lidocaine as a diluent reduces the amount of pain by approximately 75%, although some people still experience substantial pain (9).

Lidocaine acts by entering the nerve axoplasm and attaching itself within the sodium channel of the nerve (1). Only the un-ionized form of lidocaine is pharmacologically active (1). Commercially available lidocaine comes as an acidic solution to enhance its stability, but the low pH reduces the amount of active drug. Buffering lidocaine with sodium bicarbonate (1 part 7.5% sodium bicarbonate to 9 parts 1% lidocaine) increases the pH to approximately 7.4 (2). Such an increase in pH in turn increases the amount of pharmacologically active lidocaine, resulting in more effective anesthesia.

These data suggest that the use of buffered lidocaine as a diluent for ceftriaxone may potentially result in less pain on i.m. injection compared with that from the use of 1% lidocaine alone. However, no comparative trial on the use of buffered lidocaine as a diluent and the effects on injection pain has been published. In addition, another important question is whether buffered lidocaine diluent would provide satisfactory in vitro ceftriaxone stability. The specific goals of the trial described here were to investigate the comparative in vitro stability of ceftriaxone when it was reconstituted with sterile water, lidocaine, and buffered lidocaine; to examine whether the use of buffered lidocaine as a diluent results in reduced injection pain compared with the use of water or lidocaine diluents; and to compare the bioequivalences of these three formulations.

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In vitro study. Ceftriaxone (1 g; Rocephin; lot 5205) was diluted with 2 ml of sterile water, 1% lidocaine, or buffered lidocaine. The buffered lidocaine was made just prior to drug reconstitution by admixing 1 part of 7.5% sodium bicarbonate injection with 9 parts of 1% lidocaine. The stability of the reconstituted ceftriaxone was evaluated by high-performance liquid chromatography (HPLC) at 0, 24, 48, 72, and 96 h. The reconstituted drug was stored in the dark at room temperature during the analysis sequence.

In vivo study. This project was approved by the Institutional Review Board of Bassett Healthcare. Written informed consent was obtained from each subject prior to study participation.

Subjects were males and females (ages, 18 to 55 years) who were in good health, and who were within 30% of ideal body weight as determined by Metropolitan Life Insurance tables. Subjects were excluded if their serum creatinine level was >1.5 mg%, their prothrombin time was >2 s above the control values, their bilirubin level was >1.5 mg%, their serum creatinine kinase (CK) level was >225 U/liter, or their alanine aminotransferase, aspartate aminotransferase, or lactic dehydrogenase level was two or more times the upper limit of normal. Women who were premenopausal underwent urine pregnancy testing at the time of the initial screening and before each study phase. Subjects with a history of allergic reactions to lidocaine, penicillins, or cephalosporins were excluded. In addition, subjects were excluded if they had a history of any skeletal muscle disease.

Following enrollment, subjects were randomized to receive the following three treatments in a double-blind crossover fashion: (i) ceftriaxone, 1 g diluted to 2.2 ml with sterile water; (ii) ceftriaxone, 1 g diluted to 2.2 ml with 1% lidocaine; or (iii) ceftriaxone, 1 g diluted to 2.2 ml with buffered lidocaine. The volume of 2.2 ml was selected on the basis of data from a previous trial (2).

A study nurse administered the injections in the buttock using a 1.5-in. (3.8-cm), 22-gauge needle. For each subject, injection in to the buttock was randomized to left-right-left or right-left-right. A 2-week washout period was allowed between

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study phases. All injections were given between 0700 and 1000 h.

A 2-ml blood sample for ceftriaxone analysis was obtained at predose, at 5, 15, and 30 min postdose, and at 1, 2, 4, 6, 12, 14, and 24 h postdose. Blood was obtained through an indwelling intravenous catheter kept patent with 10 U of heparinized 0.9% NaCl per ml. Serum was separated and stored at −80°C until analysis.

Blood for CK level determination was obtained predose and at 4, 8, and 12 h postdose.

A linear, 100-mm visual analog scale (VAS) was used to objectively assess pain (13). The VAS was applied just prior to injection and at 0.25, 0.5, 1, 2, 4, 6, and 12 h after injection. Subjects placed a mark on a clean copy of the scale at each time period and were blinded to their previous observation. The distance along the line reflects pain intensity, from no pain (0 mm) to severe pain (100 mm). In addition, at the completion of the study, subjects were asked to subjectively assess which injection resulted in the least pain.

