Br J Clin Pharmacol 1996; **41**: 565–573

Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol

A. L. VAN STEVENINCK[1], R. GIESCHKE[2], R. C. SCHOEMAKER[1], G. RONCARI[2], B. TUK[1], M. S. M. PIETERS[1], D. D. BREIMER[3] & A. F. COHEN[1]
[1]Centre for Human Drug Research, University Hospital Leiden, The Netherlands, [2]Hoffmann-La Roche, Basle, Switzerland and [3]Centre for Bio-Pharmaceutical Sciences, Leiden, The Netherlands

1 Interaction between alcohol and bretazenil (a benzodiazepine partial agonist in animals) was studied with diazepam as a comparator in a randomized, double-blind, placebo controlled six-way cross over experiment in 12 healthy volunteers, aged 19 – 26 years.

2 Bretazenil (0.5 mg), diazepam (10 mg) and matching placebos were given as single oral doses after intravenous infusion of alcohol to a steady target-blood concentration of 0.5 g l^{-1} or a control infusion of 5% w/v glucose at 1 week intervals.

3 CNS effects were evaluated between 0 and 3.5 h after drug administration by smooth pursuit and saccadic eye movements, adaptive tracking, body sway, digit symbol substitution test and visual analogue scales.

4 Compared with placebo all treatments caused significant decrements in performance. Overall, the following sequence was found for the magnitude of treatment effects: bretazenil + alcohol > diazepam + alcohol ≥ bretazenil > diazepam > alcohol > placebo.

5 There were no consistent indications for synergistic, supra-additive pharmacodynamic interactions between alcohol and bretazenil or diazepam.

6 Bretazenil with or without alcohol, and diazepam + alcohol had marked effects. Because subjects were often too sedated to perform the adaptive tracking test and the eye movement tests adequately, ceiling effects may have affected the outcome of these tests.

7 No significant pharmacokinetic interactions were found.

8 Contrary to the results in animals, there were no indications for a dissociation of the sedative and anxiolytic effects of bretazenil in man.

Keywords bretazenil diazepam alcohol interaction performance pharmacokinetics

Introduction

Bretazenil is a tetracyclic imidazocarboxylic ester belonging to a new class of benzodiazepine receptor partial agonists [1]. It is a drug which is rapidly absorbed and has a half-life of 2.5 h. Plasma concentrations are measurable 5 to 10 min after sublingual administration [2]. Results from animal studies have indicated that the compound could be a potent anxiolytic-/anticonvulsant drug with minimal sedative or muscle relaxant effects [3]. Repeated administration of bretazenil did not produce alterations in GABA receptor binding or function [4]. Initial studies in man suggested that effective anxiolytic doses of bretazenil (0.5 to 4 mg) are about 10% of those of diazepam, and are associated with minimal sedation [1, 2]. However, sedative effects have been reported subsequently in healthy subjects after 0.2 mg bretazenil [5].

Panic disorder has been identified as an indication for the clinical use of bretazenil [6]. Concomitant use of alcohol and antianxiety drugs may occur frequently as alcohol is used to alleviate anxiety by some patients, while in others anxiety or panic attacks may be induced by alcohol [7]. Therefore, the absence of a synergistic

Correspondence: Dr A. F. Cohen, Centre for Human Drug Research, Zernikedreef 10, 2333 CL Leiden, the Netherlands

pharmacodynamic interaction between bretazenil and alcohol would be an advantage if bretazenil is to be used for this indication. For full agonist benzodiazepines interaction with alcohol is generally additive, although excessive sedation may occur in susceptible individuals [8].

An experimental design in which a steady blood alcohol concentration is established by intravenous infusion has advantages for the interpretation of interaction studies [8]. This type of study has been shown to be sensitive to demonstrate an interaction between a low dose of diazepam and alcohol [8]. In the present study, this design was used to evaluate pharmacokinetic- and pharmacodynamic interactions between bretazenil and alcohol with diazepam as a control.

Methods

The investigation was designed as a randomized, double-blind, placebo controlled, six way cross-over study in 12 healthy male volunteers. The study protocol was approved by the Ethics Review Board of the Leiden University Hospital. Thirteen subjects participated in the study after giving their informed consent. All were subjected to a full medical examination before the start of the study. Twelve subjects aged 19–26 years and weighing 65–97 kg completed the study. Of these, one subject replaced a dropout due to nausea and vomiting on two occasions.

Subjects received each of the following treatments in random order at 1 week intervals: (i) bretazenil 0.5 mg + placebo, (ii) bretazenil 0.5 mg + alcohol, (iii) diazepam 10 mg + placebo, (iv) diazepam 10 mg + alcohol, (v) placebo + placebo and (vi) placebo + alcohol. Treatments were randomized according to two 6×6 Latin squares, balanced for carry over effects of preceding treatments.

Before the start of the study subjects practised the pharmacodynamic tests during four sessions each on two occasions. A 1 h intravenous infusion of alcohol (25 g h^{-1}) was administered on the second training day to assess alcohol kinetics in each individual. One week separated the last training day and the start of the study. Subjects were not permitted coffee, tea or chocolate on study days. The use of alcohol was not allowed from the day before the tests until 24 h after drug dosage. A standard breakfast and lunch were served on study days 2.5 h before and 4 h after drug dosage, respectively.

