Flumazenil for Hepatic Encephalopathy Grade III and IVa in Patients With Cirrhosis: An Italian Multicenter Double-Blind, Placebo-Controlled, Cross-Over Study

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The rationale for use of benzodiazepine receptor antagonists is based on the so-called benzodiazepine pathogenetic hypothesis of hepatic encephalopathy (HE). To assess the efficacy of flumazenil, a specific benzodiazepine receptor antagonist, in a large and selected population of cirrhotic patients with severe HE, we conducted a double-blind, placebo-controlled, cross-over trial on 527 cirrhotic patients with HE grade III and IVa admitted to Intensive Care Units over a 5-year period; among them, 265 (132 of grade III and 133 of grade IVa) received flumazenil, whereas 262 (130 of grade III and 132 of grade IVa) received placebo. Treatment was begun within 15 minutes of randomization; the response to treatment was assessed by neurological score and by continuous electroencephalographic (EEG) recordings. Improvement of the neurological score was documented in 17.5% of grade III patients treated with flumazenil and in 14.7% of grade IVa patients, compared, respectively, with 3.8% and 2.7% of the patients of both groups treated with placebo. Improvements in EEG tracings were observed in 27.8% of grade III patients and in 21.5% of grade IVa patients, compared, respectively, with 5% and 3.3% of the patients of both groups treated with placebo. Benzodiazepines were detected in the serum of 10 patients (4 in grade III group and 6 in grade IVa group). Flumazenil is beneficial only in a selected subset of cirrhotic patients with severe HE; the applicability of this treatment to unselected patients with severe HE still remains to be determined. (HEPATOLOGY 1998;28:374-378.)

Several factors suggest that endogenous benzodiazepines and γ-aminobutyric acid may be involved in the pathophysiology of hepatic encephalopathy (HE).[1,2] Flumazenil, a specific 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 and on the applicability of this treatment to unselected patients with a different HE grade.[5-10]

Our study was planned to assess the efficacy of flumazenil in cirrhotic patients with severe HE admitted to Intensive Care Units over a 5-year period. This is the first large-scale trial that provides quantitative data on the clinical response to flumazenil in a selected population of cirrhotic patients with severe HE by a double-blind, placebo-controlled, cross-over design.

PATIENTS AND METHODS

Patient Selection. Patients with biopsy-proven cirrhosis and with HE grade III or IVa were eligible for the study. According to Grippon and Opolon,[11] HE grade III patients were considered those "in stupor, sleeping most of the time, but rousable, with incoherent speech and marked confusion," whereas HE grade IVa patients were considered those "in coma with coordinated response to painful stimuli." The study did not consider the following: 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 creatininemia $>$ two times the normal values; those with severe respiratory failure ($P_{O_2} < 60$ mm Hg and $P_{CO_2} > 50$ mm Hg); those with acidosis (pH < 7.30); those with preexisting neurological diseases; those with heart failure (New York Heart Association class III or IV); or those who received any drug for the specific treatment of HE (except lactulose).

Study Design and Randomization. This was a double-blind, placebo-controlled, cross-over study in which eight Italian Hospital and University Centers took part over a 5-year period. The study protocol was approved by the Institutional Review Boards of each Center. The patients were randomized on the basis of a computer-generated sequential list of block-randomized assignments. After a 2-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 for 3 to 5 minutes. 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 cross-over design. All patients received lactulose (30 mL every 6 hours) by nasogastric tube, whereas other specific treatments for HE such as neomycin or branched-chain aminoacids were not administered during the study periods; thereafter, these treatments could be administered. Continuous electrocardiographic monitoring was performed during both study periods, whereas blood gas analysis was performed every 3 hours up to the end of the study periods.

Clinical Assessment and Data Analysis. A modification of the Glasgow coma scale was used according to Pappas and Jones[12] for evaluation of the clinical response to treatment. This scale included the

Abbreviations: HE, hepatic encephalopathy; EEG, electroencephalography.

