The Activation of Serum Hepatocyte Growth Factor in Acute Renal Failure

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Table 1. Patient data on serum HGF and ARF treatment

HD = Intermittent hemodialysis; CHD = continuous hemodialysis. a Treatment at time of blood sampling. CHD was changed to HD in patients 2, 4 and 6 afterwards. b The day is after the occurrence of the causal accidents.

Dear Sir,

Hepatocyte growth factor (HGF) has mi-togenic activity for various epithelial cells including renal epithelial cells [1], and accelerates the tissue regeneration in acute renal failure (ARF) [2, 3]. HGF is produced as a single-chain precursor by cells of mesenchymal origin, such as tissue fibroblasts and macrophages, and is converted to a biologically active α -, β -heterodimeric molecule by proteolytic processing [4]. Although serum HGF levels are elevated in ARF patients [5], to date, there have been no reports concerning the processing conditions of increased levels of serum HGF. In order to examine proteolytic activation of HGF in ARF, we analyzed serum HGF molecular size by immunoblot analysis.

Table 1 shows the profiles of 8 ARF patients studied. The cause of ARF was crush syndrome happened in the Kobe earthquake in all cases. The ARF patients were treated by either intermittent or continuous hemodialysis, and nafamostat mesilate was used as an anticoagulant in hemodialysis. Blood sampling was performed on the 4th or 5th day after the causal accidents. Two healthy subjects and 2 patients with chronic renal failure (CRF) due to chronic glomerulonephritis were studied as controls. The CRF patients received hemodialysis treatment 3 times a week in stable conditions. The serum HGF levels determined by an enzyme immunoassay kit (Otsuka, Japan) were 0.31 and 0.50 ng/ml in CRF patients and 0.20 and 0.19 ng/ml in the healthy subjects.

The serum HGF was partially purified by heparin affinity column (HiTrap Heparin™ 1 ml, Pharmacia) [6] from 5 ml of serum from each patient. SDS-polyacrylamide gel electrophoresis was performed by Laemmli's method in 10% gel under reducing condition. Resoluted proteins were transferred to a polyvinylidene difluoride membrane elec-

trophoretically, and HGF was detected by mouse antihuman HGF α -chain monoclonal antibody (Otsuka, Japan) followed by goat antimouse IgG gold conjugates and silver enhancement. As shown in figure 1, a major band was observed at approximately 60 kD in the lanes of the ARF patients, excluding patient 8. This band corresponded to that of the recombinant human HGF, indicating an α -chain of HGF. In contrast, a major band was apparent at approximately 90 kD in the lanes of CRF patients, normal subjects, and one of the ARF patients. Based on its molecular size, the band was suspected to be a single-chain HGF before processing, and it was confirmed by the finding that the larger band disappeared and the α -chain band was enhanced after a 24-hour incubation of the serum. Thus, the serum HGF was converted

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Fig. 1. Immunoblot analysis of serum HGF. Lane A is recombinant human HGF. The serum in lane B, which is from CRF patient 2, was preincubated for 24 h.

by the processing activity in 7 of the 8 ARF patients, whereas it was not in the CRF patients or in the healthy subjects. The one exception was patient 8, who expired due to multiple organ failure. In this study the elevation of serum HGF levels observed in ARF patients was consistent with previous reports [5]. However, there was no obvious relationship between patients' serum HGF levels and their prognoses or the degree of renal damage judging from the periods of hemodialysis therapy required. Although the serum HGF level is thought to reflect the production of HGF, it is evident that this HGF level does not necessarily show the biological activity because serum HGF concentrations are determined by enzyme immunoassay in which antibodies used detect both a biologically active heterodimeric form and an inactive single-chain form.

Based on the serum HGF level, it was unlikely that the production of HGF was abnormally low in patient 8. However, the majority of serum HGF remained as a single-chain form, which could be attributed to the low activity of the HGF activator. It is possible that disseminated intravascular coagulation syndrome (DIC) may explain this low activity because the HGF activator has been reported to be stimulated by thrombin [7]. In fact, patient 8 had the highest DIC score among the patients studied.

Thus, there is a case showing a low level of HGF processing activity in ARF patients. In such a case, it is unlikely that the actions of HGF are expressed sufficiently even when the serum HGF level is adequate. Therefore, the administration of the active form of HGF will be an appropriate and effective treatment for ARF patients, particularly those with low HGF processing activity.

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