Bretazenil was given as a 0.5 mg sublingual tablet which the subjects kept under the tongue for 3 min, the remainder being swallowed with 100 ml of water. Diazepam 10 mg (Valium®) tablet was given orally with 100 ml of water. A double dummy technique was used for blinding of the treatments.

Alcohol was administered as a 5% w/v solution in 5% w/v glucose, through an indwelling catheter in a forearm vein. Infusions were given at a constant rate for 1 h, to achieve a target blood alcohol concentration of 0.5 g l^{-1}, starting 90 min before drug intake. A slower infusion rate was given subsequently to maintain this level for 4 h. The individual infusion rates were calculated according to Hartmann *et al.* [9], using pharmacokinetic parameters obtained from the pre-study infusions. Small adjustments of infusion rates were made following the first infusions of alcohol based on the measured values of breath alcohol. These adjustments were made by a separate investigator who was unblinded with regard to alcohol treatment only. The resulting infusion schedule was used on all subsequent occasions. Further adjustments were made only when blood alcohol concentrations exceeded 0.7 g l^{-1}. Similar schedules were used for the control infusions with glucose.

Three subjects were tested on each study day. Venous blood (5 ml) samples were taken into glass Vacutainer® tubes containing potassium oxalate/sodium fluoride as an anticoagulant, before drug administration, and at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300 and 360 min after. After centrifugation for 15 min at 2500 ***g*** plasma was separated and transferred to glass tubes and stored at -35° C until analysis. The measurement of breath alcohol was performed at -90, -60, -30, 15, 60, 105, 150, 195, 270, 330 and 360 min. Pharmacodynamic tests were performed at –90, -60, 15, 60, 105, 150, 195, 270 and 330 minutes. Blood pressure and heart rate were measured at -120, 0, 60, 240 and 360 min.

Pharmacodynamic tests

The following tests were used: (i) a 10 min adaptive tracking test, (ii) registration of smooth pursuit- and saccadic eye movements, (iii) a 2 min measurement of anterio-posterior body sway, standing with eyes closed, (iv) a 2 min measurement of anterio-posterior body sway, standing with eyes closed on an unstable foam surface, (v) a 90 s digit symbol substitution test (DSST) and (vi) visual analogue scales. Recording and analysis of eye movements were performed with a microcomputer-based system for sampling and analysis of eye movements. Body sway was measured with an apparatus (TNO/NIPG, Leiden, the Netherlands) similar to the Wright ataxiameter (Wright, 1971). All tests were performed as described previously [8]. The visual analogue scales described by Bond & Lader [10] were used with composite scores for alertness, mood and calmness. Blood pressure and heart rate were measured with an oscillometric blood pressure monitor (Nihon Kohden, MPV 7201), with subjects seated for 2 min prior to measurement.

Alcohol and drugs analysis

Plasma concentrations of bretazenil were assayed by capillary gas chromatography. The inter-assay precision at 2.5 ng ml^{-1} was 7.8%. The limit of quantification was 100 pg ml^{-1} [11]. Plasma diazepam concentrations were measured by h.p.l.c. The mean intra-assay variability ranged from 4.2 to 6.9% over the concentration range of 50 to 1500 ng ml^{-1}. The detection limit was

50 ng ml^{-1} [12]. Alcohol concentrations in breath were measured using a Lion Alcoholmeter AE-D3 (Lion Laboratories Ltd, South Glamorgan, UK), calibrated at regular intervals to a standard gas mixture equivalent to 0.8 g l^{-1} alcohol. The alcohol detection limit was 0.01 g l^{-1}. The assay was linear over a range from 0–4 g l^{-1}.

Pharmacokinetic analysis

Values of t_{max} and C_{max} were noted directly from the data. AUC(0, 6 h) values of bretazenil and diazepam were estimated using the linear trapezoidal rule. Estimates of the elimination half-lives of bretazenil were obtained by fitting a two compartment model to the plasma drug concentration-time curves. Elimination half-lives were not calculated for diazepam because the period of sampling was too short. Pharmacokinetic parameters for alcohol were calculated by fitting a two compartment model with Michaelis Menten kinetics to the concentration-time curves, with K_m fixed at 0.03 g l^{-1} [9]. All pharmacokinetic analyses were performed using the software package Siphar (Version 4.0, Simed, Créteil, France).

Statistics

The influence of alcohol on the pharmacokinetic parameters of bretazenil and diazepam was evaluated by use of paired *t*-tests. Pharmacodynamic interactions between the drugs and alcohol were evaluated for bretazenil and diazepam separately, according to a 2×2 factorial interaction model on the average response over the period from 0 to 195 min after drug administration. The factorial model provides three estimates (reported with 95% confidence intervals) which answer three questions:

1. How large is the sum of the individual treatment effects relative to the effect of the combination treatment. Interactions are synergistic (supra-additive) if the effect of combined treatment with alcohol and drug is larger than the sum of the effects of the single treatments. Interactions are negative if the effect of the combined treatment is smaller than the sum of the effects of single treatments.
2. How large is the drug effect averaged over treatments with and without alcohol.
3. How large is the alcohol effect averaged over treatment with and without drug.

