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Flumazenil for hepatic coma in patients with liver cirrhosis: an Italian multicentre double-blind, placebo-controlled, crossover study

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Several factors suggest that endogenous benzodiazepines and gamma-amino-butyric acid may be involved in pathophysiology of hepatic encephalopathy (HE). Contrasting opinions exist on the therapeutic efficacy of flumazenil in the treatment of HE. This study was planned to assess the efficacy of flumazenil by a double-blind, placebo-controlled, crossover design in a large and selected population of cirrhotic patients in stage 4a HE admitted to intensive care units over a 4-year period. Out of 236 patients selected for the study, 132 received flumazenil, whereas 131 patients received placebo. Improvement of the neurological score was documented in 31 patients (23%) of flumazenil group and in two patients (1.5%) of placebo group ($p < 0.001$) during the first study period, whereas during the crossover period, improvement of the neurological score was documented in seven patients (5.3%) of the flumazenil group and in none of the placebo group ($p = 0.022$). Improvements in EEG tracings were observed in 44 patients (33.3%) of flumazenil group and in five patients (3.8%) of placebo group ($p < 0.001$) during the first study period; during the crossover period, improvements in EEG tracings were observed in 10 patients (7.5%) of the flumazenil group and in two patients (1.5%) of the placebo group ($p = 0.040$). The presence of benzodiazepines was detected in the serum of three responders and in two non-responders. The presence of diazepam and NN-desmethyl diazepam was documented in two responders; prior intake of synthetic diazepam was later confirmed in these patients. The results of our study suggest that flumazenil is beneficial only in a selected subset of cirrhotic patients with severe HE; the applicability of this treatment to unselected patients with hepatic coma or to cirrhotic patients with less severe HE still remains to be determined.

Keywords: flumazenil; hepatic encephalopathy; liver cirrhosis

INTRODUCTION

Several factors suggest that endogenous benzodiazepines and gamma-amino-butyric acid (GABA) may be involved in pathophysiology of hepatic encephalopathy (HE).[1,2] Flumazenil, a benzodiazepine antagonist, has been used in the treatment of intoxication patients with coma[3] and has shown diagnostic utility in coma patients with suspected poisoning.[4] Contrasting opinions exist on the therapeutic efficacy of flumazenil in the treatment of HE.[5–9]

The aim of our study was to assess the efficacy of flumazenil in a large and selected population of cirrhotic patients in hepatic coma admitted to intensive care units over a 4-year period by a double-blind, placebo-controlled, crossover design.

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MATERIALS AND METHODS

Patient selection

Patients in hepatic coma with biopsy-proven liver cirrhosis were eligible for inclusion in the study. According to Grippon and Opolon[10] and Cadranel *et al.*[9] the study considered patients in stage 4a HE: 'patients in coma with coordinated response to painful stimuli'. The study has not considered the patients under 18 years of age; those who received synthetic benzodiazepines in the preceding 4 days; those with a recent heavy alcohol abuse (in the preceding month); those with levels of creatininaemia > two times the normal values; those with severe respiratory failure ($P_{O_2} < 60$ mmHg and $P_{CO_2} > 50$ mmHg); those with acidosis (pH < 7.30); those with pre-existing neurological diseases; those with heart failure (NYHA class III or IV) and those who received any drug for the specific treatment of HE (except lactulose).

Study design and randomization

This was a double-blind, placebo-controlled, crossover study in which seven Italian hospital and university centres took part over a 4-year period. In each centre the patients were randomized on the basis of a computer-generated sequential list of block-randomized assignments. After a 3-hour stabilization period, patients were randomly assigned to receive flumazenil (1 mg in 20 ml saline solution) or an identical volume of placebo solution (NaCl 0.9%) by intravenous infusion in 3–5 min. For each patient, two sets of identical ampoules (active drug or placebo) were prepared to be administered in a random order according to the randomization crossover design. All patients received lactulose (30 ml four times daily) by nasogastric tube, whereas other specific treatments for HE such as neomycin or branched-chain amino acids were not administered during the study periods.

