0270-9139/86/0603-0400$02.00/0
HEPATOLOGY
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Vol. 6, No. 3, pp. 400–405, 1986
Printed in U.S.A.

Effects of a New Loop Diuretic (Muzolimine) in Cirrhosis with Ascites: Comparison with Furosemide

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Muzolimine is a loop diuretic with both the dose-dependent increasing effectiveness of loop diurectics and the long-lasting effect of thiazides. This is a potential advantage in the treatment of ascites in advanced cirrhosis since these patients have a low tolerance to sudden reductions of blood volume.

Equivalent single, oral doses of furosemide (40 mg) and muzolimine (30 mg) were given to 10 cirrhotic patients with ascites and reduced renal perfusion (glomerular filtration rate = 30 to 75 ml per min). The study was preceded by 4 days of equilibration (dietary sodium 40 mmoles per day), and the drugs were alternated via a single-blind, cross-over protocol after a wash-out period of 3 days. Renal function was monitored under basal conditions and after diuretic administration through 4-hr clearance periods for 24 hr. The renin-aldosterone axis was evaluated before diuretic administration and after 8 and 24 hr. Muzolimine led to a 12-hr cumulative diuresis [AUC_{0-12} = 2.52 ± 0.42 (S.E.) ml per min] and natriuresis (5.14 ± 1.05 mmoles per hr), which were comparable to those of furosemide (2.85 ± 0.29 ml per min and 6.75 ± 1.63 mmoles per hr). Its effect, however, was distributed over a longer period (8 hr) than furosemide (4 hr). Muzolimine activity mainly differed from furosemide because of: significantly lower 12-hr potassium excretion (AUC_{0-12} = 0.28 ± 0.82 vs. 2.69 ± 0.46 mmoles per hr; $p < 0.005$); greater sodium/chloride excretion ratio (0.45 ± 0.08 vs. 0.26 ± 0.06; $p < 0.025$), and absence of rebound phenomena. These were clearly evident with furosemide, involving both the glomerular filtration rate and renal ion excretions from the third clearance period. Finally, plasma renin activity significantly increased 24 hr after furosemide (from 1.66 ± 0.40 to 2.35 ± 0.60 ng per ml per hr; $p < 0.05$), whereas it was not affected by muzolimine administration. These findings suggest that muzolimine use in the treatment of ascites in cirrhosis may be followed less easily by complications, such as diuretic-induced uremia and metabolic alkalosis, as compared to other loop diuretics.

Antimineralcorticoids are the drugs of choice for the medical treatment of cirrhotic ascites (1, 2). Their efficacy, however, can be impaired when renal perfusion is reduced (3). Loop diuretics are thus employed in addition to antimineralcorticoid drugs. The administration of these diuretic agents to cirrhotic patients can easily be followed by short- and long-term side effects, including hypovolemia, renal failure, hepatic encephalopathy, potassium and magnesium depletion and hypochloremic alkalosis (1, 4).

Muzolimine is a new nonsulfonamide diuretic drug of the pyrazolinone group (5). Animal and human experiments have demonstrated that it has the dose-dependent increasing capacity of loop diuretics as well as the long-lasting effect of thiazides (6–8). It also appears to induce less renal potassium excretion than furosemide in normal man (7, 9).

In this study, equivalent doses of furosemide and muzolimine were given to patients with cirrhosis and ascites, with moderately reduced renal perfusion. Our aim was to evaluate the effectiveness of muzolimine in these patients and to verify possible differences in the side effects produced by the two drugs.

PATIENTS AND METHODS

Ten male patients with cirrhosis and ascites in positive sodium balance were studied. The diagnosis was established by laparoscopy and liver biopsy. Six of the patients had alcoholic cirrhosis, 2 had postnecrotic cirrhosis and 2 had cryptogenic cirrhosis. All had normal mental state or minimal encephalopathy, total serum bilirubin levels <3.5 mg per dl, serum albumin levels between 2.6 and 3.7 gm per dl and prothrombin activity of 50 to 90% of normal. Ascites, which had first appeared 4 to 15 months earlier, was documented by clinical means and ultrasonography. Their glomerular filtration rate ranged from 30 to 75 ml per min. No patient had heart failure, intrinsic renal disease, hypertension, diabetes or gastrointestinal bleeding and shock within the 4 months preceding the study. In no case was ascites refractory to the combined regimen of low sodium diet and spironolactone (200 to 500 mg per day) plus muzolimine (30 to 60 mg per day) performed after the completion of the study.