In the case of a significant interaction, the effect of for instance alcohol depends on whether or not drug is present. In this case, estimates 2 and 3 provide average effects which are not the same as comparing the all-placebo treatment with the drug-only or alcohol-only treatment.

Blood pressure and heart rate at 1 h after drug intake were compared between treatments by repeated measures analysis of variance. In the case of significant treatment effects, contrasts between active treatments and placebo were evaluated by use of paired *t*-tests and are reported with 95% confidence intervals. The statistical analyses were performed using SPSS/PC + V4.0.1 statistical software (SPSS Inc., Chicago, IL, USA).

Results

One subject was replaced because of nausea and vomiting following alcohol-placebo and alcohol-diazepam treatments. The new subject received the same order of treatments as the subject who was replaced. All other subjects completed the study without any major adverse events.

Pre-study alcohol disposition

The average maximum breath alcohol concentration was 0.39 g l^{-1} (range 0.21–0.57 g l^{-1}). Mean ($\pm$s.d.) disposition parameters were: $V_{max} = 9.6 \pm 2.7$ g h^{-1}, $V_1 = 8.9 \pm 3.0$ l, $k_{12} = 14.8 \pm 8.1$ h^{-1} and $k_{21} = 2.5 \pm 0.5$ h^{-1}. From individually determined pharmacokinetic parameters, the infusion rates were calculated. Mean ($\pm$s.d.) rates were 36 ± 7.5 g h^{-1} (range: 26.4–50.0 g h^{-1}) for the loading infusion, and 9.2 ± 2.6 g h^{-1} (5.9–13.4 g h^{-1}) for the maintenance infusion.

Alcohol concentrations

The average breath alcohol concentrations during pseudo steady state were 0.47 g l^{-1} (range: 0.29–0.70 g l^{-1}) after alcohol alone, 0.48 g l^{-1} (0.31–0.70 g l^{-1}) after alcohol + bretazenil and 0.45 g l^{-1} (0.33–0.68 g l^{-1}) after alcohol + diazepam. The average time courses of breath alcohol concentrations are shown in Figure 1a and b. In one subject an extra adjustment of the infusion rate was made during the pseudo steady state interval because his breath alcohol concentration exceeded 0.7 g l^{-1} during treatment with alcohol + bretazenil.

Pharmacokinetics of bretazenil

No significant differences in t_{max}, C_{max}, $t_{\frac{1}{2}}$ and AUC(0, 6 h) values of bretazenil were found after combined treatment with alcohol or placebo. The mean ($\pm$s.d.) values for the alcohol and placebo treatments were, respectively $t_{max} = 1.5 \pm 0.6$ h and 1.4 ± 0.4 h, $C_{max} = 5.4 \pm 1.8$ ng ml^{-1} and 5.0 ± 2.0 ng ml^{-1}, $t_{\frac{1}{2}} = 2.3 \pm 0.5$ h and 2.2 ± 0.6 h, and AUC(0, 6h) $= 19.2 \pm 5.7$ ng ml^{-1} h and 17.6 ± 5.3 ng ml^{-1} h.

Pharmacokinetics of diazepam

There were no significant differences in t_{max}, C_{max} and AUC(0, 6h) of diazepam after combined treatment with alcohol or placebo. The mean ($\pm$s.d.) values for the

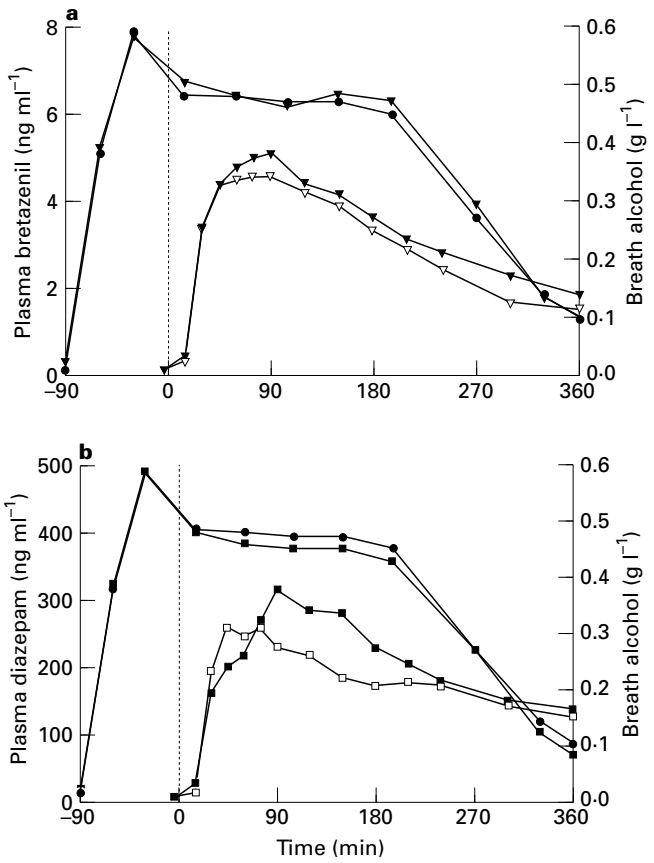

Figure 1 Average plasma concentrations of bretazenil (a; lower curves, left axis), diazepam (b; lower curves, left axis) and mean breath alcohol concentrations (a,b; upper curves, right axis). ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo.