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assessment of the following parameters: verbal ability, eye-opening, pupillary light reflex, corneal reflex, spontaneous eye movements, oculocephalic reflex, motor response, and pattern of respiration. According to this scale, the best possible score was 27 and the worst was 8.[8,12] The neurological score was assessed by two investigators of each Center and by one clinical monitor of the coordinating Center of the study (Department of Emergency Medicine, University "La Sapienza," Rome). Electroencephalographic (EEG) tracings were analyzed and scored blindly by two independent neurologists who had no knowledge of the clinical response to drug. EEG scoring was based on the following classification[9,13]: Grade I: irregular background activity (theta and alpha); Grade II: continuous theta activity with bursts of delta waves; Grade III: prevalent delta activity with polyphasic transients, sharp and slow wave complexes; Grade IVa: continuous delta activity, abundant sharp and slow wave complexes (EEG reactivity present); Grade IVb: slower activity (delta and some polyphasic transients with EEG reactivity absent); Grade IVc: discontinuous activity with silent periods; Grade V: no activity. The neurological and EEG scores were reported on the patients' charts. Each chart, provided with a computer-generated code of identification, was purged of information that might identify the branch of randomization and analyzed in a blinded fashion by an independent investigator using a computerized database.

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.[14]

End-points and Drug Administration. The primary end-point of the study was the improvement in the clinical neurological functions, assessed by both neurological and EEG score during the study periods. The mortality rate between groups with a different HE grade was the secondary end-point of the study. Treatment was begun within 15 minutes of randomization; neurological assessment was performed 10 minutes before and then every 10 minutes up to 3 hours after drug injection. Continuous EEG tracings were recordered for 15 minutes before and 10 minutes after drug admnistration. After the first study period, patients received the other study medication (active drug or placebo) if they were still in grade III or IVa; neurological and EEG scores were then recorded as in the first study period for a further 3 hours.

Statistical Analysis. To detect a difference of 10% in the rate of clinical response between groups with a test power of 90% ($\alpha = 0.01$; $\beta = 0.10$, two-tailed test), 130 patients were needed in each group. Categorical data were analyzed using the χ^2 test with Yates' correction; continuous data were expressed as means ± SD and analyzed using the *t* test for independent samples.[15] The relative risk with 95% CI for the rate of mortality betwen groups of patients with a different grade of HE was also calculated.

Informed Consent. The research was performed 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

Enrollment and Characteristics of the Patients

From January 1993 to December 1997, of 1,882 cirrhotic patients admitted to the study Centers, 527 (262 in HE grade III and 265 in HE grade IVa) fulfilled the selection criteria and entered the trial. Two hundred sixty-five (132 in grade III and 133 in grade IVa) were assigned to receive flumazenil, whereas 262 (130 in grade III and 132 in grade IVa) were assigned to receive placebo. The groups of patients with different HE grade were similar with regard to age, gender, pathogenesis of cirrhosis, and severity of the liver disease. Patients' characteristics at randomization and events precipitating HE in the groups of patients selected for the study are reported in Table 1.

Patients with sepsis received antibiotics: ceftazidime was administered in 7 patients of the flumazenil group (3 in grade III and 4 in grade IVa) and in 8 patients of the placebo group (3 in grade III and 5 grade Iva); ceftriaxone was administered in 8 patients of the flumazenil group (4 in grade III and 4 in grade IVa) and in 9 patients of the placebo group (4 in grade III and 5 in grade Iva); ciprofloxacin was administered in 7 patients of the flumazenil group (4 in grade III and 3 in grade IVa) and in 6 patients of the placebo group (3 in grade III and 3 in grade IVa).

Neurological Response

The neurological score documented in the two groups of patients with different HE grade during the first treatment period and during the cross-over period is shown in Fig. 1A and 1B.

