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The effect of flumazenil on subclinical psychometric or neurophysiological alterations in cirrhotic patients: a double-blind placebo-controlled study

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Summary. It is not yet clear if benzodiazepine receptor ligands, implicated in the pathophysiology of hepatic coma, also have a role in subclinical cognitive or neurophysiological alterations in cirrhotic patients. Therefore, we carried out a double-blind, placebo-controlled study to evaluate the effectiveness of flumazenil, a benzodiazepine antagonist, on brainstem auditory evoked responses and on the number connection test in cirrhotic patients with subclinical neurophysiological or cognitive alterations. Thirteen cirrhotic subjects with subclinical neurophysiological or cognitive alterations were studied. A total of 3 mg of flumazenil or saline was infused intravenously. Before and after the infusion, the number connection test was administered and brainstem auditory evoked responses recorded. After 72 h, patients were crossed over. Flumazenil did not influence brainstem auditory evoked responses or the number connection test. A screening test for benzodiazepines was negative in all subjects. We conclude that benzodiazepine receptor ligands have a negligible role, if any, in the pathophysiology of subclinical neurophysiological or cognitive alterations of cirrhotic patients.

Key words: auditory evoked responses, flumazenil, hepatic encephalopathy, number connection test.

Introduction

Hepatic encephalopathy may result from the action of benzodiazepine receptor ligands (Basile *et al*., 1991). Indeed, a benzodiazepine antagonist, flumazenil, was successfully used in the treatment of overt hepatic encephalopathy in several uncontrolled studies and case reports (Bansky *et al*., 1985, 1989; Scollo-Lavizzari & Steinmann, 1985; Burke, 1988; Grimm *et al*., 1988; Ferenci *et al*., 1989; Pidoux *et al*., 1989; Franco *et al*., 1990; Viel *et al*., 1990). Double-blind randomized studies seemed to confirm the effectiveness of flumazenil in acute hepatic coma, at least in 14Ð66% of patients (Pomier-Layrargues *et*

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al., 1994; Cadranel *et al.,*1995; van Der Rijt *et al*., 1995; Groeneweg *et al*., 1996; Gyr *et al*., 1996).

A relevant percentage (7–70%) of cirrhotic patients without overt hepatic encephalopathy display psychometric or neurophysiological alterations (Sood *et al*., 1989; Weissenborn *et al*., 1990; Van der Rijt & Schalm, 1992; Quero *et al*., 1996). Usually, such alterations are considered to be the expression of a latent, or subclinical, phase of hepatic encephalopathy (Gitlin, 1988; Mehndiratta *et al*., 1990; Bley, 1996; Quero *et al*., 1996), even if a firm demonstration of this assumption does not exist.

The role of benzodiazepine receptor agonists in the pathophysiology of subclinical neurophysiological or cognitive alterations in cirrhotic patients is not well defined. Gooday *et al.* (1995) suggested that such substances may have a pathophysiological role because flumazenil improved simple reaction time and cognitive speed in cirrhotic patients with subclinical cognitive alterations. However, the role of benzodiazepine receptor ligands on the alterations of the number connection test and evoked potentials, widely used tools to detect the so-called subclinical hepatic encephalopathy in cirrhotic patients without overt hepatic encephalopathy, has not yet been defined.

Therefore, we performed a randomized, double-blind, placebo-controlled, crossover trial to test the efficacy of flumazenil on brainstem auditory evoked responses and on the number connection test in cirrhotic patients with subclinical alterations of these tests.

Subjects and methods

The study comprised 13 patients (aged 54±7 years, 10 men, three women) with liver cirrhosis. The diagnosis was clinical in three patients (based on history, biochemical and sonographic findings and gastroscopy evidence of oesophageal varices) and biopsy-proven in 10 patients. Ten patients had alcoholic cirrhosis, one mixed aetiology (alcohol and virus-related) cirrhosis and two post-hepatitic cirrhosis. Two were class A, three class B and eight class C, according to the Pugh–Child classification. None had surgical portosystemic shunt or transjugular portosystemic shunt**.**

None of the patients had (a) heart, respiratory (P_{O_2}<mmHg, P_{CO_2}>50 mmHg or both) or renal failure (serum creatinine values 150 μmol l^{-1}); (b) psychiatric or neurological illness; (c) auditive threshold more than 25 dbHl between 1000 and 4000 Hz; (d) overt hepatic encephalopathy. None took psychotropic drugs in the preceding 2 weeks or alcohol in the preceding month, which was documented by an interview with the patient and confirmed by a relative.

In any case, before the beginning of the experiment, all patients underwent benzodiazepine screening (Emit-dau technique, Dupont, detection limit corresponding to 0·3 μg ml^{-1} diazepam).