The nature of the study was explained to the patients and informed consent was obtained in every case. The study was designed and performed according to the principles of the Declaration of Helsinki.

Protocol of the Study. The study was preceded by an equilibration period lasting 4 days, during which diuretic administration was stopped and a diet of 2,000 calories, 40 mmoles of sodium and 80 mmoles of potassium was given. Previous diuretic treatments consisted of spironolactone alone (up to 200 mg per day) in four cases or spironolactone plus furosemide

Received April 15, 1985; accepted September 15, 1985.
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(up to 80 mg) in the remaining cases. Steroids, amines, antihypertensive drugs and prostaglandin synthesis inhibitors were also avoided.

The patients were studied on the fifth day and on the ninth day using a single-blind, cross-over protocol after overnight fasting. Equivalent single oral doses (8) of furosemide (40 mg) and muzolimine (30 mg) were randomly administered on the first experimental day and were then alternated. During the 4 hr before the diuretic administration and for the following 12 hr (divided into 4-hr clearance periods), urine was collected to test urine volume, electrolyte excretion, urine creatinine and osmolality. The measurements were repeated after 24 hr. Blood was collected from an antecubital vein to test serum electrolyte concentration and osmolality under basal conditions, at the midpoints of the urine collections and after 24 hr. Blood samples for plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were collected in iced tubes under basal conditions and 8 and 24 hr after diuretic administration. These collections were performed after at least 2 hr of bed rest.

Plasma ammonia, phenylalanine and tyrosine concentrations were evaluated under basal conditions 6 and 24 hr after diuretic administration.

Determination. PRA and PAC were determined as described elsewhere (10). Normal values from our laboratory: PRA = 0.68 to 1.99 ng per ml per hr; PAC = 32 to 150 pg per ml. The glomerular filtration rate (GFR) was determined as endogenous creatinine clearance with the usual formula $U_{creat}/P_{creat} \times V$. The plasma and urine concentrations of creatinine were measured by Jaffé's colorimetric method (Boehringer-Mannheim GmbH, Mannheim, Germany). Serum and urine concentrations of sodium, potassium and chloride were determined by direct potentiometry with ion-selective electrodes. Calcium, inorganic phosphorum and magnesium were assayed by colorimetric methods (calcium: *o*-cresophtalein, without deproteinization; phosphorum: NH_4 molybdate-vanadate; magnesium: calmagite). Plasma and urine osmolality were determined by freezing-point analysis. Osmolar and ion clearance were calculated as $U_x/P_x \cdot V$. Negative free-water clearance ($T^c_{H_2O}$) was obtained from $C_{osm} - V$. Fractional clearances were calculated as $(U_x/P_x \cdot V)/GFR \cdot 100$.

Plasma ammonia concentration was evaluated by an enzymatic UV method (Boehringer-Mannheim GmbH, Mannheim, Germany). Values were expressed as μmoles per liter (normal values = 14.7 to 55.3 μmoles per liter). Readings on the spectrophotometer were down to 334/340 nm (Enzymatic PH 200-Biotesting, Firenze, Italy). Plasma phenylalanine and tyrosine concentrations were evaluated by fluorometric methods using commerical kits from Sigma Ltd. (St. Louis, Mo.) Plasma concentrations were expressed as milligrams per deciliter (normal values: phenylalanine = 0.6 to 2.7 mg per dl; tyrosine = 1.3 to 3.5 mg per dl).

Statistical Analysis. Results were expressed as mean ± S.E. Cumulative 12-hr ion excretion and fractional clearances were determined by calculating the areas under the curves ($AUC_{0\text{-}12}$).

The significance of the differences between basal conditions and during diuretic effect was tested by Student's t test for paired data.

