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SHORT COMMUNICATIONS

PROTEIN BINDING OF LIDOCAINE IN CANINE SERUM AND PLASMA: EFFECTS OF AN ACIDIC pH AND THE TECHNIQUE OF STUDY

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INTRODUCTION

Local anaesthetic toxicity studies using various animal models have been reported.[1–4] The findings of these studies, however, are difficult to apply to the human clinical situation. In order to compare results, the disposition of the drug in question must be known in each species used. One parameter needed to understand the drug disposition is protein binding.

Rapid and sensitive analytical techniques for quantitative determination of amide local anaesthetics are available. Free local anaesthetic concentrations present within serum or plasma, however, have been determined using equilibrium dialysis, rather than by direct assay. This method, while reliable, requires a minimum of 3–4 h for free amide local anaesthetic in plasma or serum to reach equilibrium with the dialysis buffer at 37°. Ultrafiltration appears to be more appropriate than dialysis techniques because it can be carried out rapidly without the addition of potentially competitive buffer components[5] or dilution of plasma or serum electrolytes.[6]

The purpose of this investigation was to determine the plasma and serum protein binding of lidocaine in the dog with doses equal to those used in man. Since the central nervous system toxicity effect of local anaesthetics increases

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with respiratory acidosis,[4] the influence of a moderately acidic pH on the protein binding of lidocaine was also examined. We also considered the applicability of ultrafiltration to the determination of free lidocaine concentrations at physiologic and acidic pH values.

METHODS

Plasma (containing 10 u ml^{-1} heparin) and serum were obtained from mature dogs of mixed breed and either sex. The plasma and serum were respectively pooled, separated into 10 ml aliquots and frozen (−20°) until used.

Equilibrium dialysis

Protein binding was determined using a Spectrapor Equilibrium Dialyzer (Spectrum Medical Industries Inc., New York, N.Y.) equipped with 1 ml Teflon cells. The cell halves were separated by a Spectrapor Dialysis Membrane No. 2 (Spectrum) pretreated in accordance with the manufacturer's instructions. Molecular weight exclusion of this membrane is 12–14000. One millilitre aliquots of plasma or serum adjusted to the appropriate pH with 1 N HCl or NaOH at 37° were dialysed against an equal volume of isotonic Sorenson's phosphate buffer at the appropriate pH. Sorenson's phosphate buffer was made isotonic by the addition of NaCl to a concentration of 0·5 per cent (w/v).

The extent of protein binding was studied over a range of five concentrations, namely 1, 2, 5, 10, and 20 μg ml^{-1}. Two pH values were studied. These were physiologic pH for the dog (7·36) and a pH representing moderately severe acidosis (6·96). Six replications were completed for each drug concentration at each pH.

The plasma or serum used in the equilibrium dialysis experiments initially contained the desired concentration of lidocaine. The addition of drug to the protein side resulted in a shorter time interval needed to reach equilibrium as compared to placing the drug in the buffer.[7] The initial concentration of the local anaesthetic was determined by gas chromatography before dialysis (see Analysis section). Preliminary experiments verified that equilibrium was achieved within 3 h. For the present study, dialysis cells were rotated at 20 rpm in a water bath maintained at 37°. Following dialysis, the protein (plasma or serum) and the buffer of each cell were removed for analysis.

Ultrafiltration

Determination of protein binding in canine serum was performed using an Amicon Micropartition System (Amicon Corporation, Danvers, Massachusetts), equipped with a YMT ultrafiltration membrane (Amicon). One millilitre aliquots of serum adjusted to the appropriate pH with 1 N HCl or NaOH were subjected to ultrafiltration at 30°.

Protein binding was studied at the same lidocaine concentrations and pH values as described in the equilibrium dialysis section. Six replications were

completed for each drug concentration at each pH. The canine serum containing the desired concentration of amide local anaesthetic was allowed to equilibrate overnight at 4° with mixing. The concentration of local anaesthetic contained in the serum was determined by gas chromatography at the time of ultrafiltration. This was accomplished by centrifugation at 2050 × g for 40 min at 30° using a clinical centrifuge (International Equipment Company, Needham, Massachusetts), equipped with a Model 803 angle head centrifuge rotor (IEC). Preliminary experiments determined that the YMT membrane did not show any non-specific adsorption of lidocaine. Following centrifugation, the ultrafiltrate was removed for analysis.

Analysis

Determination of lidocaine concentrations was performed by gas chromatography using a modification of the method of Mather and Tucker.[8] A Hewlett Packard Model 5840 gas chromatograph (Hewlett Packard, Avondale, Pennsylvania), equipped with a six-foot glass column packed with 3 per cent OV-17 on Gas Chrom Q and a nitrogen specific detector was used. Calibration curves were constructed from standard concentrations analysed with each set of samples. The coefficient of variation of the assay method was 2–5 per cent at $5\,\mu g\,ml^{-1}$ and 12–15 per cent at $10\,ng\,ml^{-1}$.

Statistical analysis

All data comparisons were tested for homogeneity of independent variances (F test). Data passing the F test underwent a parametric analysis of variance followed by a Scheffe's critical value test for multiple comparisons. Data which failed the F test were tested using a non-parametric analysis of variance followed by a Dunn's critical value test for multiple comparisons. A $p<0{\cdot}05$ was considered the minimum level of significance.

RESULTS

Equilibrium dialysis

Decreasing the pH from 7·36 to 6·96 caused a significant decrease ($p<0{\cdot}05$) in the per cent plasma protein binding at all concentrations studied with the exception of $1\,\mu g\,ml^{-1}$ (Table 1). A similar decrease in pH, however, did not result in a significant change in lidocaine serum protein binding. On comparing lidocaine protein binding in serum and plasma, a significantly smaller extent ($p<0{\cdot}005$) of binding in plasma was observed over the range of $1–5\,\mu g\,ml^{-1}$.