Analysis of ceftriaxone levels in serum was performed in duplicate by using reversed-phase, ion-pairing HPLC by a modification of the method of Patel et al. (8). An isocratic high-performance liquid chromatograph was equipped with a reversed-phase C_{18} column (5-μm particles; 2.5 cm by 4.6 mm [internal diameter]) and a variable-wavelength detector set at 280 nm. The mobile phase consisted of 500 ml of acetonitrile, 10 ml of 1 M phosphate buffer (pH 7), 3 g of hexadecyltrimethylammonium bromide, and water to 1 liter. The retention time of ceftriaxone was 6 to 7 min, with a flow rate of 1 ml/min. Serum samples were either diluted 1:10 with cold methanol or filtered through a Millipore Ultraspec-MC membrane filter (molecular weight limit, 10,000) to remove serum proteins. The lower limit of assay quantitation was 5 μg/ml for the diluted sample and 0.5 μg/ml for the filtered sample. This assay has a detection range of 0.5 to 500 μg/ml and interday and intraday coefficients of variation of 6.1 and 5.8%, respectively, at 25 and 100 μg/ml.

Pharmacokinetic analysis was performed by first graphically analyzing the data to determine the time to the peak concentration (T_{max}) and the peak concentration (C_{max}). The area under the curve from time zero to infinity ($AUC_{0-\infty}$) was determined by the trapezoidal rule. Total body clearance (CL/F) was determined by the equation CL/F = dose/$AUC_{0-\infty}$. The terminal elimination rate constant was determined by nonlinear regression analysis of the terminal portion of the serum concentration-versus-time curve. Bioavailability (F) was calculated as the ratio of $AUC_{0-\infty}$ for lidocaine or buffered lidocaine divided by $AUC_{0-\infty}$ for the water phase.

Statistical analyses were performed by using version 6.08 of SAS software (11). Prestudy sample size calculation with the data of Patel et al. (9) suggested that 12 subjects would be needed to detect a 20% difference in a change of pain on injection and a 20% difference in AUC between the two lidocaine phases.

$AUC_{0-\infty}$, CL/F, C_{max}, and T_{max} were analyzed by analysis of variance for repeated measures. Post hoc Scheffe's test was applied if any statistically significant differences were found ($P \leq 0.05$).

Bioequivalence was determined by using $AUC_{0-\infty}$. After log transformation of the data, the two, one-sided test procedure was applied (12). Westlake's 90% confidence intervals were also determined (18). The Westlake 90% confidence interval criterion of 80 to 125% for bioequivalence was applied (18).

Visual analog scale data and CK data were analyzed by using a generalized linear model analysis of variance with Scheffe's test for significant differences (9). All data are presented as means ± standard deviations.

TABLE 1. Pharmacokinetics of 1 g of ceftriaxone diluted with sterile water, 1% lidocaine, and buffered lidocaine[a]

Study phase	AUC (μg · h/ml)	CL/F (ml/min/1.73 m^2)	C_{max} (μg/ml)	T_{max} (h)
Sterile water	377 ± 165.2	49.9 ± 7.2	40.6 ± 10.0	3.8 ± 1.1
Lidocaine (1%)	342.7 ± 138.7	41.8 ± 14.1	41.9 ± 10.6	3.2 ± 2.1
Buffered lidocaine	327.8 ± 112	48.3 ± 21.8	40.9 ± 17.6	4.2 ± 2.0

[a] Westlake's 90% confidence interval: water versus lidocaine, 102% < F < 106%; water versus buffered lidocaine: 84.4% < F < 93%; lidocaine versus buffered lidocaine: 80.5% < F < 89.9%.

No difference in the stability of any of the three diluents was noted over the 96-h period for the in vitro trial. All diluents retained >90% of the ceftriaxone activity over the time period studied.

A total of 12 subjects (9 females and 3 males) were enrolled in the in vivo trial. All subjects completed the entire trial. The average age of the subjects was 35.7 ± 6.8 years. The average weight of the subjects was 72.6 ± 14.1 kg.

The pharmacokinetic parameters for the three phases are provided in Table 1. No significant differences in any parameter were seen for the three study phases. Both the lidocaine and buffered lidocaine phases were bioequivalent to the sterile water phase. The bioavailability of lidocaine compared with that of water was 90.9%, and that of buffered lidocaine compared with that of water was 86.9%. Power analysis showed that we had a 90% chance of detecting a 20% difference in AUC between each phase.

