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Age does not influence the serum protein binding of bupivacaine

B. TH. VEERING, A. G. L. BURM, M. P. R. R. GLADINES & JOH. SPIERDIJK
Department of Anesthesiology, Leiden University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands

The serum protein binding of bupivacaine was studied in 74 subjects, 39 males and 35 females, aged 20–90 years, without evidence of acute or chronic inflammatory disease or malignancy. Subjects were drug free for at least 1 month. The free fractions of bupivacaine did not change with age in either males or females. This is in keeping with the lack of effect of age on AAG concentrations. Free fractions of bupivacaine were slightly higher in females as compared with males. The previously observed decline in clearance of bupivacaine with age probably reflects a concomitant decline in the metabolic activity of hepatic enzymes.

Keywords serum protein binding bupivacaine age

Introduction

Bupivacaine, a long acting local anaesthetic, is frequently used to provide analgesia in surgical patients. Like other local anaesthetic drugs, it has a relatively narrow safety margin.

Bupivacaine is predominantly eliminated from the body by hepatic metabolism (Tucker *et al.*, 1977) and its plasma clearance decreases with age (Veering *et al.*, 1987a,b, 1988, 1991). Since bupivacaine has a relatively low hepatic extraction ratio (Tucker *et al.*, 1977), the observed age-related decline in clearance is most likely due to a change in drug metabolizing hepatic enzyme activity and/or serum protein binding of bupivacaine, rather than from an alteration in the liver blood flow (Wilkinson & Shand, 1975).

Bupivacaine is highly bound to serum proteins (approximately 90%). In the plasma drug concentration range associated with safe and effective perineural administration (up to 2 $\mu g\,ml^{-1}$, 0.007 $mmol\,l^{-1}$) binding occurs mainly to high affinity, low capacity sites on α_1-acid glycoprotein (AAG) (Denson *et al.*, 1984; Tucker *et al.*, 1970).

This study examined the effect of age on the protein binding of bupivacaine at serum concentrations corresponding to those obtained after epidural administration (Veering *et al.*, 1987a).

Methods

The study was approved by the Committee on Medical Ethics of the University Hospital. On the day of admission, informed consent was obtained from 74 patients, aged 20–90 years, who were scheduled for a minor surgical, orthopaedic or gynaecological procedure. Only subjects who had to undergo elective operations, such as sterilization, elective plastic cosmetic surgery, or minor elective orthopaedic procedures were enrolled. The patients (39 males and 35 females) had not taken any drugs (including over-the-counter and recreational drugs) for at least 1 month before the study. Medical examination and standard laboratory tests showed that none of the subjects had diseases or conditions known to modify the concentrations of AAG or human serum albumin (HSA). Moderate and heavy smokers, smoking more than 10 cigarettes per day, were excluded from the study. Twenty-two subjects (15 males and 7 females) smoked between 3 and 10 cigarettes daily.

Blood samples (10 ml) were collected by venepuncture using unheparinized glass tubes. Samples were obtained at a fixed time (17.00 h) before dinner. Serum was obtained by centrifugation of clotted blood at 2800 *g* for 10 min and was separated immediately and stored at −20° C for subsequent analysis. HSA and AAG concentrations were measured by rate nephelometry (Sternberg, 1977). The coefficient of variation of these assays was $\leq 5\%$ in the concentration ranges encountered in this study.

The binding of bupivacaine in serum was determined by equilibrium dialysis with a Dianorm® dialysis system, equipped with twenty Teflon® dialysis cells (Diachema), (volumes: plasma compartment 1 ml, buffer compartment 2 ml), which were rotated in a water bath at 37° C for 4 h. The cell membranes had a molecular weight cut-off of 10,000 Daltons. The serum pH was adjusted to 7.4 with 3–4 μl 8% w/v phosphoric acid ml^{-1} of spiked serum (Ponganis & Stanski, 1985). Subsequently, 0.6 ml serum was dialyzed against 1.5 ml of an isotonic phosphate

Correspondence: Dr B. Th. Veering, Department of Anesthesiology, Leiden University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands

buffer (107 mM Na_2PO_4 and 24 mM KH_2PO_4; pH = 7.4). The extent of protein binding was measured in triplicate at a bupivacaine concentration of 0.5 μg ml^{-1} serum. Control experiments demonstrated that equilibrium was achieved within 3 h, that binding of bupivacaine to the membrane or the cell walls was minimal (<5%) and that the serum pH after dialysis did not differ significantly from 7.4.

Following dialysis, the concentrations of bupivacaine in the dialysate were measured by capillary gas chromatography as described by Burm *et al.* (1982) with some modifications. Thus the gas chromatograph (Hewlett Packard 5710) was equipped with a fused silica column (length 10 m, internal diameter 0.32 mm, stationary phase [CP wax 57CB]) and a falling needle solid injection system (Chrompack). The coefficient of variation of the assay was <6% at drug concentrations encountered in this study.