RESULTS

The results are reported in Tables 1 and 2 and in Figures 1 to 3.

No significant differences were found between basal values preceding either protocol apart from fractional sodium clearance (furosemide = 0.42 ± 0.1%; muzolimine = 0.22 ± 0.04%; $p < 0.025$). Furosemide increased urine volume and ion excretion during the first clearance period (0 to 4 hr). The effect of muzolimine was smoother; peak values were lower than with furosemide, but the diuretic activity was still evident during the second clearance period (up to 8 hr) (Figure 1). $AUC_{0\text{-}12}$ of sodium, chloride, magnesium and calcium excretions induced by the two drugs did not differ significantly. However, the $AUC_{0\text{-}12}$ $U_{Na}V/AUC_{0\text{-}12}$ $U_{Cl}V$ ratio was higher with muzolimine (0.45 ± 0.08 vs. 0.26 ± 0.06; $p < 0.025$) (Figure 2).

As expected, the glomerular filtration rate significantly increased after furosemide, while muzolimine had no effect on this parameter. Rebound phenomena were only seen with furosemide. They affected the glomerular filtration rate (from the third clearance period: 8 to 12 hr), urine volume (at the fourth clearance period: 20 to 24 hr) and sodium, chloride and magnesium excretions (Figures 1 and 3).

Urine potassium excretion was close to zero with muzolimine and significantly lower than with furosemide (Table 2). In order to avoid possible influences of hemodynamic factors on urine ion excretion, $AUC_{0\text{-}12}$ of

TABLE 2. PRA and aldosterone concentrations under basal conditions and after diuretics

	Drug[a]	Basal values	8 hr	24 hr
PRA	F	1.7 ± 0.4	1.9 ± 0.5	2.4 ± 0.6[b]
(ng/ml/hr)	M	1.9 ± 0.4	1.7 ± 0.3	1.7 ± 0.5
PAC	F	246 ± 64	232 ± 56	256 ± 67
(pg/ml)	M	273 ± 67	255 ± 69	266 ± 67

[a] F = furosemide; M = muzolimine.
[b] $p < 0.05$; comparison vs. basal values.

TABLE 1. 12-hr areas under curves of the parameters studied

	F	M		F	M
UV (ml/min)	2.8 ± 0.3	2.5 ± 0.4	C_{osm} (ml/min)	6.0 ± 0.8	5.6 ± 1.0
$U_{Na}V$ (mmoles/hr)	6.8 ± 1.7	5.1 ± 1.0	$T^c_{H_2O}$ (ml/min)	2.8 ± 0.5	2.9 ± 0.5
U_KV (mmoles/hr)	2.7 ± 0.5	0.3 ± 0.8[a]	$C_{osm}/GFR \times 100$	11.8 ± 2.9	16.0 ± 4.4
$U_{Cl}V$ (mmoles/hr)	30.0 ± 6.8	16.0 ± 3.3	$T^c_{H_2O}/GFR \times 100$	6.1 ± 1.5	6.4 ± 2.0
$U_{Mg}V$ (mmoles/hr)	2.9 ± 0.4	2.8 ± 0.7	$C_{Na}/GFR \times 100$	1.4 ± 0.3	1.6 ± 0.4
$U_{Ca}V$ (μmoles/hr)	91.1 ± 26.2	68.4 ± 34	$C_K/GFR \times 100$	19.5 ± 7.1	11.0 ± 5.7
U_PV (mmoles/hr)	1.7 ± 0.7	1.0 ± 0.5[b]	$C_P/GFR \times 100$	27.0 ± 7.5	21.9 ± 6.8

The abbreviations used are: F = furosemide; M = muzolimine; UV = urine volume; U_xV = ion excretion; C_{osm} = osmolar clearance; $T^c_{H_2O}$ = negative free water clearance; $C_x/GFR \times 100$ = fractional clearance.
[a] $p < 0.01$.
[b] $p < 0.005$.