In terms of muscle damage and pain, no significant differences in CK values were seen between any of the three phases. When examining the VAS, pain on injection was less with buffered lidocaine, but it was not statistically different from pain on injection with lidocaine (Fig. 1). Both buffered lidocaine and lidocaine caused significantly less pain than the water diluent immediately after injection (78 and 50% reductions in pain scores, respectively). The duration of injection pain was significantly different with the water diluent compared with that with the other two phases up to 1 h after injection. Sub-

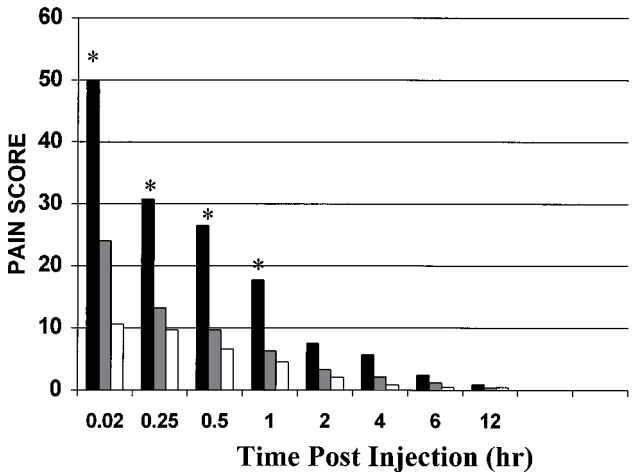

FIG. 1. Pain rating on a 0 to 100 visual analog scale following i.m. injection of ceftriaxone with three diluents. *, $P < 0.05$ versus lidocaine or buffered lidocaine. ■, sterile water; ▨, 1% lidocaine; □, buffered lidocaine.

jectively, 10 of the 12 subjects rated the buffered lidocaine phase as causing the least pain immediately after the injection.

Ceftriaxone is a broad-spectrum cephalosporin with a long half-life in serum that allows once-daily dosing. Although the drug can be administered i.m., ceftriaxone diluted with sterile water causes significant injection pain. The manufacturer therefore recommends the use of 1% lidocaine as a diluent (9). Buffered lidocaine has been shown to result in better anesthesia than 1% lidocaine alone in various circumstances (4, 5, 7, 10, 14, 16, 17, 19). The purpose of our trial was to determine if the use of buffered lidocaine as a diluent resulted in less injection pain for ceftriaxone versus the use of lidocaine or sterile water while maintaining bioequivalence. In addition, we investigated the in vitro stabilities and bioequivalences of the three formulations to establish whether ceftriaxone diluted with buffered lidocaine can be stored at room temperature between reconstitution and administration.

The in vitro stabilities of the three ceftriaxone diluent groups were similar after storage over a 96-h period (having >90% activity) at room temperature in the dark. Therefore, any of the three can be confidently used in a clinical setting which requires the preparation and storage of ceftriaxone at least 72 h prior to administration.

In our healthy volunteers, we found no difference in the bioequivalence of the three diluents. Therefore, any of the diluents can be used, and adequate concentrations of drug are maintained in serum.

No significant differences in CK values were noted between the three groups. This demonstrates that the use of lidocaine or buffered lidocaine does not reduce muscle damage, as measured by CK level determination. Injection pain, as measured by the VAS, was significantly different between the sterile water group and the two lidocaine groups. Although buffered lidocaine was associated with the least injection pain, the difference did not reach statistical significance, as measured by the VAS, compared with 1% lidocaine alone.

Compared with sterile water, 1% lidocaine as a diluent affords a significant reduction in injection pain (9). While comparative clinical data indicate that buffered lidocaine has a greater anesthetic effect than 1% lidocaine (4, 5, 7, 10, 14, 16, 17, 19), our objective data suggest that the use of buffered lidocaine offers little advantage over the use of 1% lidocaine for the reconstitution and administration of ceftriaxone, despite a trend of a greater reduction in injection pain and a subjective finding of less pain in 10 of 12 subjects given the drug with buffered lidocaine. While our study was designed to detect a 20% reduction in injection pain, it is possible that buffered lidocaine offers a greater reduction in pain compared with 1% lidocaine, but the number of subjects in the current study was too small to detect this difference.

Lidocaine (1%) can be easily used as a ceftriaxone diluent. On the basis of the results of the current study, it is the diluent of choice. While ceftriaxone diluted with buffered lidocaine is bioequivalent to 1% lidocaine and has in vitro stability equal to that of 1% lidocaine, it is more inconvenient to use since it must be prepared. A trial with a larger number of subjects may show a significant difference in injection pain between 1% lidocaine and buffered lidocaine.