Ultrafiltration

Decreasing the serum pH from 7·36 to 6·96 resulted in a significant decrease ($p<0{\cdot}001$) in lidocaine binding at all concentrations with the exception of $20\,\mu g\,ml^{-1}$ (Table 1). The per cent lidocaine protein binding determined by

Table 1. Per cent binding of lidocaine in canine plasma and serum

Initial concentration (μg/ml)	Plasma E.D.*			Serum E.D.			Serum U.F.†		
	7·36‡	6·96‡	p¶	7·36	6·96	p	7·36	6·96	p
1	46±6§	35±17	NS‖	83±4	86±3	NS	87±1	71±4	0·001
2	47±6	36±8	<0·05	87±3	83±2	NS	71±2	63±2	0·001
5	51±9	31±13	<0·05	71±9	66±14	NS	56±2	50±2	0·001
10	48±3	31±15	<0·05	42±19	45±7	NS	59±1	49±2	0·001
20	42±14	28±7	<0·05	36±8	38±18	NS	46±1	45±1	NS

* Equilibrium dialysis.
† Ultrafiltration.
‡ pH.
§ Mean ± S.D. ($n = 6$).
‖ Not significant ($p > 0·05$).
¶ Level of significant difference.

ultrafiltration and equilibrium dialysis for serum resulted in a significant difference ($p < 0·005$) between the two methods. Ultrafiltration at physiologic pH resulted in a lower per cent serum protein binding than equilibrium dialysis at concentrations of 2 and 5 $\mu g\,ml^{-1}$ ($p < 0·001$). At an acidic pH (6·96), ultrafiltration resulted in a significantly lower ($p < 0·001$) serum protein binding for lidocaine at all concentrations with the exception of 10 $\mu g\,ml^{-1}$.

DISCUSSION

On comparing the differences in protein binding using plasma and serum, we found a smaller extent of binding for lidocaine in plasma. The lower binding of lidocaine in canine plasma may have resulted from an influence of heparin, which was used as an anticoagulant. Heparin has been reported to form complexes with various inorganic ions (i.e. Ca^{2+}) and with α_1-acid glycoprotein. This may lead to alterations in protein conformation resulting in changes in the *in vitro* protein binding.[9, 10] A heparin effect on protein binding *in vitro* has been reported for diazepam (albumin binding) and propranolol (α_1-acid glycoprotein binding) using proteins from plasma from human sources.[10]

Since equilibrium dialysis could possibly alter lidocaine serum protein binding, another method was indicated. Ultrafiltration was used to examine lidocaine serum binding. On comparing the results obtained from these two methods, a significantly lower ($p < 0·005$) lidocaine binding was obtained with ultrafiltration. This lower binding resulted in a higher free fraction as measured by ultrafiltration. With ultrafiltration, an acidic pH resulted in a significant decrease ($p < 0·001$) in the lidocaine canine serum binding for concentrations of 10 $\mu g\,ml^{-1}$ or less (Table 1).

The differences between the two methods (equilibrium dialysis and ultrafiltration) demonstrated in this study may be attributed to the difference in experimental conditions. For example, equilibrium dialysis was performed at 37° whereas ultrafiltration was performed at 30°. Temperature may affect binding by altering the type or number of binding site(s) on a protein.[11] However, an influence of temperature appears to be unlikely in this case since data on human lidocaine binding reported at 37° by Routledge *et al.*[12] are in agreement with those reported by Tucker *et al.*[13] at 4°.

A further possibility is that when equilibrium dialysis is performed, changes occur in the concentrations of ions critical to maintaining protein conformation. Furthermore, ions in concentrations not normally found within serum or plasma do move into the protein side and may compete with drugs for binding sites or alter protein conformation.[6] This occurs since the dialysis fluid, usually buffer with or without sodium chloride, does not contain the same ionic composition as the serum or plasma. This suggests that in order to achieve a true free fraction of a drug using equilibrium dialysis, the dialysing fluid should contain the same ionic composition as the serum or plasma. Due to the logistics of correcting the ionic composition of the dialysing fluid (electrolyte analysis) and the time to reach equilibrium, ultrafiltration appears to be an attractive alternative.

Ultrafiltration, however, has limitations. Among these limitations are uptake of small molecules by the membrane (non-specific adsorption of drug) and the change in protein concentration with volume filtered. Non-specific adsorption of the drug must be determined before using ultrafiltration. In this study, preliminary experiments determined that the concentrations of lidocaine before and after ultrafiltration were within the experimental error of the method of analysis. Concentration of the protein during ultrafiltration has been reported not to alter the equilibrium established between the drug and protein.[5]

Another consideration is that ultrafiltration in this study resulted in a lower coefficient of variation than equilibrium dialysis. Since absolute values are not attainable, the error associated with an estimation of this value becomes important. One question addressed by this study is the relative change that occurs in protein binding with an acidic pH. Ultrafiltration appears to be a more sensitive method for determining small changes in protein binding.

In summary, the results of this study indicate that lidocaine protein binding will decrease with decreases in pH in canine plasma, independent of the technique used. Lidocaine protein binding in canine serum, however, resulted in two different observations dependent on the method used. Equilibrium dialysis suggests little change in lidocaine serum binding with a decrease in pH, whereas ultrafiltration indicates a decreased binding.

This study, therefore, suggests that the pH effect of lidocaine protein binding in the canine is dependent on the technique and the blood fraction (serum or plasma) used for the study. Additionally, ultrafiltration appears to be the

method of choice for lidocaine binding studies on the basis of its reproducibility (lower coefficient of variation) and logistical considerations.

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