Clinical assessment

A modification of the Glasgow Coma Scale was used according to Pappas and Jones[11] for evaluation of the clinical response to treatment. According to this scale, the best possible score was 27 and the worst was 8.[8] Electroencephalographic (EEG) grading was scored blindly by two independent observers according to a modification of Fisher's classification.[9,12]

Concentrations of benzodiazepines

Blood samples were screened for the presence of benzodiazepines in the serum of the patients selected for the study by thin-layer chromatography (detection level > 11 mg/l). Gas-chromatography mass spectrometry was used for identification of diazepam and NN-desmethyl diazepam in blood samples as described by Falkner *et al.*[13]

End-point and drug administration

The end-point of the study was improvement in clinical neurological functions, assessed by both neurological and EEG score. Treatment was begun within 15 min of randomization; neurological assessment was performed 10 min before and then every 10 min up to 3 hours after drug injection. Continuous EEG tracings were recorded for 15 min before and 10 min after drug administration. After the first study period, patients received the other study medication (active drug or placebo) if they were still in stage 4a HE. Neurological and EEG scores were then recorded as in the first study period for a further 3 hours.

Statistical analysis

In order to detect a difference of 10% in the rate of clinical response between groups with a test power of 90% ($\alpha = 0.05$; $\beta = 0.10$, two-tailed test), 130 patients were needed in each group. The patients' charts, provided with a computer-generated code of identification, were purged of information that might identify the branch of randomization and analysed in a blinded fashion by an independent investigator using a computerized database. Non-categorical data have been analysed using the chi-squared test with Yates's correction; continuous data have been expressed as mean ± standard deviation and analysed using the *t*-test for independent samples.[14] The relative risk (RR) with 95% confidence interval (95% CI) for the rate of mortality between groups was also calculated.

Informed consent

The research has been carried out in accordance with the Helsinki Declaration. The study protocol was explained to at least one relative of each patient selected for the study. The patient's relative and the physician were not aware of the nature of the treatment being administered. In all cases, written informed consent was obtained from a patient's relative.

RESULTS

Enrolment and characteristics of the patients

From January 1993 to February 1997 out of 1568 cirrhotic patients admitted to the study centres, 236 (15%) fulfilled the selection criteria and entered the trial; 132 patients were assigned to receive flumazenil, whereas 131 patients received placebo. The two groups of patients were similar

Table 1. Patient characteristics at randomization

Characteristics	Flumazenil ($n = 132$)	Placebo ($n = 131$)	p value[a]
Males	92	93	0.924
Females	40	38	0.924
Age (mean value)	53	55	0.418
Alcoholic cirrhosis	59	60	0.955
Posthepatitic cirrhosis	72	70	0.955
Cryptogenic cirrhosis	1	1	0.481
Child-Pugh grade B	20	19	0.979
Child-Pugh grade C	112	112	0.979
Previous portacaval shunting	14	13	0.983
Initial neurological score (mean value)	17	18	0.755
EEG grade III	120	118	0.984
EEG grade IVa	6	7	0.989
EEG grade IVb	6	6	0.778
Precipitating factors			
Haemorrhage	85	86	0.933
Sepsis	12	11	0.985
Dehydration	2	2	0.620
Surgery	26	25	0.976
None	7	7	0.795

[a] Chi-squared test with Yates's correction and t-test for independent samples.

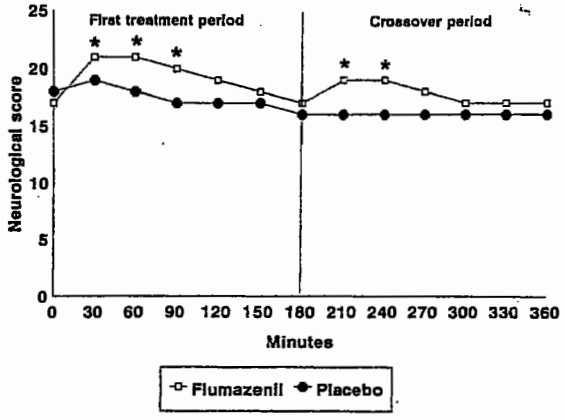

Fig. 1. Mean value of neurological score documented in the two study groups during the first treatment period and during the crossover period. *$p < 0.001$ vs. placebo.