In all patients the I-V interpeak latency (Fig. 1) of the brainstem auditory evoked potentials (auditory stimulus 21 and 70 Hz, Brain Atlas III, Bio-logic) was higher than two SD above the reference values of our laboratory (Martini *et al*., 1991) and/or they per-

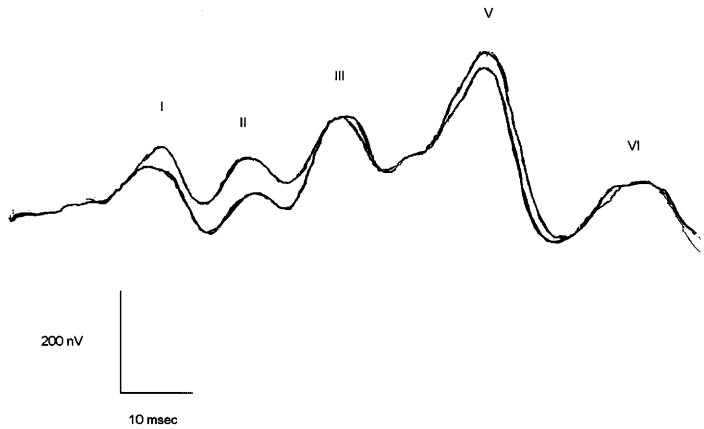

Fig. 1. Normal acoustic brainstem evoked potentials.

formed the number connection test (NCT) in more than 30 s. The NCT is the most widely used neuropsychological tool in the detection of subclinical hepatic encephalopathy. Twenty-five numbered circles, from 1 to 25, are printed on a sheet of paper (Conn, 1977). The task is to connect them in consecutive order with a pencil as quickly as possible. One out of four versions of the test was randomly sorted and performed after filling a demonstration form. The test was graded according to Conn's criteria for the calculation of the index of portosystemic encephalopathy, a widely used tool to assess the severity of hepatic encephalopathy (Conn *et al*., 1977): NCT≤30 s=score 0; NCT 31–50 s=score 1; NCT 51–80 s=score 2; NCT 81–120 s=score 3; NCT 120 s=score 4. Of the patients that we studied, five had score 1, four score 2 and four score 3.

Patients were randomly and double-blindly assigned to flumazenil or placebo (saline infusion) treatment. After taking a blood sample for benzodiazepine screening, a bolus of 10 ml of flumazenil (1 mg) or 10 ml of placebo was infused, followed by four boluses of 5 ml of flumazenil (0·5 mg) or placebo every 30 min, thus reaching a total dose of 3 mg of flumazenil in 2 h. Thirty minutes before and within 30 min after the infusion patients underwent the number connection test (using different versions of the test) and auditory evoked potentials.

After a 3-day washout, patients were crossed over and all the above steps were repeated.

There was no ethics committee in our faculty while the study was being performed (1994), but the protocol was submitted and approved by the Senior Staff Committee of our Department. All patients gave their informed consent to the study.

STATISTICS

The comparison between flumazenil and placebo was carried out by two-way ANOVA for repeated measure (factors: between = treatment, within = period). The sample size (13 subjects) was calculated fixing = 0·05 and = 0·20, according to the treatment that

could reduce the I–V interval or the NCT to at least one standard deviation of the values of the controls of our laboratory as effective.

Results are reported as means ± standard deviation.

Results

The screening test for benzodiazepines was negative in all patients.

The I–V interpeak latency and the number connecton test did not appear to be influenced by the treatment ($F_{1,22} = 0{\cdot}01$ $P = 0{\cdot}92$ and $F_{1,22} = 0{\cdot}13$ $P = 0{\cdot}71$ respectively), by the sequence with which the treatment was selected ($F_{1,22} = 1{\cdot}49$ $P = 0{\cdot}23$ and $F_{1,22} = 0{\cdot}91$] $P = 0{\cdot}35$, respectively), or by the interaction between treatment and sequence (Fig. 2).

The mean difference (flumazenil–placebo) in the interval I–V before treatment was 0·05 ± 0·14 ms (95% CI from –0·04 to 0·14); after treatment it was 0·001 ± 0·11 ms (95% CI from –0·08 to 0·08). The mean difference (flumazenil–placebo) in the NCT before treatment was 5 ± 3 s (95% CI–2 to 12); after treatment it was 3 ± 7 s (95% CI–12 to 18).