alcohol and placebo treatments, respectively, were: $t_{max} = 1.6 \pm 0.7$ h and 1.3 ± 0.9 h, $C_{max} = 367 \pm 107$ ng ml^{-1} and 318 ± 35 ng ml^{-1}, and AUC(0, 6h) = 1172 ± 241 ng ml^{-1} h and 1046 ± 167 ng ml^{-1} h.

Pharmacodynamics

Changes in performance were demonstrated for all active treatments. Overall the effects were largest for bretazenil + alcohol, followed by diazepam + alcohol, bretazenil, diazepam and alcohol alone. The effects of bretazenil + alcohol, diazepam + alcohol and bretazenil alone were generally large, causing subjects to fall asleep during the adaptive tracking test, eye movement registrations and occasionally during body sway measurements. In these cases ceiling effects may have caused underestimation of interactions. The average effects are summarised in Table 1 for bretazenil and in Table 2 for diazepam. The average time courses for adaptive tracking, saccadic peak velocity, body sway, DSST and subjective alertness are shown in Figures 2 to 6. Figure 7 shows data from subject 4, illustrating ceiling effects.

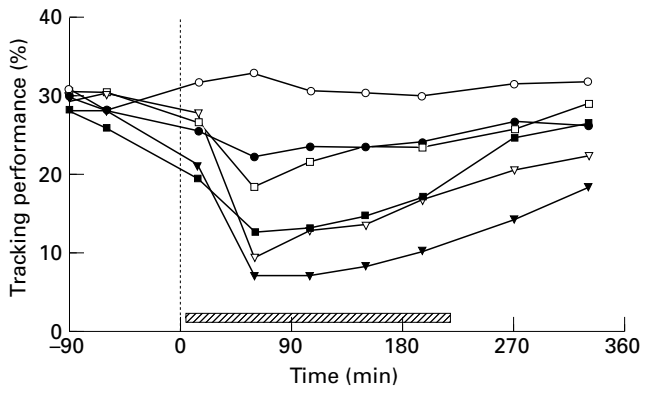

Figure 2 Average adaptive tracking performance. ○ = glucose 5% i.v. + placebo, ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

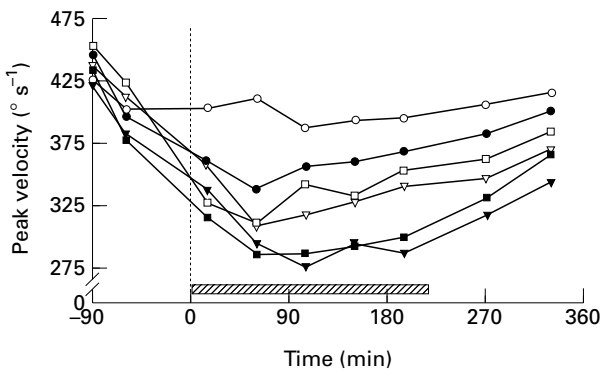

Figure 3 Average saccadic peak velocity. ○ = glucose 5% i.v. + placebo, ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

Alcohol effects

Two estimates of alcohol effect were obtained, one from the factorial analysis of the bretazenil × alcohol interaction (Table 1), and one from the factorial analysis of the diazepam × alcohol interaction (Table 2). The results from both analyses were comparable. Significant detrimental effects of alcohol were found for the following parameters: adaptive tracking performance, smooth pursuit (alcohol + diazepam analysis only), saccadic peak velocity, saccadic latency, body sway, body sway on an unstable surface, DSST and subjective alertness. In the alcohol + bretazenil analysis, alcohol caused a slight but significant decrease in the standard deviation of adaptive tracking, i.e. a reduction in the variability of the performance.

Bretazenil effects

Bretazenil caused a decrease in subjective alertness as well as decreases in the performance of all objective

Table 1 Pharmacodynamic data; drug effects and interaction terms in a 2 × 2 factorial analysis of the alcohol/bretazenil interaction (95% confidence intervals in parentheses).

