Atrial Natriuretic Factor in Ascites of Patients with Cirrhosis of the Liver or with Malignant Neoplasms

*Alexander L. Gerbes, **Angelika M. Vollmar, *Yining Xie, and †Rainer M. Arendt

*Departments of Medicine *II and **I, Klinikum Grosshadern, and †Institute of Pharmacology, Veterinary School, University of Munich, Munich, Federal Republic of Germany*

The presence of atrial natriuretic factor (ANF)-like immunoreactivity was demonstrated in ascitic fluid (AF) of 14 patients with cirrhosis of the liver (LC) and 10 patients with malignant neoplasms (MN). ANF concentrations (mean ± SEM, fmol/ml) in AF of LC/MN were 2.4 ± 0.5/3.3 ± 0.5, significantly ($p < 0.001$) lower than the corresponding concentrations in plasma (P) (15.5 ± 2.6/13.0 ± 2.9 fmol/ml). High performance gel permeation and reverse phase chromatography of ANF immunoreactivity in AF showed correspondence to ANF_{99-126}. ANF concentrations in AF were significantly ($p < 0.01$) correlated with concentrations in P in LC/MN ($r = 0.66/0.90$). Thus, elevations of ANF concentrations in P after infusion of AF would not seem to be due to high ANF content of AF.

Increases of diuresis and natriuresis have been observed after intravenous reinfusion of ascitic fluid in patients with ascites due to cirrhosis of the liver and in patients with malignant neoplasms (1,2). Recently, marked elevations of ANF plasma concentrations after reinfusion were reported and were considered to contribute to the ensuing diuresis (3–5). Although the pathophysiological role of ANF in volume regulation has not been fully elucidated in cirrhosis (6,7) and has not received much attention in malignant ascites, the mechanism of ANF increase after ascites infusion deserves attention. The reinfused ascitic fluid itself might contribute to the observed rises of ANF plasma concentrations rather than volume-stimulated ANF release alone.

ANF has been demonstrated in various fluids of the human body, such as plasma (8), urine (9), and cerebrospinal fluid (10). Ascitic fluid, however, has not been examined as yet for the occurrence of ANF. In this study, the presence of ANF in ascitic fluid was investigated.

SUBJECTS AND METHODS

Twenty-four patients with ascites were investigated. Fourteen had biopsy-proven liver cirrhosis (age 42 to 81, mean 61 ± 4 years) and 10 had primary or metastatic malignancy in the abdomen or pelvis (age 42 to 80, mean 64 ± 4 years).

Determination of ANF by Radioimmunoassay (RIA)

Simultaneously obtained samples of ascitic fluid and peripheral venous blood were drawn into precooled polystyrene tubes containing 500 KIU aprotinin and 1 mg sodium ethylenediaminetetraacetic acid (EDTA) per ml. Samples were centrifuged at 4°C, and the supernatant fluid was immediately frozen and stored at −80°C. Extraction of ascites aliquots as well as RIA procedures were performed in the same way as for plasma samples; procedures have been detailed elsewhere (8). Briefly, samples were extracted by adsorption to Amberlite XAD-2 adsorbent resin. The final titer of the C-terminal-directed antibody Toni III was 1:120,000, cross-reactivity to rat pro-ANF was 48% and the assay sensitivity was 0.5 fmol α-human ANF/tube. The 50% binding intercept of the standard curve was 10 fmol.

Chromatographic Analysis of ANF Immunoreactivity

Ascitic fluid extracts of six patients were subjected to high performance gel permeation chromatography (HPGPC) and reverse phase high performance chromatography (RP-HPC).

HPGPC

Lyophilized ascitic fluid (10 ml) was dissolved in 25-μl column eluens and applied to a Spherogel TM TSK, 2000 SW column (10 μm, 7.5 × 300 mm; Beckman Instruments), eluted with 0.09% trifluoroacetic acid (TFA) containing 0.005 M Na_2SO_4, 0.002 M NaH_2PO_4, and 30% acetonitrile (flow rate, 0.3 ml/min). Calibration was carried out with bovine serum albumin (BSA) (V_0), vitamin B_{12} (V_t), rat pro-ANF_{2-126}, and α-human ANF_{99-126}. Immunoreactive ANF (IR-ANF) fractions (0.6 ml), detected by RIA, were pooled and lyophilized.

RP-HPC

An aliquot of the pooled IR-ANF fractions of the HPGPC run was redissolved in 25 μl of 0.1% TFA and loaded on a HPLC C-18 ODS Ultrasphere TM column (5 μm, 2 × 150 mm; Beckman Instruments), according to ref. 11. Elution was carried out with a linear gradient of acetonitrile (10 to 80%, 45 min) in 0.1% TFA (flow rate, 0.2 ml/min). Calibration of the column was performed with synthetic atriopeptin I and III, human ANF_{99-126}, and rat pro-ANF_{2-126}. Fractions (0.4 ml) were assayed for ANF immunoreactivity.

Statistical Evaluation

Differences between ascitic and plasmatic ANF concentrations were compared by paired *t*-test; the Pearson correlation coefficient was determined by the usual linear least squares test. Data are presented as means ± SEM.