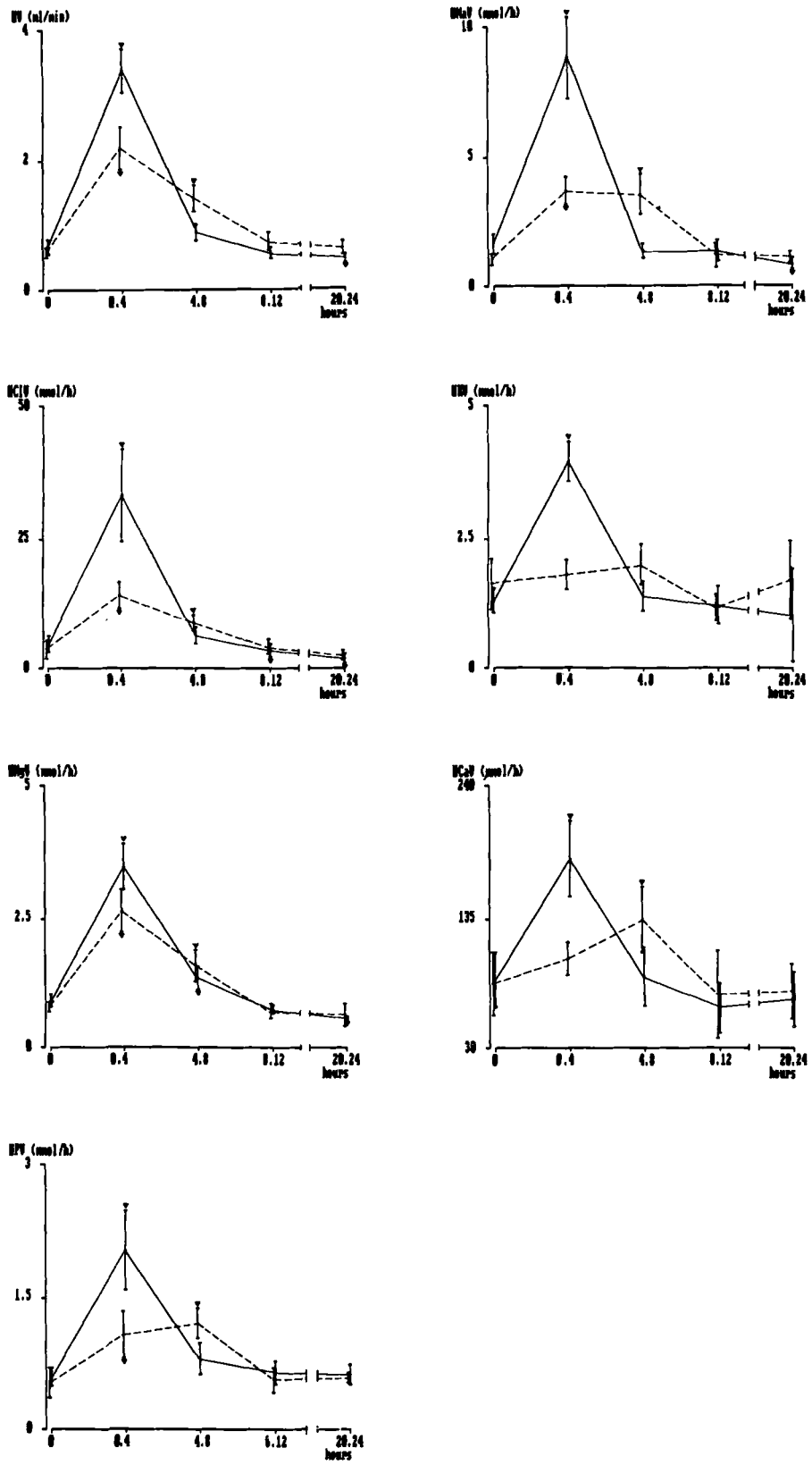

FIG. 1 Time course of diuresis (UV) and ion excretions (U_xV) after furosemide (——) and muzolimine (– – –) administration. ▼ = $p < 0.05$ or less vs. basal (0) values. The *horizontal axis* refers to the clearance periods (hours from diuretic administration). Mean ± S.E. are reported.

fractional C_{Na} and C_K were also compared. No significant differences between treatments were found. As for potassium, a higher phosphate excretion occurred after furosemide, but no difference in AUC_{0-12} fractional clearance was detected (Table 1).