	Alcohol[a]	*Bretazenil*[b]	*Interaction*[c]
Tracking performance (%)	−6.4 (−8.9, −3.8)***	−14.1 (−16.6,−11.5)***	−1.0 (−3.8, 1.8)¶
s.d. of tracking performance (%)	−1.0 (−2.0, −0.1)*	−1.0 (−2.4, 0.4)	1.1 (0.3, 1.8)*,¶
Smooth pursuit performance (%)	−4.1 (−9.5, 1.4)	−5.9 (−10.6, −1.2)*	2.3 (−1.1, 5.6)¶
Saccadic peak velocity (°/s)	−38 (−61, −16)**	−65 (−83, −48)***	−3 (−22, 17)¶
Saccadic latency (ms)	13 (7, 20)**	21 (14, 27)***	−5 (−9, −1)*,¶
Saccadic inaccuracy (%)	−0.7 (−1.7, 0.4)	1.6 (0.3, 2.9)*	−0.2 (−1.7, 1.3)¶
Body sway AP eyesclosed(mm min^{-1})	159 (39, 279)*	362 (224, 499)***	−51 (−154, 52)
Body sway AP foam (mm min^{-1})	245 (52, 439)*	485 (357, 613)***	−106 (−284, 73)
Digit symbol substitution (*n*)	−4.0 (−7.3, −0.6)*	−10.6 (−13.5, −7.7)***	1.2 (−1.5, 3.8)
Visual analogue scale 'alertness' (mm)	−7.0 (−11.3, −2.7)**	−9.0 (−14.1, −3.8)**	−3.2 (−5.2, −1.1)**
Visual analogue scale 'mood' (mm)	−3.1 (−6.3, 0.1)	−0.8 (−4.4, 2.8)	−0.1 (−1.5, 1.4)
Visual analogue scale 'calmness' (mm)	−1.0 (−4.0, 2.0)	2.0 (−0.6, 4.6)	−0.7 (−4.8, 3.3)

* $P<0.05$, ** $P<0.01$, *** $P<0.001$. ¶: ceiling effects may have affected results (see text).

a) Alcohol effect: ((A + AB) − (P + B))/2, b) Bretazenil effect: ((B + AB) − (P + A))/2, c) Interaction: ((A + B) − (P + AB))/2, where A = alcohol infusion, B = bretazenil, AB = alcohol and bretazenil, and P = placebo.

performance tests (Table 1). Marked sedation was observed, with subjects tending to fall asleep during the tests on some occasions. The effects of bretazenil were comparable with those occurring with the combination of diazepam and alcohol.

Effects of combined alcohol and bretazenil

Following bretazenil + alcohol subjects were highly sedated and often unable to perform the tests adequately. Although the measured effects were generally additive, ceiling effects are likely to have affected the results of adaptive tracking and eye movement analysis. A significant synergistic interaction was found for saccadic latency. Significant negative interactions were found for the standard deviation of adaptive tracking performance and for subjective alertness (Table 1).

Diazepam effects

Diazepam caused considerable sedation as indicated by significant decreases in subjective alertness and in all performance parameters except smooth pursuit eye movements. For all parameters except saccadic peak velocity the average effects of diazepam were less than those observed after bretazenil. The lack of a difference for saccadic peak velocity may have been caused by a ceiling effect of bretazenil.

Effects of combined alcohol and diazepam

Alcohol + diazepam caused large decreases in subjective alertness and in all objective measures of performance. As a result the performance of tests was often inadequate. As for bretazenil, the measured effects of diazepam and alcohol were generally additive. A significant synergistic interaction was found for smooth pursuit eye movements.

Haemodynamic effects

Bretazenil caused significant decreases in diastolic blood pressure, whereas diazepam increased heart rates but did not affect blood pressure. Increased heart rates and decreases in diastolic blood pressure were observed after alcohol alone, after alcohol + bretazenil and after alcohol + diazepam. The haemodynamic effects are summarised in Table 3.

Table 2 Pharmacodynamic data; drug effects and interaction terms in a 2 × 2 factorial analysis of the alcohol/diazepam interaction. (95% confidence intervals in parentheses).

	Alcohol	*Diazepam*	*Interaction*[1]
Tracking performance (%)	−7.3 (−10.2, −4.5)***	−8.4 (−10.5, −6.3)***	0.0 (−2.9, 2.9)¶
s.d. of tracking performance (%)	−0.3 (−1.2, 0.5)	0.3 (−0.5, 1.1)	0.4 (−0.3, 1.0)¶
Smooth pursuit performance (%)	−5.8 (−10.9, −0.6)*	−2.0 (−5.7, 1.7)	3.9 (0.5, 7.4)*,¶
Saccadic peak velocity (°/s)	−42 (−60, −23)***	−65 (−82, −49)***	0 (−20, 20)¶
Saccadic latency (ms)	17 (7, 28)**	11 (3, 18)*	−9 (−18, 0)¶
Saccadic inaccuracy (%)	0.0 (−0.9, 0.9)	2.2 (0.9, 3.4)**	−0.9 (−1.8, 0.1)¶
Body sway AP eyesclosed(mm min^{-1})	140 (11, 269)*	215 (128, 301)***	−32 (−144, 80)
Body sway AP foam (mm min^{-1})	224 (67, 382)**	265 (121, 408)**	−85 (−278, 108)
Digit symbol substitution (*n*)	−3.0 (−5.1, −1.0)**	−5.1 (−6.9, −3.2)***	0.2 (−2.4, 2.8)
Visual analogue scale 'alertness' (mm)	−7.8 (−11.7, −4.0)**	−6.2 (−9.9, −2.5)**	−2.3 (−6.0, 1.4)
Visual analogue scale 'mood' (mm)	−1.6 (−4.2, 0.9)	−0.9 (−2.6, 0.9)	−1.5 (−4.1, 1.0)
Visual analogue scale 'calmness' (mm)	−0.2 (−3.0, 2.6)	0.7 (−1.1, 2.5)	−1.5 (−5.7, 2.6)

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, ¶: ceiling effects may have affected results (see text).

a) Alcohol effect: ((A + AD) − (P + D))/2, b) Diazepam effect: ((D + AD) − (P + A))/2, c) Interaction: ((A + D) − (P + AD))/2, where A = alcohol infusion, D = diazepam, AD = alcohol and diazepam and P = placebo.