Grade III Group. Improvement of the neurological score was documented in 35 patients of the flumazenil group and in 6 patients of the placebo group ($\chi^2 = 22.17$; $P < .001$) during the first study period, whereas during the cross-over period, improvement of the neurological score was documented in 11 patients of the flumazenil group and in 4 patients of the placebo group ($\chi^2 = 2.64$; $P = .104$). In responders, the neurological score improved within 5 minutes (range, 2-6 minutes) in both study periods. Improvements in EEG tracings were observed in 51 patients of the flumazenil group

TABLE 1. Patient Characteristics at Randomization

Characteristics and Precipitating Factors of HE	Grade III		Grade IVa	
	Flumazenil (n = 132)	Placebo (n = 130)	Flumazenil (n = 133)	Placebo (n = 132)
Males	88	90	93	94
Age (yr)	56 ± 11.5	48 ± 20	53 ± 12	55 ± 13.5
Alcoholic cirrhosis	55	53	52	51
Posthepatitic cirrhosis	75	77	80	80
$HBsAg^+$ and $HCV\text{-}Ab^-$	10	11	12	11
$HBsAg^-$ and $HCV\text{-}Ab^+$	60	63	64	66
$HBsAg^+$ and $HCV\text{-}Ab^+$	5	3	4	3
Cryptogenetic cirrhosis	2	1	1	1
Child-Pugh grade B	18	19	20	20
Child-Pugh grade C	114	112	113	112
Previous portacaval shunting	9	7	14	13
Neurological score	20 ± 3.1	20.2 ± 2.5	17 ± 3.3	18 ± 3
EEG grade III	129	130	121	119
EEG grade IVa	3	1	6	7
EEG grade IVb	0	0	6	6
pH	7.41 ± 0.04	7.40 ± 0.02	7.45 ± 0.03	7.43 ± 0.06
P_{O_2}	88 ± 12	90 ± 11.3	92.3 ± 12.4	93.1 ± 10.5
P_{CO_2}	25.3 ± 4.2	27.3 ± 3.1	23.2 ± 2.2	24.2 ± 2.7
Hemorrhage	91	88	86	87
Sepsis	10	12	12	11
Dehydration	1	1	2	2
Surgery	22	24	26	25

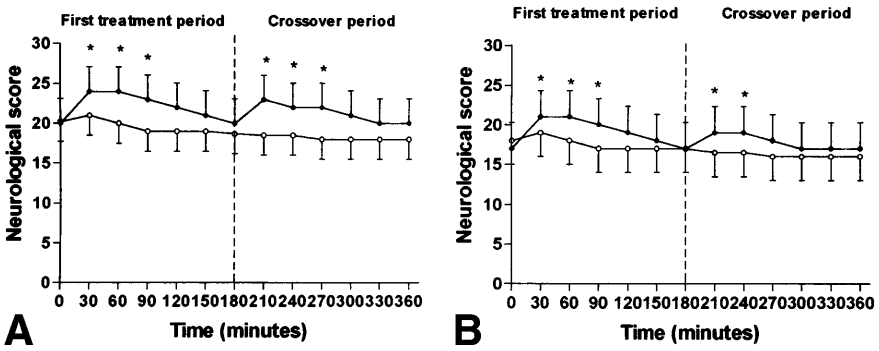

FIG. 1. Neurological score (mean ± SD) documented in the patients of the grade III group (A) and in those of the grade IVa group (B) during the first treatment period and during the cross-over period. (●), flumazenil; (○), placebo. *P < .001 vs. placebo.

and in 8 patients of the placebo group ($\chi^2 = 37.77$; $P < .001$) during the first study period; during the cross-over period, improvements in EEG tracings were observed in 22 patients of the flumazenil group and in 5 patients of the placebo group ($\chi^2 = 10.85$; $P < .001$) (Fig. 2). In responders, EEG improved within 3 minutes (range, 2-4 minutes) in both study periods.

