PROPOFOL INHIBITS ENZYMATIC DEGRADATION OF ALFENTANIL AND SUFENTANIL BY ISOLATED LIVER MICROSONES IN VITRO

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SUMMARY
We have studied the effect of propofol on the enzymatic degradation of alfentanil and sufentanil utilizing isolated liver microsomes obtained from pig and human liver. Propofol inhibited dose-dependently the oxidative metabolic degradation of alfentanil and sufentanil by both microsomal preparations. The calculated concentration of propofol causing 50% inhibition of metabolic degradation (IC50) was 32.6 μmol litre⁻¹ for alfentanil and 22.1 μmol litre⁻¹ for sufentanil in pig liver microsomes. Similar values of inhibitory activity of propofol (IC50 values 62.8 and 52.9 μmol litre⁻¹, respectively) were observed using human microsomes prepared from liver taken from an organ transplant donor. We suggest that propofol in clinically relevant concentrations interferes with oxidative metabolic degradation of alfentanil and sufentanil in the microsomal fraction of pig and human liver.

KEY WORDS

Alfentanil and sufentanil are used widely in combination with propofol for total i.v. anaesthesia. Their pharmacokinetic profiles suggest little cumulative effects in therapeutic dose ranges. Clinical experience suggests that, when propofol and alfentanil are used together to provide anaesthesia, the specific activity of each drug is potentiated [1]. Current data indicate that the disposition kinetics of propofol are unaffected in the presence of therapeutic plasma concentrations of another structurally related drug, fentanyl [2]. However, alfentanil appears to exhibit pharmacokinetic changes in the presence of propofol [1]. The liver is the main organ responsible for metabolic elimination of alfentanil and sufentanil. The liver extraction ratio of alfentanil is in the range 0.3–0.5. Thus the clearance of alfentanil and sufentanil might be influenced significantly by changes in hepatic microsomal enzyme function or interaction with propofol at the same enzymatic system level during propofol anaesthesia [3]. We have investigated, therefore, the effects of propofol on the degradation of alfentanil and sufentanil in vitro using isolated microsomal fractions obtained from pig and human liver.

METHODS AND RESULTS
Microsomes were prepared from the livers taken from two male Landrace pigs (22–25 kg). A sample of human liver was obtained from a kidney transplant donor at the Groote Schuur Hospital using established procedure provided for organ transplantation. Pooled microsomal fractions were isolated using differential ultracentrifugation [4] and resuspended in sucrose 0.1 mol litre⁻¹ with Tris hydrochloride 0.1 mol litre⁻¹ (pH 7.4). Samples were stored in small aliquots at —80 °C until enzymatic experiments were performed in vitro [4]. Briefly, the final incubation mixture (0.25 ml) contained 50 μl of microsomal protein suspension as the source of the drug metabolizing enzymes (cytochrome P450 90 μg per 20–50 pmol), 150 μl of Tris buffer 0.05 mol litre⁻¹ (pH 7.5), 25 μl of MgCl₂ 0.06 mol litre⁻¹, 150 μl of Tris buffer 0.05 mol litre⁻¹ (pH 7.5), 25 μl of reduced beta-nicotinamide adenine dinucleotide phosphate-tetrasodium salt (NADPH) 0.004 mol litre⁻¹ (freshly prepared for each assay) and the drug substrate (alfentanil or sufentanil in a final concentration of 10 μmol litre⁻¹).

Various concentration of pure propofol diluted in methanol or equal volumes of methanol only (solvent controls) were added to the reaction vials and evaporated to dryness before each experiment. After incubation of the above reactants at 37 °C for 45 min, the reaction was stopped by addition of 1 ml of NaOH 0.5 mol litre⁻¹. The concentration of remaining substrate (alfentanil or sufentanil) after incubation with microsomes was determined with a specific capillary gas chromatographic method and nitrogen-phosphorus detection. The sensitivity limit of this method was 0.5 nmol per injection [5]. The final results (concentrations of remaining drug substrate) were calculated using a calibration curve for known concentration of drug standards in the incubation mixture (with heat-inactivated microsomes). IC50 values (the concentration of propofol causing 50%
inhibition of metabolic degradation of drug substrate relative to that in the presence of evaporated methanol solvent only) were determined by interpolation of semilog plots of at least eight concentrations of propofol (0.5–300 μmol litre⁻¹) against percentage inhibition of alfentanil or sufentanil degradation from the experimental mixture. All enzymatic experiments were performed in five separate experimental sessions using pooled microsomal fractions from three different pig liver samples and for human microsomal studies using the pooled microsomal fractions from the donor liver sample.

When alfentanil and sufentanil were incubated with microsomal cytochrome P450 in the presence of the NADPH system, the oxidative reaction rates for its oxidative catalysis were drug substrate 0.47 and 0.32 nmol (nmol P450⁻¹) min⁻¹, respectively, in pig liver microsomes and drug substrate 0.91 and 0.69 nmol (nmol P450⁻¹) min⁻¹, respectively, in human microsomes. Propofol inhibited dose-dependently the oxidative metabolic degradation of alfentanil and sufentanil by microsomal preparations from both pig and human liver. Propofol 300 μmol litre⁻¹ inhibited completely enzymatic degradation of alfentanil and sufentanil both in human and pig liver microsomes (fig. 1) Concentrations of propofol as small as 0.5 μmol litre⁻¹ did not interfere with the oxidative degradation of alfentanil and sufentanil in the same experimental conditions (not shown).

REFERENCES

Fig. 1. Inhibition by propofol of oxidative metabolism of alfentanil and sufentanil by liver microsomes in vitro. Each point represents the mean (SD) of five separate experiments. IC₅₀ values for propofol were determined by interpolation of semilog plots against percentage inhibition of alfentanil and sufentanil degradation. Pig liver: mean (SD) IC₅₀ values of propofol were 32.6 (8.3) μmol litre⁻¹ for alfentanil (A) and 22.1 (7.9) μmol litre⁻¹ for sufentanil (+). Human liver: mean (SD) IC₅₀ values were 62.8 (16.4) μmol litre⁻¹ for alfentanil (●) and 52.9 (15.4) μmol litre⁻¹ for sufentanil (●).