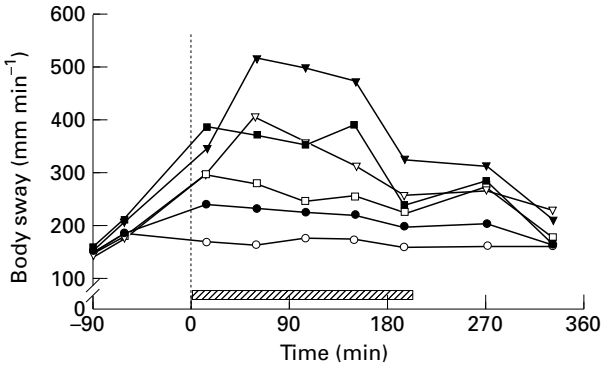

Figure 4 Average body sway (eyes closed). ○ = glucose 5% i.v. + placebo, ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

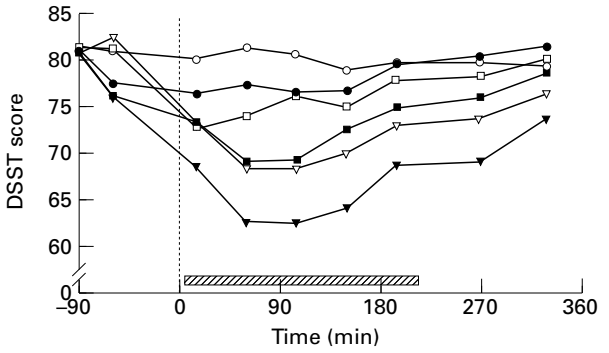

Figure 5 Average digit symbol substitution (DSST) scores. ○ = glucose 5% i.v. + placebo, ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

Discussion

The present study investigated the pharmacokinetic and pharmacodynamic interactions between bretazenil (0.5 mg) or diazepam (10 mg), and alcohol at breath concentrations of about 0.5 g l^{-1}. Although diazepam (5 mg) appeared optimal for the evaluation of pharmacodynamic interactions with alcohol in a previous study [8], a higher dose was selected in the present study to allow a comparison with a larger body of existing data. The average pseudo-steady state alcohol concentrations were closer to target levels in the present study, compared with a previous investigation [8], because of

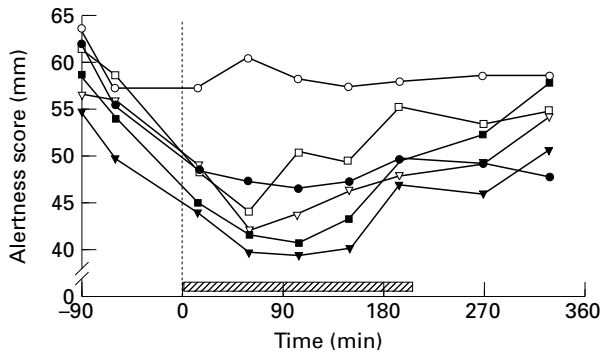

Figure 6 Average scores for subjective alertness. ○ = glucose 5% i.v. + placebo, ▽ = glucose 5% i.v. + bretazenil, ▼ = alcohol i.v. + bretazenil, □ = glucose 5% i.v. + diazepam, ■ = alcohol i.v. + diazepam, ● = alcohol i.v. + placebo. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

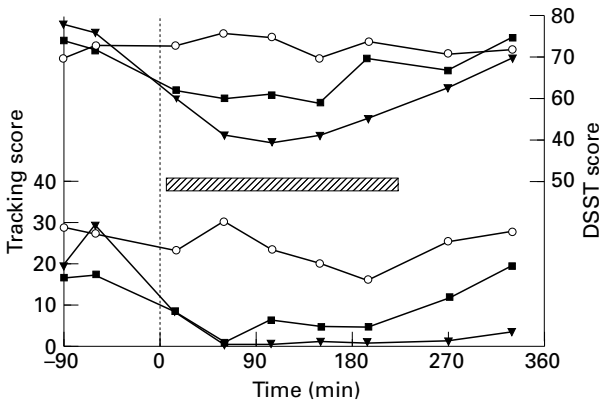

Figure 7 Subject 4; adaptive tracking performance (lower curves) and digit symbol substitution scores (upper curves). ○ = glucose 5% i.v. + placebo, ▼ = alcohol i.v. + bretazenil, ■ = alcohol i.v. + diazepam. Horizontal bar indicates interval with constant breath alcohol i.v. levels. Ceiling effects reduce the discrimination between treatments at high effect levels in the adaptive tracking test but not in the digit symbol substitution test. The vertical dashed line indicates the time of administration of bretazenil, diazepam or placebo. The horizontal bar indicates the interval with constant breath alcohol levels.

additional adjustments of the infusion rates based on the measured breath alcohol. Individual alcohol concentrations varied considerably, since small errors in the predicted infusion rates cause large deviations in the resulting alcohol levels. However, pseudo-steady alcohol levels were stable in all subjects, which is required for a reliable interpretation of drug interactions [8].