RESULTS

Serial dilutions of ascites were parallel to the standard curve. Recoveries of 7.8 and 15.6 fmol synthetic human α-ANF added to samples of ascites and plasma before extraction were 84% and 67% in ascites and 75% and 65% in plasma. Nonspecific binding, determined in the RIA without antiserum, was less than 5% in both ascites and plasma. In patients with cirrhosis, ascitic ANF concentrations were 2.4 ± 0.5 fmol/ml, significantly ($p < 0.001$) lower than ANF concentrations in plasma (15.5 ± 2.6 fmol/ml). In patients with malignancies, ANF levels in ascites were 3.3 ± 0.5 fmol/ml, significantly ($p < 0.001$) lower than levels in plasma (13.0 ± 2.9 fmol/ml). ANF concentrations in ascites were significantly correlated to ANF plasma concentrations in cirrhosis ($r = 0.66$, $p < 0.01$) as well as in malignancies ($r = 0.90$, $p < 0.01$) (Fig. 1).

When preextracted ascitic fluid was chromatographed on HPGPC and RP-HPC, immunoreactive ANF was detected as a single peak coeluting with synthetic human ANF_{99-126}. No significant amount of higher-molecular-weight ANF-like material such as precursor pro-ANF was detected.

DISCUSSION

This study demonstrates the presence of ANF in ascitic fluid of patients with cirrhosis of the liver and with malignant neoplasms. Extraction procedures as well as characteristics of our highly sensitive and specific radioimmunoassay for determination of ANF in plasma have been previously described (8). Application of the extraction procedure to aliquots of ascitic fluid yielded high recovery rates, not different from those obtained in plasma. Validity of the RIA for ANF measurement in ascites was demonstrated by parallelity of serial dilution curves with the standard curve and by the absence of significant binding interference.

In an attempt to characterize further ANF immunoreactivity in ascites, the

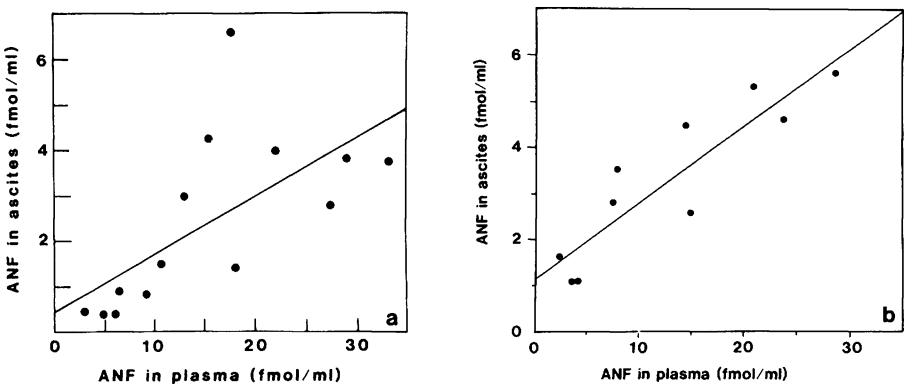

FIG. 1. Significant correlation of ANF concentrations in ascites and in plasma of 14 patients with cirrhosis of the liver ($r = 0.66$, $p < 0.01$) (**a**) and 10 patients with malignant neoplasms ($r = 0.90$, $p < 0.01$) (**b**).

molecular weight pattern was investigated by HPGPC and reverse phase HPLC. Although trace amounts of higher-molecular-weight ANF in plasma of patients with cirrhosis have been reported (8), ANF immunoreactivity in ascites coeluted with synthetic ANF_{99-126}, the main circulating form in human plasma. However, because of the low concentrations of ANF in ascites and a 48% cross-reactivity of our antibody to pro-ANF (8), the presence of small amounts of higher-molecular-weight ANF in ascites cannot be excluded.

ANF concentrations in ascites were significantly correlated with ANF plasma concentrations. This correlation as well as characterization of ascitic ANF as ANF_{99-126}, the major circulating form of ANF, may indicate plasmatic origin of ascitic ANF. ANF concentrations in ascites were significantly lower than in plasma. Thus, elevations of ANF levels in plasma after the infusion of ascitic fluid (3–5) seem not to be due to increased levels of ANF in ascitic fluid but rather due to increased release of ANF after the hemodynamic changes of infusion.

ACKNOWLEDGMENTS

Anita Friedrich, Veit Gülberg, and Fabian Höpker are thanked for their support. Parts of this study were supported by a grant from the funds of the Münchner Medizinische Wochenschrift (head: Prof. Dr. Eyer).

REFERENCES

1. LeVeen, H. H., Christoudias, G., Ip, M., et al. (1974): *Ann. Surg.*, 180:580–591.
2. Gough, I. R. (1984): *Cancer*, 54:2226–2230.
3. Burghardt, W., Wernze, H., and Diehl, K. L. (1986): *Klin. Wochenschr.*, 64(Suppl. VI):103–107.
4. Campbell, P., Blendis, L. M., Skorecki, K., et al. (1986): *Hepatology*, 6:1120.
5. Witte, C. L., Martinez, A. P., and Witte, M. H. (1987): *N. Engl. J. Med.*, 316:487.
6. Gerbes, A. L., Arendt, R. M., Ritter, D., et al. (1985): *N. Engl. J. Med.*, 313:1609–1610.
7. Gerbes, A. L., Arendt, R. M., and Paumgartner, G. (1987): *J. Hepatol.*, 5:123–132.
8. Arendt, R. M., Gerbes, A. L., Ritter, D., et al. (1986): *Klin. Wochenschr.*, 64(Suppl. 6):97–102.
9. Marumo, F., Sakamoto, H., Ando, D., et al. (1986): *Biochem. Biophys. Res. Commun.*, 137:231–236.
10. Marumo, F., Masuda, T., and Ando, K. (1987): *Biochem. Biophys. Res. Commun.*, 143:813–818.
11. Vollmar, A. M., Arendt, R. M., and Schulz, R. (1987): *Eur. J. Pharmacol.*, 143:315–321.