Of 46 patients who showed improvement of the neurological score in both study periods, 26 had alcoholic cirrhosis, 18 had posthepatitic cirrhosis ($\chi^2 = 1.216$; $P = .270$ vs. alcoholic cirrhosis), and 2 had cryptogenetic cirrhosis ($\chi^2 = 19.959$; $P < .001$ vs. alcoholic cirrhosis; $\chi^2 = 11.696$; $P < .001$ vs. posthepatitic cirrhosis). Of 73 patients who showed improvement of EEG score, 41 had alcoholic cirrhosis, 30 had posthepatitic cirrhosis ($\chi^2 = 1.629$; $P = .202$ vs. alcoholic cirrhosis), and 1 had cryptogenetic cirrhosis ($\chi^2 = 36.583$; $P < .001$ vs. alcoholic cirrhosis; $\chi^2 = 24.263$; $P < .001$ vs. posthepatitic cirrhosis).

Grade IVa Group. Improvement of the neurological score was documented in 31 patients of the flumazenil group and in 3 patients of the placebo group ($\chi^2 = 24.36$; $P < .001$) during the first study period, whereas during the cross-over period, improvement of the neurological score was documented in 8 patients of the flumazenil group and in none of the placebo group ($\chi^2 = 6.37$; $P = .012$). In responders, the neurological score improved within 10 minutes (range, 6-15 minutes) in both study periods. Improvements in EEG tracings were observed in 45 patients of the flumazenil group and in 6 patients of the placebo group ($\chi^2 = 34.71$; $P < .001$) during the first study period; during the cross-over period, improvements in EEG tracings were observed in 12 patients of the flumazenil group and in 3 patients of the placebo group ($\chi^2 = 4.59$; $P = .032$) (Fig. 2). In responders, EEG improved within 9 minutes (range, 5-12 minutes) in both study periods.

Of 39 patients who showed improvement of the neurological score in both study periods, 21 had alcoholic cirrhosis, 17 had posthepatitic cirrhosis ($\chi^2 = 0.255$; $P = .613$ vs. alcoholic cirrhosis), and 1 had cryptogenetic cirrhosis ($\chi^2 = 17.120$; $P < .001$ vs. alcoholic cirrhosis; $\chi^2 = 12.939$; $P < .001$ vs. posthepatitic cirrhosis). Of 57 patients who showed improvement of EEG score, 31 had alcoholic cirrhosis, 25 had posthepatitic cirrhosis ($\chi^2 = 0.499$; $P = .480$ vs. alcoholic cirrhosis), and 1 had cryptogenetic cirrhosis ($\chi^2 = 27.970$; $P < .001$ vs. alcoholic cirrhosis; $\chi^2 = 21.396$; $P < .001$ vs. posthepatitic cirrhosis).

Benzodiazepine Detection

In the grade III group, the presence of benzodiazepines was detected in the serum of 3 responders and in 1 nonresponder. In the grade IVa group, benzodiazepines were detected in the serum of 4 responders and in 2 nonresponders. The presence