Bretazenil, bretazenil + alcohol, and diazepam + alcohol often caused subjects to fall asleep during adaptive tracking and eye movements. Under these circumstances, judgement of interactions is difficult: ceiling effects occur, tests yield extreme results when subjects fall asleep, and sleepiness is influenced by the challenge of the test. Thus, while subjects were often too sedated to adequately perform the rather monotonous adaptive tracking or eye movement tests, they were usually still able to perform the more demanding digit symbol substitution test. Judgement of pharmacodynamic interactions is further impaired by the fact that they may be restricted to specific performance parameters, depending on typical sensitivities of individual subjects [8].

Most tests showed no signs of an interaction between diazepam and alcohol. There was a clear effect of diazepam + alcohol on smooth pursuit, while diazepam or alcohol alone had no apparent effect. This last result is not in accordance with the conclusions of other studies that report impairment of smooth pursuit eye movements with benzodiazepines [13, 14].

Bretazenil + alcohol caused a synergistic interaction for saccadic latency, but not for other measures of saccadic eye movements. The reduction in variability (standard deviation) of the adaptive tracking performance can be attributed to extreme sleepiness during this rather tedious test. The negative interaction for subjective alertness may indicate a poor judgement of performance following bretazenil + alcohol. By contrast, subjects had less difficulty staying awake during more stimulating tasks such as body sway and digit symbol substitution. No interactions were found for these test measurements.

Therefore a pharmacodynamic interaction between bretazenil and alcohol could not be consistently detected, although the two substances combined caused marked sedation in the present study, as did bretazenil alone. This is in contrast to the results of animal experiments, which consistently showed that bretazenil is an anxiolytic and anticonvulsant drug with minimal muscle relaxant- and sedative effects [1, 3]. Results of the present study do not suggest a similar dissociation of effects in man at a dose of 0.5 mg. There may be differences between species in benzodiazepine receptor reserve or different receptor subtypes in the neurone pools involved in the effects [1, 15, 16]. Alternatively, a full agonist metabolite

Table 3 Haemodynamic effects at $t = 60$ min, compared with placebo (95% confidence intervals). BP-syst = Systolic blood pressure, BP-diast = Diastolic blood pressure.

	Alcohol	*Bretazenil*	*Diazepam*	*Bretazenil + alcohol*	*Diazepam + alcohol*
Heart rate (beats min^{-1})	7 (4, 10)***	NS	9 (2, 15)*	8 (2, 15)*	11 (5, 16)**
BP-syst (mmHg)	NS	NS	NS	NS	NS
BP-diast (mmHg)	−6 (−10, −3)**	−4 (−7, −1)*	NS	−4 (−7, −2)**	−4 (−6, −1)*

BP-syst = Systolic blood pressure, BP-diast = Diastolic blood pressure.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS: not significant.

of bretazenil may be generated in man. Oxidative and reductive processes are involved in the metabolism of bretazenil and several metabolites have been demonstrated *in vitro* [11]. The insufficient predictive value of animal models used in the development of anxiolytic drugs appears to be a more general problem for which no satisfactory solution has been found [17].

In a previous study, Saletu *et al.* [5], reported that sedation following an oral dose of 0.2 mg bretazenil was less than that observed after 10 mg diazepam. However, clinical studies have indicated 0.5 mg bretazenil as a minimal dose for the treatment of panic attacks [6]. In view of the marked sedative effects of bretazenil and bretazenil + alcohol, there seems to be no advantage of bretazenil over existing anxiolytic drugs. Although alcohol and benzodiazepine (partial-) agonists may be less sedative in patients with panic disorder than in healthy controls [18, 19], bretazenil 1.0 mg impaired psychomotor performance more than diazepam 20 mg in experienced users of psychoactive drugs [20].

The effects of alcohol on haemodynamic parameters were striking as these are not normally expected after low doses [21]. However, this may be explained by the fact that alcohol concentrations were kept at 0.5 g l^{-1} for a prolonged period of time. Haemodynamic effects, which are normally expected after pre-anaesthetic or hypnotic doses of benzodiazepines [21], were also found after bretazenil 0.5 mg and diazepam 10 mg in the present study. Although the effects were small and of no clinical consequence, it should be noted that they occurred in a population with normally very stable haemodynamics. The cardiovascular effects of diazepam and bretazenil are in agreement with a proposed reduction of cardiac output or vasodilation [21].