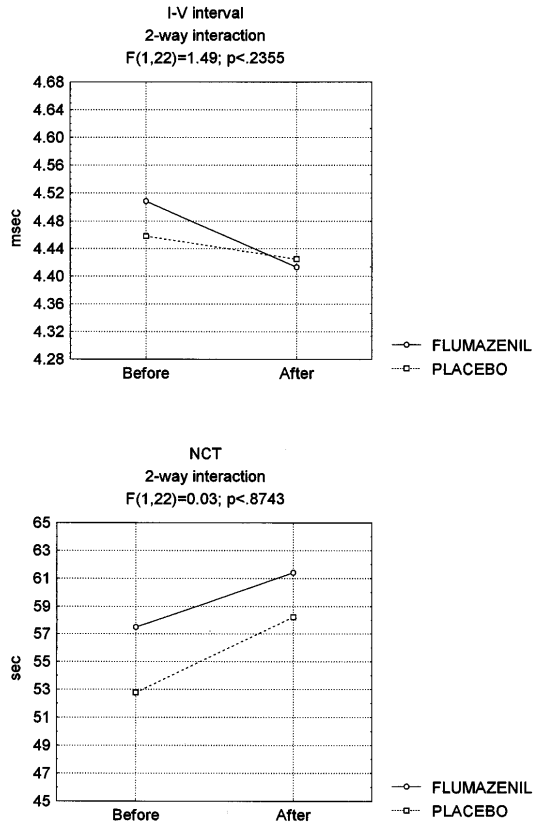

Fig. 2. Comparison of flumazenil vs. placebo on the I–V interval of brainstem acoustic evoked potentials and number connection test.

Table 1. Comparison of flumazenil or placebo treatment on the I–V interval in cirrhotic patients with prolonged basal I–V intervals

Flumazenil		Placebo		Treatment	Period	Interaction
Before	After	Before	After	$F_{1,8}=0{\cdot}007$	$F_{1,8}=3{\cdot}425$	$F_{1,8}=0{\cdot}630$
4·91±0·38*	4·79±0·48	4·86±0·45	4·79±0·52	$P=0{\cdot}93$	$P=0{\cdot}10$	$P=0{\cdot}63$

*Mean ±SD in ms.

Table 2. Comparison of flumazenil or placebo treatment on NCT >50 sincerely (class 2 according to Conn's grading)

Flumazenil		Placebo		Treatment	Period	Interaction
Before	After	Before	After	$F_{1,10}=0{\cdot}046$	$F_{1,10}=1{\cdot}001$	$F_{1,10}=0{\cdot}367$
76±24*	79±34	67±21	82±39	$P=0{\cdot}83$	$P=0{\cdot}34$	$P=0{\cdot}56$

*Mean ±SD in s.

In addition, considering only the five patients with prolonged basal I–V interval (>2 SD of controls), or the six patients with NCT 50 s (at least class 2 of Conn's grading), no effect was found (Tables 1 and 2).

Discussion

Evoked potentials, and particularly interpeak latency of brain stem auditory evoked potentials, are a sensitive tool in the detection of hepatic encephalopathy (Mehndiratta *et al*., 1990). Previous alcohol consumption may influence evoked potentials (Porjesz & Begleiter, 1987), however it should be pointed out that it is not yet clear if alcohol *per se* influences evoked responses, or if they are influenced by liver disease caused by alcohol consumption. In any case, in our series acute alcohol toxicity was excluded because the patients had abstained for at least 1 month before the test.

In addition, the number connection test is a reliable index of central nervous system dysfunction in liver disease (Sood *et al*., 1989; McCrea *et al*., 1996), possibly reflecting visuo-motor impairment (Moss *et al*., 1992) or attentional dysfunction (McCrea *et al*., 1996).

Even if positive results with flumazenil were obtained in a subgroup of patients with overt hepatic encephalopathy (Pomier-Layrargues *et al*., 1994; Van der Rijt *et al*., 1995; Gyr *et al*., 1996), we did not find significant reductions of the I–V interpeak latency in acoustic brainstem evoked potentials or the number connection test with flumazenil in the cirrhotics without overt hepatic encephalopathy, considered either together or in the subgroup with prolonged I–V interval or number connection test. A type II error can be excluded with $P<0{\cdot}20$ if the effect is at least equal to 1 SD; however, a negligible effect of flumazenil on the I–V interval cannot be excluded because a slight reduction

(1·7%) in the I–V interval was found. However, it was so small that the study should have enrolled about 290 subjects to attain sufficient power to avoid a β-error with $P = 0{\cdot}20$.

Indeed, a reduction in event-related auditory P300 potentials in cirrhotic patients without overt hepatic encephalopathy was found in an uncontrolled study by Bruha *et al.* (1994) and a reduction in reaction times by Gooday *et al.* (1995) in a placebo-controlled trial. This latter study, however, did not show any effect on the symbol digit substitution test, suggesting that more complex cognitive functions needing attention and visuo-motor integration are unaffected, a finding that is well in keeping with the absence of any effect on the number connection test in our study.

Overall, our findings suggest that benzodiazepine receptor ligands had a minor role, if any, in the pathophysiology of subclinical central nervous system dysfunction of the cirrhotic patients that we studied. The data may also suggest, however, that much more caution than usual should be paid in the interpretation of subclinical neurophysiological or neuropsycological alterations (at least as far as the grading of the number connection test is concerned) in cirrhotic patients. Indeed, subclinical cognitive or neurophysiological alteration could be a rather aspecific finding with a multifactorial aetiology and pathophysiology, so that the concept of subclinical hepatic encephalopathy may need further insight.

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