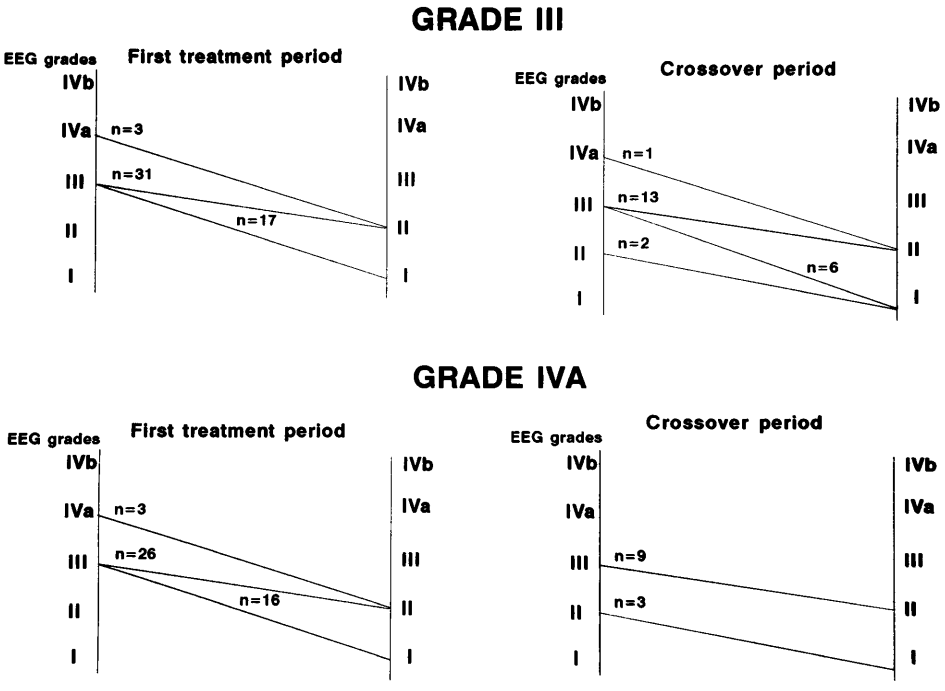

FIG. 2. Effect of flumazenil on EEG grading in responders documented during the first treatment period and during the cross-over period in the patients of the grade III group and in those of the grade IVa group.

of diazepam and NN-desmethyl diazepam was documented in 1 responder of the grade III group and in 2 responders of the grade IVa group; prior intake of synthetic diazepam, not reported at the time of enrollment, was later confirmed in these patients.

Side-Effects and Clinical Outcome

No side-effects occurred during either flumazenil or placebo infusion in both groups of patients. Child-Pugh grade, baseline neurological and EEG score, pH, P_{O_2}, and P_{CO_2} were similar in responders and in nonresponders during the study periods in both groups of patients; a previous portacaval shunting did not significantly influence either the time or the rate of response to drug.

Grade III Group. In the 72 hours following flumazenil infusion, each of 35 responders in the first study period remained either at a stable level of neurological and EEG scores or they improved; in addition, the 11 responders in the cross-over period remained at a stable level of neurological and EEG score in the following 72 hours. In the other patients who did not respond to flumazenil in both study periods, HE improved spontaneously in most cases within 24 to 48 hours after randomization. Among them, HE relapsed in 32 patients within 6 days (range, 5-8 days) after flumazenil infusion with subsequent spontaneous recovery within 24 hours. Seven nonresponders (3 in the flumazenil group and 4 in the placebo group) showed a worsening of the neurological and EEG scores and subsequently died within 4 days (range, 2-6 days) after randomization for hypovolemic shock (5 patients) or for septic shock (2 patients). Total mortality rate in the grade III group was 2.7% (2.3% in the flumazenil group and 3.1% in the placebo group; relative risk: 0.74; 95% CI: 0.17 to 3.24; $P = .983$).

Grade IVa Group. In the 72 hours following flumazenil infusion, 25 of 31 responders (80.6%) in the first study period remained either at a stable level of neurological and EEG scores or they improved. In the other 6 patients, HE relapsed 48 hours after flumazenil infusion with subsequent spontaneous recovery within 24 to 36 hours; the 8 responders in the cross-over period remained at a stable level of neurological and EEG scores in the following 72 hours. In the other patients who did not respond to flumazenil in both study periods, HE improved spontaneously in most cases within 48 to 72 hours after randomization. Among them, HE relapsed in 57 patients within 4 days (range, 3-5 days) after flumazenil infusion with subsequent spontaneous recovery within 24 to 48 hours. Twenty-three nonresponders (10 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), hypovolemic shock (3 patients), or for lactic acidosis (2 patients). Total mortality rate in the grade IVa group was 8.65% (7.5% in the flumazenil group and 9.8% in the placebo group; relative risk: 0.76; 95% CI: 0.35 to 1.68; $P = .648$).