In the present study, bretazenil 0.5 mg caused greater sedation than diazepam 10 mg. There were no pharmacokinetic interactions between alcohol and bretazenil. There were also no clear pharmacodynamic interactions, although these may be obscured by the marked sedation caused by the combination of bretazenil and alcohol. There was no evidence for a dissociation of sedative and anxiolytic effects in man of bretazenil 0.5 mg, which appears to be the preferred dose for treatment of anxiety- and panic disorder [1, 2].

The authors thank Hoffmann La-Roche Ltd for financial support, for supplying bretazenil, Valium and matching placebo tablets and for the analysis of bretazenil and diazepam plasma concentrations. We also wish to thank Drs H. C. R. Brandenburg for preparing the infusions and Anandy Reints for her skilful assistance during the conduct of the study.

References

1 Haefely W, Martin JR, Schoch P. Novel anxiolytics that act as partial agonists at benzodiazepine receptors. *Trends Pharmacol Sci* 1990; **11**: 452–456.

2 Potokar J, Nutt DJ. Anxiolytic potential of benzodiazepine receptor partial agonist. *CNS Drugs* 1994; **1**: 305–315

3 Potier MC, Prado de Carvalho L, Venault P, Chapouthier G, Rossier J. Demonstration of the partial agonist profiles of Ro 16–6028 and Ro 17–1812 in mice in vivo. *Eur J Pharmacol* 1988; **156**: 169–172.

4 Marley RJ, Gallager DW. Chronic diazepam treatment produces regionally specific changes in GABA-stimulated chloride influx. *Eur J Pharmacol* 1989; **159**: 217–223.

5 Saletu B, Grünberger J, Linzmayer L. On the central effects of a new partial benzodiazepine agonist Ro 16–6028 in man: pharmaco–EEG and psychometric studies. *Int J clin Pharmacol Ther Toxicol* 1989; **27**: 51–65.

6 Katschnig H, Merz WA, Alf C. Attack–controlled discontinuous administration of RO 16–6028 as a new strategy in the treatment of panic disorder: Results of a placebo controlled double blind study. *Psychopharmacology* 1988; **96 (suppl)**: 253.

7 Cowley DS. Alcohol abuse, substance abuse, and panic disorder. *Am J Med* 1992; **92 (suppl 1A)**: 41S–48S.

8 Van Steveninck AL, Gieschke R, Schoemaker HC *et al.* Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. *Psychopharmacology* 1993; **110**: 471–478.

9 Hartmann D, Flückiger A, Ziegler WH, Timm U, Zell M. Experimental model for the investigation of kinetic and/or dynamic interactions between drugs and ethanol in humans. *J Pharmacol Sci* 1988; **77**: 299–301.

10 Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J med Psychol* 1974; **47**: 211–218.

11 Timm U, Fischer G, Zell M, Zumbrunnen R. Determination of the partial benzodiazepine receptor agonist Ro 16–6028 in plasma by capillary gas chromatography with nitrogen–selective detection after conversion into the ethyl ester derivative. *J Chromatogr* 1989; **494**: 119–134.

12 Cotler S, Puglisi CV, Gustafson JH. Determination of diazepam and its major metabolites in man and in the cat by high–performance liquid-chromatography. *J Chromatogr* 1981; **222**: 95–106.

13 Bittencourt PRM, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol* 1983; **15**, 259–262.

14 Van Steveninck AL, Kroon JM, Schoemaker HC, Pieters MSM, Breimer DD, Cohen AF. A study comparing the sensitivities of adaptive tracking, eye movement analysis and visual analogue lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* 1991; **50**: 172–180.

15 Haefely W. Partial agonists of the benzodiazepine receptor: from animal data to results in patients. In *Chloride channels and their modulation by neurotransmitters and drugs*, eds Biggio G, Costa E, New York: Raven Press, 1988: 275–292.

16 Kemp JA, Wafford K, Horne AL, Marshall GR, Whiting P. Functional determination of benzodiazepine receptor ligand efficacy. *J Psychopharmacol* 1992; **BAP/EBPS Abstracts, A33**: 132.

17 Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B. Animal models of anxiety and the development of novel anxiolytic drugs. *Prog Neuro Psychopharmacol* 1991; **15**: 205–212.

18 Roy Byrne PP, Cowley DS, Greenblatt DJ, Shader RI, Hommer D. Reduced benzodiazepine sensitivity in panic disorder. *Arch Gen Psychiat* 1990; **47**: 534–538.

19 Nutt DJ, Glue P, Lawson C, Wilson S. Flumazenil provocation of panic attacks. Evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch Gen Psychiat* 1990; **47**: 917–925.

20 Sellers EM, Busto U, Kaplan HL. Clinical abuse liability of partial and full agonist benzodiazepines: a comparison of bretazenil, diazepam and alprazolam. In *Biological Psychiatry (Proc. of the 5th World Congress of Biol.Psych.)*, eds Racagni G, *et al*., Amsterdam: Elsevier Science Publishers, 1991: 709–712.

21 Rall TW. Hypnotics and sedatives. In *The pharmacological basis of therapeutics*, eds Goodman Gilman A, Goodman LS, Rall TW, Murad F, New York: Macmillan, 1990: 345–382.

(*Received 13 July 1994, accepted 1 February 1996*)