Under basal conditions, a $T^c_{H_2O}$ was present. Both drugs led to higher relative increases in C_{osm} than in urine volume. As a result, an increase in $T^c_{H_2O}$ occurred (Figure 3). No significant differences in fractional $T^c_{H_2O}$ and C_{osm} between the two treatments were seen (Table 1).

Plasma potassium concentration significantly de-

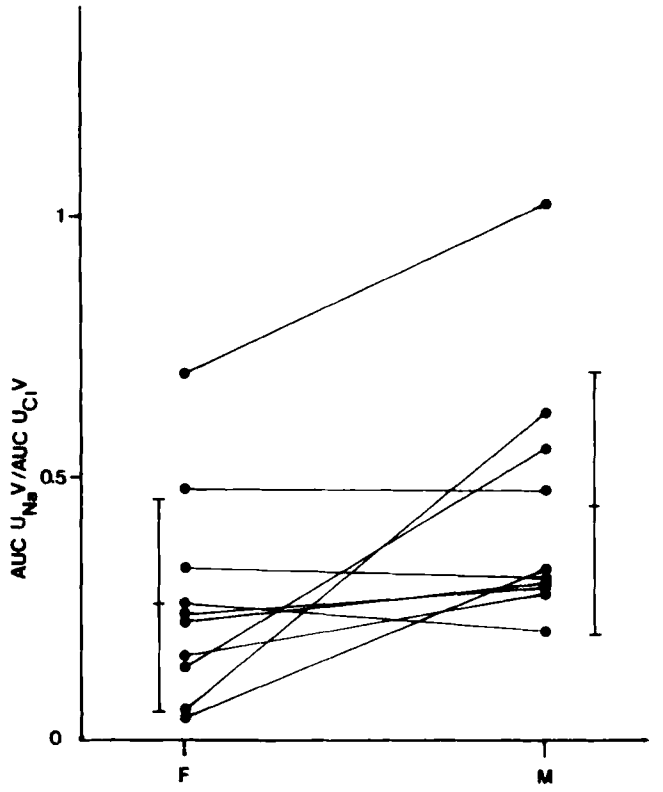

FIG. 2. The ratio between 12-hr cumulative excretions (AUC_{0-12}) of sodium and chloride in furosemide (F)- and muzolimine (M)-treated cirrhotics. Mean ± S.D. are reported.

creased 24 hr after furosemide administration. In contrast, it decreased slightly but significantly during the period of muzolimine activity, subsequently returning to basal values. No changes in plasma sodium, calcium and magnesium concentrations occurred during either protocol.

Muzolimine administration was not followed by changes in PRA or PAC, whereas PRA significantly increased 24 hr after furosemide. PAC also proved unchanged with the latter drug (Table 2).

With both diuretics, plasma ammonia concentration significantly increased after 6 hr (furosemide = 41 ± 7 to 69 ± 10 μmoles per liter, $p < 0.01$; muzolimine = 39 ± 8 to 70 ± 6 μmoles per liter, $p < 0.001$), returning afterwards to basal values (furosemide = 44 ± 5 μmoles per liter; muzolimine = 45 ± 7 μmoles per liter). Basal values for plasma phenylalanine and tyrosine were: furosemide = 1.6 ± 0.2 and 1.4 ± 0.2 mg per dl, respectively; muzolimine = 1.7 ± 0.4 and 1.4 ± 0.3 mg per dl, respectively. No significant changes occurred after either treatment.