Free fractions of bupivacaine (*fu*) were determined from the concentrations C_s of bupivacaine in serum before dialysis and the concentration C_d in dialysate after dialysis using the following equation (Tozer *et al.*, 1983):

$$fu = \frac{C_d}{C_s - RC_d}$$

where R is the ratio between the volumes of buffer and serum before dialysis.

The values of *fu*, HSA and AAG concentrations, were examined using multiple and simple linear regression and correlation analysis with age, sex and light smoking/non smoking as independent variables, and *t*-tests. When $P < 0.05$ differences were considered to be statistically significant.

Results

HSA and AAG concentrations in 68 of the 74 patients studied were reported previously in this journal (Veering *et al.*, 1990). It was shown that HSA and AAG concentrations were independent of sex and (light) smoking habits. HSA concentrations decreased with increasing age, whereas AAG concentrations did not change with age. The same conclusions were obtained on evaluating the data from all 74 patients.

Simple and overall coefficients of determination for the free fraction of bupivacaine in serum as a function of age, sex and smoking habit are shown in Table 1 and Figure 1.

Table 1 Simple and overall coefficients of determination for the free fraction of bupivacaine in serum (*fu*) as a function of age, sex and smoking habit

Dependent variable	*Independent variable*	*Simple* r^2	*Overall* r^2
fu	Age + sex + smoking	—	0.101
	Age + sex	—	0.074
	Age + smoking	—	0.098
	Age	0.003	—

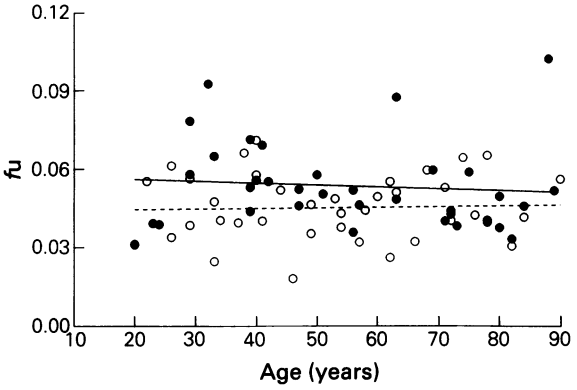

Figure 1 The relationship between age and the free fraction of bupivacaine in serum in 39 male (○——○) and 35 female (●——●) subjects.

The free fractions of bupivacaine were independent of (light) smoking habits and age but were higher in females (mean ± s.d.: 0.053 ± 0.016) as compared with males (0.045 ± 0.012) ($P < 0.05$).

Discussion

Our data indicate that the serum binding of bupivacaine (concentration 0.5 μg ml^{-1}) is independent of age (uncomplicated by disease). This is consistent with the observations of Davis *et al.* (1985), Veering *et al.* (1990) and Verbeeck *et al.* (1984) that AAG concentrations show little or no changes with age. Although albumin concentrations decrease with increasing age, this does not appear to influence the protein binding of bupivacaine at low concentrations.

The findings suggest that the decline in plasma clearance of bupivacaine with age (Veering *et al.*, 1987a,b, 1988, 1991) cannot be explained by changes in protein binding. Also, since bupivacaine has a relatively low hepatic extraction ratio, age-related changes in liver blood flow would be expected to have little effect on the clearance of this drug. Therefore, the observed decline in the clearance of bupivacaine is best explained by a decrease in intrinsic hepatic metabolism. Whether there is a decreased activity of the hepatic enzymes involved in the metabolism of bupivacaine or a decrease in capacity, because of the reduced size of the liver, is not clear.

Studies of hepatic enzyme activities in humans are sparse (Schmucker *et al.*, 1990). Limited evidence from studies of liver tissue specimens with normal histology does not indicate any age related change in specific activities of several cytochromes P-450 (Schmucker *et al.*, 1990; Woodhouse *et al.*, 1984). The decrease in hepatic mass with age (Wynne *et al.*, 1989) might partially account for the diminished elimination of drugs with low intrinsic clearance, including bupivacaine.

The finding of a small sex-difference in the free fraction of bupivacaine between males and females not taking oral contraceptives is probably of minor clinical significance. The reason for this difference in binding has not been established, but it may reflect hormonal influences. Thus, Routledge *et al.* (1981) reported that free fractions of lignocaine were higher in females taking contra-

ceptives than in those not taking contraceptives. This could be attributable entirely to changes in AAG concentration caused by oestrogens (Gleichmann *et al.*, 1973). Although Routledge *et al.* (1981) found no significant difference in free fraction between males and females (not taking contraceptives), their numbers of subjects were small in contrast to ours.

We conclude that ageing, uncomplicated by disease, does not influence the serum binding of bupivacaine. Therefore, the previously observed decline in total plasma clearance of bupivacaine with age (Veering *et al.*, 1987a,b, 1988, 1991) probably reflects a concomitant decline in hepatic enzyme activity or capacity.

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