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REFERENCES

1. **Bartfield, J. M., P. J. Homer, D. T. Ford, and P. Sternklar.** 1992. Buffered lidocaine as a local anesthetic: an investigation of shelf life. Ann. Emerg. Med. **21:**16–19.
2. **Bradley, J. S., L. S. Compagiannis, W. E. Murray, M. A. Acosta, and G. L. Tsu.** 1992. Pharmacokinetics and safety of intramuscular injection of concentrated ceftriaxone in children. Clin. Pharm. **11:**961–964.
3. **Buckley, F. P., G. D. Neto, and B. R. Fink.** 1985. Acid and alkaline solutions of local anesthetics: duration of nerve block and tissue pH. Anesth. Analg. **64:**477–482.
4. **Christoph, R. A., L. Buchanan, K. Begalla, and S. Schwartz.** 1988. Pain reduction in local anesthetic administration through pH buffering. Ann. Emerg. Med. **17:**117–120.
5. **DiFazio, C. A., H. Caron, K. R. Grosslight, J. C. Moscicki, W. R. Bolding, and R. A. Johng.** 1986. Comparison of pH adjusted lidocaine solutions for epidural anesthesia. Anesth. Analg. **65:**70–76.
6. **Foster, T. S., R. P. Shrewsbury, and J. D. Coonrod.** 1980. Bioavailability and pain study of cefamandole nafate. J. Clin. Pharmacol. **20:**526–533.
7. **McKay, W., R. Morris, and P. Mushlin.** 1987. Sodium bicarbonate alternates pain on skin infiltration with lidocaine, with or without epinephrine. Anesth. Analg. **6:**572–574.
8. **Patel, I. H., S. Chen, M. Parsonnet, M. R. Hackman, M. A. Brooks, J. Konikoff, and S. A. Kaplan.** 1981. Pharmacokinetics of ceftriaxone in humans. Antimicrob. Agents Chemother. **20:**634–641.
9. **Patel, I. H., R. E. Weinfeld, J. Konikoff, and M. Parsonnett.** 1982. Pharmacokinetics and tolerance of ceftriaxone in humans after single dose intramuscular administration in water and lidocaine diluents. Antimicrob. Agents Chemother. **21:**957–962.
10. **Sapin, P., R. Petrozzi, and G. J. Dehmer.** 1991. Reduction in injection pain using buffered lidocaine as a local anesthetic before cardiac catheterization. Catheterization Cardiovasc. Diagn. **23:**100–102.
11. **SAS Institute.** 1985. SAS user's guide. Statistics. SAS Institute, Cary, N.C.
12. **Schuirmann, D. J.** 1987. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J. Pharmacokinet. Biopharm. **15:**657–680.
13. **Scott, J., and E. C. Huskisson.** 1976. Graphic representation of pain. Pain **2:**175–184.
14. **Siler, J. N., and H. Rosenberg.** 1992. Lidocaine hydrochloride versus lidocaine bicarbonate for epidermal anesthesia in outpatients undergoing orthoscopic surgery. J. Clin. Anesth. **2:**296–300.
15. **Sonneville, P. F., K. S. Albert, H. Skeggs, H. Genner, K. C. Kwan, and C. M. Martin.** 1977. Effect of lidocaine on the absorption, disposition and tolerance of intramuscularly administered cefoxitin. Eur. J. Clin. Pharmacol. **12:**273–279.
16. **Stewart, J. H., S. E. Chinn, G. W. Cole, and J. A. Klein.** 1990. Neutralized lidocaine with epinephrine for local anesthesia. II. J. Dermatol. Surg. Oncol. **16:**842–845.
17. **Stewart, J. H., G. W. Cole, and J. A. Klein.** 1989. Neutralized lidocaine with epinephrine for local anesthesia. J. Dermatol. Surg. Oncol. **15:**1081–1083.
18. **Westlake, W. J.** 1988. Bioavailability and bioequivalence of pharmaceutical formulations, p. 329–352. *In* W. J. Westlake (ed.), Biopharmaceutical statistics for drug development. Marcel Dekker, Inc., New York.
19. **Zahl, K., M. Jordan, J. McGroaty, B. Sorensen, and A. W. Gotta.** 1991. Penibulbar anesthesia, effect of bicarbonate on mixtures of lidocaine, bupivicaine, and hyaluronidase with or without epinephrine. Ophthalmology **98:** 239–242.