DISCUSSION

The most interesting aspect of muzolimine pharmacodynamics in normal subjects is its smoother action compared with furosemide and other loop diuretics (6–8, 11–13). This was also confirmed by us in cirrhotic patients. Due to reduced effective plasma volume (14, 15) and cardiovascular responsiveness to vasoactive stimuli (14, 16), patients with advanced cirrhosis have a decreased tolerance to sudden reductions in blood volume. This is likely to occur with fast-acting diuretics such as furosemide. In fact, the excess fluid to be eliminated is mainly compartmentalized as ascites, the removal of which may be as low as 200 ml per day (17). This explains why rebound phenomena on the glomerular filtration rate and urine ion excretions along with renin-angiotensin system activation occurred in this study only after furosemide. A possible rebound with muzolimine during the 12-hr to 20-hr period cannot be excluded with certainty. Even in this case, it should have been small and transient so that no influence on PRA was seen up to 24 hr. Partial dissociation between PRA and PAC or urine aldosterone glucuronide excretion has been reported in cirrhosis after drug-induced changes of renin-angiotensin system activity (10, 18). This could account for the steadiness of PAC observed in this study after furosemide administration. If these properties of muzolimine are confirmed after long-term treatment, this could be regarded as a potential advantage over other loop diuretics. In fact, volume depletion (19) and renin-angiotensin system activation (10, 20) are the main factors leading to diuretic-induced uremia in cirrhosis.

Muzolimine has been reported to induce a lower potassium-wasting effect than other loop diuretics (7–9). This was confirmed in our patients although they had markedly increased PAC. The secondary enhancement of renin-aldosterone axis is the main mechanism leading to diuretic-induced potassium depletion (21), but this cannot be taken into account in our study. The smoother diuretic activity of muzolimine could have induced a lower potassium secretion by the distal convoluted tubule by maintaining a lower tubular flow rate. In fact, potassium secretion in this portion of the nephron is a direct function of luminal flow rate (22). Moreover, since plasma potassium concentration significantly decreased during muzolimine activity, the reduced filtered load of potassium may have helped to lower actual urine excretion of the ion. In fact, the inhibition of potassium reabsorption by loop diuretics may increase distal potassium delivery (23).

The transient influence of muzolimine on plasma potassium concentration not accountable by renal excretion has also been described in normal man (9). The meaning of this finding is unclear. Diuretic-induced alkalosis of such a degree to shift potassium to the intracellular compartment seems unlikely in our patients. Moreover unmetabolized muzolimine does not act on Na^+,K^+-ATPase or other transport ATPases in rat salivary duct epithelium (24) nor on Na^+,K^+ furosemide-sensitive co-transport in human red cells (25). However, possible interactions by active metabolites of the drugs with cellular active ion transport systems cannot be excluded at present.

That chloresis strikingly exceeded natriuresis after both drugs was due to marked hyperaldosteronism. The higher sodium/chloride excretion ratio seen after muzolimine suggests an effect on the tubular KCl co-transport system (26). This finding, along with the low potassium excretion, may reduce the likelihood of diuretic-induced metabolic alkalosis with its prolonged use.

Diuretic agents that act on the medullary segment of Henle's loop typically inhibit "free water" production. It