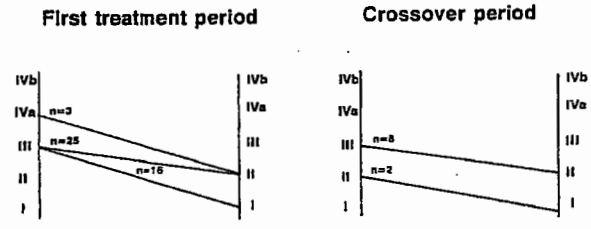

Fig. 2. Effect of flumazenil on EEG grading in responders during the first treatment period and during the crossover period.

with regard to age, gender, pathogenesis of cirrhosis and severity of liver disease. Patients characteristics at baseline and events precipitating HE in the two groups of patients are reported in Table 1. Patients with sepsis received antibiotics (ceftriaxone in nine patients of flumazenil group and eight patients of placebo group, ciprofloxacin in three patients of flumazenil group and in three patients of placebo group).

Neurological response

The neurological score (mean value) documented in the patients of both groups in the first treatment period and in the crossover period is shown in Fig. 1. Improvement of the neurological score was documented in 31 patients (23%) of flumazenil group and in two patients (1.5%) of placebo group ($p < 0.001$) during the first study period, whereas during the crossover period, improvement of the neurological score was documented in seven patients (5.3%) of flumazenil group and in none of placebo group ($p = 0.022$). In responders, the neurological score improved within 10 min (range 6–15 min) in both study periods. Improvements in EEG tracings were observed in 44 patients (33.3%) of flumazenil group and in five patients (3.8%) of placebo group ($p < 0.001$) during the first study period; during the crossover period, improvements in EEG tracings were observed in 10 patients (7.5%) of flumazenil group and in two patients (1.5%) of placebo group ($p = 0.040$). The effect of flumazenil on EEG grading in patients responding to drug in both study periods is shown in Fig. 2. In responders EEG improved within 9 min (range 5–12 min) in both study periods.

Benzodiazepines detection

The presence of benzodiazepines was detected in the serum of three responders and in two non-responders. The presence of diazepam and NN-desmethyl diazepam was documented in two responders; prior intake of synthetic diazepam was later confirmed in these patients.

Side effects and clinical outcome

No side effects occurred during either flumazenil or placebo infusion. Child-Pugh grade, initial neurological and EEG scores, prothrombin time, serum creatinine level, pH, P_{O_2} and P_{CO_2} were similar in responders and in non-responders; furthermore, these factors did not influence significantly the time of response to drug. In the 72 h following flumazenil infusion 23 of 31 responders (74.2%) in the first study period remained either at stable level of neurological and EEG scores or improved. In the other eight patients HE relapsed 48 h after flumazenil infusion with subsequent spontaneous recovery within 24 h; the seven responders in the crossover period remained at stable level of neurological and EEG scores in the following 72 h. In the other patients who did not respond to flumazenil in both study periods, HE improved spontaneously in most cases within 48–72 h after randomization. Among them, HE relapsed in 56 patients within 4 days (range 3–5 days) after flumazenil infusion with subsequent spontaneous recovery within 24–48 h. Twenty-two patients (nine in the flumazenil group and 13 in the placebo group) showed no improvement in the neurological and EEG scores and, subsequently, died within 3 days (range 2–4 days) after randomization for septic shock (18 patients), hypovolaemic shock (two patients) and lactic acidosis (two patients). Total mortality rate was 8.3% (6.8% in flumazenil group and 9.9% in placebo group; RR: 0.69; 95% CI: 0.30–1.55; $p = 0.492$).

DISCUSSION

The rationale for use of specific antagonists of central brain benzodiazepine receptors in the treatment of HE is based on the so-called benzodiazepine hypothesis of the pathogenesis of HE.[15] Central brain benzodiazepine receptors are coupled to a chloride ionophore on postsynaptic membranes and modulate the opening of chloride channels induced by the activation of postsynaptic GABA receptors located on the adjacent sites.[8,15] Initially, it was suggested that central brain benzodiazepine receptors were increased in animal models of HE; subsequently, it was demonstrated that these receptors were unchanged in animals and human subjects with HE.[8] Therefore, it is likely that in HE brain benzodiazepine antagonists improve neurological status not by way of action on altered benzodiazepine receptors *per se*, but by displacement of ligands from these receptors (synthetic pharmaceutical benzodiazepines or endogenous benzodiazepines).[8,16]