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.[16] 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 γ-aminobutyric acid receptors located on the adjacent sites.[8,16] 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).[17]

Single reports and five open trials 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.[5-7,18-25]

In our large-scale trial, improvement of the neurological score was documented in 17.5% of grade III patients treated with flumazenil (26.5% in the first treatment period and 8.4% in the cross-over period) and in 14.7% of grade IVa patients (23.3% in the first treatment period and 6% in the cross-over period), compared with 3.8% of grade III patients (4.6% in the first treatment period and 3% in the cross-over period) and with 2.7% of grade IVa patients treated with placebo. Improvements in EEG tracings were observed in 27.8% of grade III patients (38.6% in the first treatment period and 16.9% in the cross-over period) and in 21.5% of grade IVa patients (33.8% in the first treatment period and 9% in the cross-over period), compared, respectively, with 5% of grade III patients (6.1% in the first treatment period and 3.8% in the cross-over period) and with 3.3% of grade IVa patients (4.5% in the first treatment period and 2% in the cross-over period) treated with placebo.

As regards the etiology of cirrhosis, the rate of response was 11.3% in patients with alcoholic cirrhosis (12.6% in grade III patients and 10% in grade IVa patients) and 9.15% in patients with nonalcoholic cirrhosis (10% in grade III patients and 8.3% in grade IVa patients). A previous portacaval shunting in both groups of cirrhotic patients did not significantly influence the rate of response to drug. Furthermore, the treatment with flumazenil did not influence the mortality rate in both groups of patients compared with placebo.

The neurological score improved by 21.7% (20% in grade III patients and 23.5% in grade IVa patients) within 30 minutes after administration of flumazenil up to 90 minutes (+12.5% and +23.5%, respectively, compared with placebo), falling to baseline value after 160 to 180 minutes in both groups. In the placebo group, the neurological score reduced by 6.4% at 60 to 180 minutes (7.4% in grade III patients and 5.5% in grade IVa patients), especially in patients having hemorrhage and sepsis as events precipitating HE, without significant difference compared with baseline value. 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.[26,27] 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 65.7% of patients with an improvement of the neurological score

(68.4% of grade III patients and 63% of grade IVa patients) also showed an improvement of EEG score, whereas 34.3% of patients with an improvement of EEG score (36.9% of grade III patients and 31.5% of grade IVa patients) did not show improvement of the neurological score. The higher sensitivity of EEG score may justify the difference observed between neurological and EEG scores in detecting an early improvement of the neurological functions as a response to drug.[10] During the clinical follow-up, the neurological functions improved in most patients of both groups within 1 to 3 days, suggesting that the absence of efficacy of the drug in nonresponders (especially in those of grade IVa) was caused by the fact that the drug was used at preterminal stages of HE 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 nonspecific 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, because improvement of neurological and EEG scores occurred, respectively, in 91.5% (93.4% in the grade III group and 89.7% in the grade IVa group) and in 94.3% (95.8% in the grade III group and 92.9% in the grade IVa group) of responders with no detectable blood levels of benzodiazepines. These findings, which are in agreement with those reported by Pomier-Layrargues et al.[8] and by us in a previous preliminary trial,[10] 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.[8,10,28] When used in the treatment of benzodiazepine overdose, the drug works within minutes[3,4,17]; late improvement in hepatic coma most likely represents spontaneous evolution rather than drug efficacy.[8-10] The time of administration of the drug may justify the difference observed in the rate of clinical response between patients receiving flumazenil directly after randomization and those receiving it during the cross-over period. A possible inability by flumazenil for displacement of agonist ligands from benzodiazepine receptors over time may be suggested (e.g., for changes in the molecular structure of ligands). However, this hypothesis is speculative; a better knowledge of the cellular effects of flumazenil is required to answer this 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 performed as early as possible (possibly within 3 hours from the onset of the symptoms), preferably in patients with grade III and in those with a history of benzodiazepine intake. In the patients who respond within 15 minutes on EEG grading, or who have positive benzodiazepine 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 severe HE still remains to be determined.

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