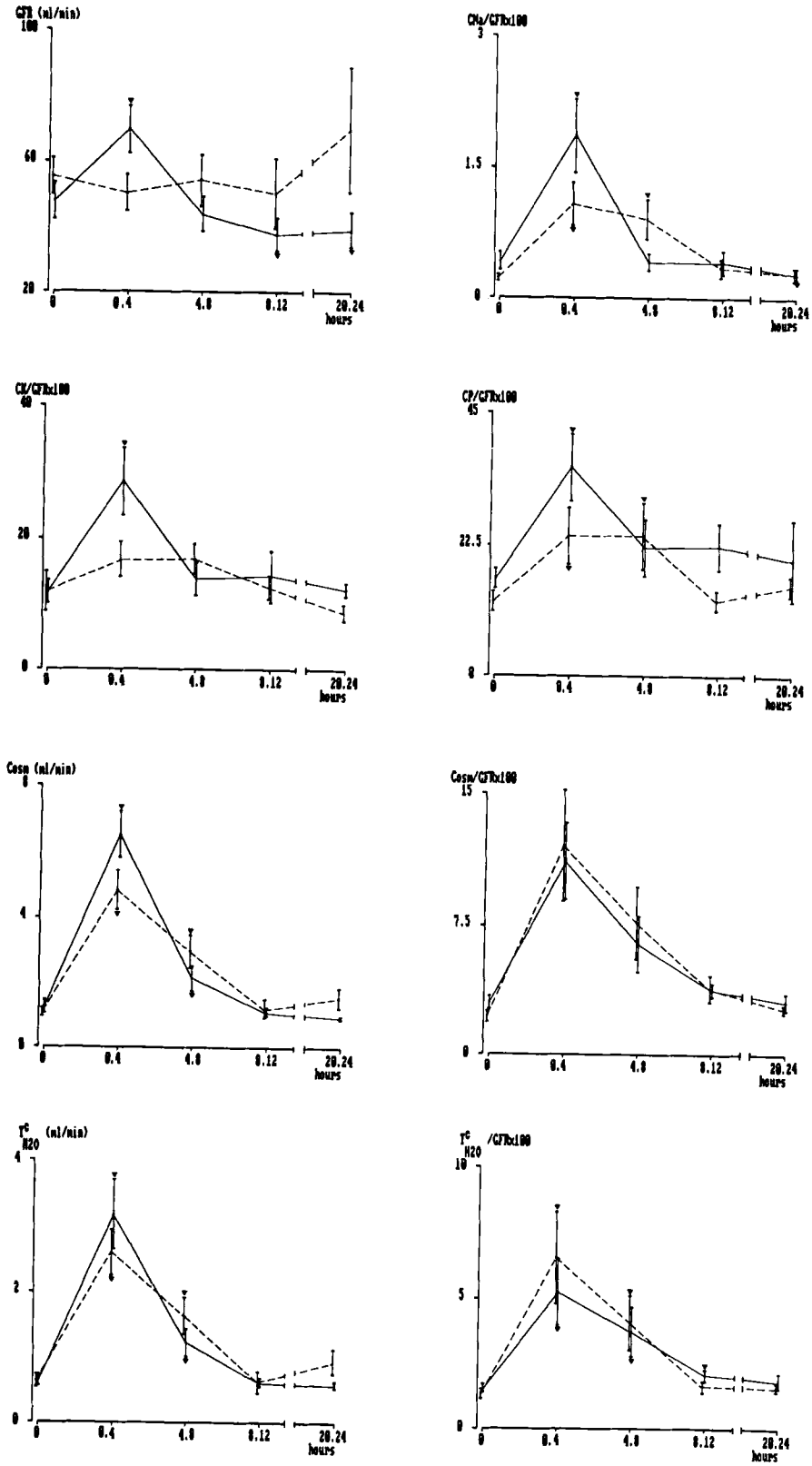

FIG. 3 The time course of glomerular filtration rate (GFR), osmolar (C_{osm}) and negative free water ($T^c_{H_2O}$) clearances and fractional clearances (C_x/GFR × 100). Symbols are the same as in Fig. 1.

should be noted, however, that hydropenic studies evaluating the effects of diuretics on $T^c_{H_2O}$ are performed under osmotic diuresis induced by mannitol infusion (27). It should also be taken into account that markedly increased plasma arginine-vasopressin levels have been reported in cirrhotics with advanced disease (28). These considerations may explain why the diuresis of our patients increased less than C_{osm} in relation to basal values. Dilutional hyponatremia is, therefore, facilitated by such drugs in cirrhosis (10).

The remaining results show that calcium and magnesium depletions can be equally expected with both furosemide and muzolimine.

Both drugs also induced similar effects on plasma

ammonia, phenylalanine and tyrosine concentrations, which may be involved in the development of hepatic encephalopathy (29).

The effects of both diuretics on proximal renal tubule were comparable. Although furosemide led to a higher phosphorum excretion than did muzolimine, no differences were found comparing phosphorum fractional clearance, which can reflect diuretic activity on this portion of the nephron (30).

In conclusion, our comparison of muzolimine and furosemide administration in advanced cirrhosis shows that the former drug has the following characteristics: absence of rebound phenomena, activation of the renin-angiotensin system and potassium wasting effect, along with a milder derangement in the sodium/chloride excretion ratio. These pharmacodynamic properties may be beneficial in the clinical use of this drug. However, these results were obtained through an acute pharmacodynamic protocol and cannot be extrapolated to chronic administration.

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