Single reports and five open trials[5–7,17–24] have reported the treatment of 29 cirrhotic patients during 32 episodes of HE by intravenous infusion of flumazenil. A transient or sustained improvement in HE was achieved in 22 cases (68.7%). Klotz and Walker[7] observed no improvement in hepatic encephalopathy in a double-blind crossover study of two cirrhotic patients. Similarly, in an open controlled study of 25 patients, Marsepoil *et al.*[24] did not find that flumazenil had any positive effect. In contrast, Pomier-Layrargues *et al.*[8] in a randomized double-blind, placebo-controlled, crossover study of 21 cirrhotic patients found a positive effects in six of 13 patients (46.2%), whereas no improvement in neurological symptoms occurred during placebo administration. Cadranel *et al.*[9] in a double-blind, placebo-controlled study found that flumazenil administration had a significant, although moderate, effect both on the EEG and on clinical grading in 12 of 18 patients (66%) compared with none of placebo group.

In our study improvement of the neurological score was documented in 23% of flumazenil group and in 1.5% of placebo group during the first study period, whereas during the crossover period improvement of the neurological score was documented in 5.3% of the flumazenil group and in none of the placebo group. Improvements in EEG tracings were observed in 33.3% of the flumazenil group and in 3.8% of the placebo group during the first study period; during the crossover period, improvements in EEG tracings were observed in 7.5% of the flumazenil group and in 1.5% of the placebo group.

The neurological score improved by 23.5% within 30 min after administration of flumazenil up to 90 min (+14.3% compared with placebo; $p < 0.001$), falling to baseline value after 160–180 min. In the placebo group the neurological score reduced by 5% at 60–180 min, especially in patients having haemorrhage and sepsis as events precipitating HE, without significant difference compared with baseline value ($p = 0.176$). The short half-life of flumazenil may, in part, justify the curve of the neurological score observed in our study population. From this point of view, the administration

of flumazenil for longer periods could be required to sustain normalization of the neurological score. Although the short half-life of flumazenil explains its safety in patients without liver failure, increased plasmatic half-life and the cerebral retention of ^{11}C-flumazenil have been documented in cirrhotic patients.[25,26] Therefore, careful monitoring of side effects is warranted when using large doses or the continuous infusion of flumazenil.[9]

Comparison of clinical and EEG scores showed that 70.3% of patients with an improvement of the neurological score showed also an improvement of EEG score, whereas 29.6% of patients with an improvement of EEG score did not show improvement of the neurological score. The higher sensitivity of EEG score may justify the difference observed between neurological and EEG score in detecting an early improvement of the neurological functions as response to drug. During the clinical follow-up, hepatic coma was found to be reversed in most patients within 1 to 3 days, suggesting that the absence of efficacy of the drug in non-responders was due to the fact that the drug was used at preterminal stages of hepatic coma in these patients.

It is difficult to affirm whether the rate of response to flumazenil represents a specific effect of the drug on HE or a non-specific excitation of the central nervous system, as it is possible to observe in poisoning or metabolic coma.[3,4] In our study the efficacy of flumazenil was not related to the presence or absence of benzodiazepines in the blood, since improvement of neurological and EEG score occurred, respectively, in 92.1% and 94.4% of responders with no detectable blood levels of benzodiazepines. These findings, which are in agreement with those reported by Pomier-Layrargues *et al.*,[8] suggest that mechanisms alternative to the action on blood-borne benzodiazepines must be invoked, such as the action of flumazenil on benzodiazepine receptor agonist ligands synthesized *in situ* in brain in liver failure or some other intrinsic action of flumazenil.[27] When used in the treatment of benzodiazepine overdose, the drug works within minutes;[4] late improvement in hepatic coma most likely represents spontaneous evolution rather than drug efficacy.[9] A better knowledge of the cellular effects of flumazenil is required to answer this important question.

The results of our study suggest that flumazenil is beneficial only in a selected subset of cirrhotic patients with severe HE; the administration of the drug should be done as early as possible (possibly within 3 h from the onset of the symptoms). In the patients who respond within 15 min on EEG grading, or who have positive benzodiazepines screening, flumazenil infusion could be performed for longer periods with a careful monitoring of side effects. The applicability of this treatment to unselected patients with hepatic coma or to cirrhotic patients with less severe HE still remains to be determined.

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