プロテインチップアレイを用いた急性肝不全生体肝移植患者におけるバイオマーカーの検討

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Original (Short Communication)

Inquiries About Biomarkers of Acute Liver Failure in Patients Who Underwent Living Donor Liver Transplantation Using a Protein Chip Array

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Abstract

The causative agent of hepatic encephalopathy (HE) has not been identified with certainty. The recovery of consciousness in patients with acute liver failure (ALF) who underwent liver transplantation (LT) is sometimes drastic ; therefore, we thought that the causative agents of HE would change markedly peri-operatively in these patients. We examined the biomarkers including new agents in the serum of patients using the ProteinChip® System 4000 (Ciphergen Biosystems, Yokohama, JAPAN).

Sixteen samples were obtained from four patients with ALF who underwent living donor LT (LDLT) at four time points ; pre-operative, one post-operative day (1POD), 3POD, and 7POD. We used three chips made by the Biomek2000 robot. All duplicated samples were assayed and analyzed using the CiphergenExpress™ data manager. We divided the peri-operative changes in the intensity of identified peaks into seven patterns. The number of peaks whose intensity shows significant changes peri-operatively reached 755.

Of course, it is difficult to determine each structure in all 755 peaks ; therefore, we should narrow down the candidates for causative agents of HE in further studies. Our own results suggest that many difficulties lie ahead in determining the causative agent of HE.

Key Words : biomarkers, acute liver failure, living donor liver transplantation, protein chip array, perioperative change

Introduction

Ammonia, short-and medium-chain fatty acids, amino acid disturbances, glutamate (GLU), and γ-aminobutyric acid (GABA) have been reported to play important roles in hepatic encephalopathy (HE)[1)]. The causative agent of HE has not been identified with certainty, however. The recovery

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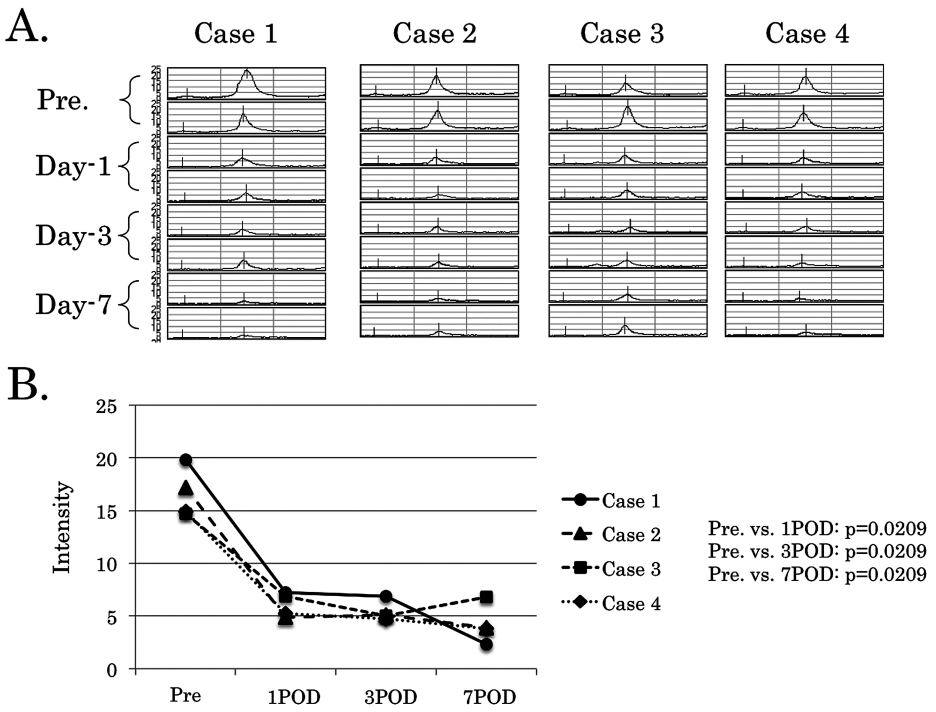


Fig. 1 The typical pattern of changing in the intensity of identified peak in our analyses (IMAC 30, Fraction 1) was demonstrated in Figure 1. The identified peaks at 2743 m/z are shown by short lines at duplicated samples at each point in all 4 patients (Fig. 1A). The pre-operative intensity of identified peaks was significantly higher compared to that of 1-, 3-, and 7POD in each 4 patient (p=0.0209) (Fig. 1B).

of consciousness in patients with acute liver failure (ALF) who underwent liver transplantation (LT)[2] is sometimes drastic ; therefore, we thought that the causative agents of HE would change markedly peri-operatively in these patients.

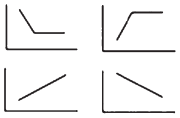
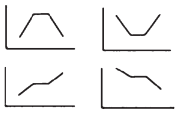
Method

We examined the biomarkers including new agents in the serum of patients using the ProteinChip® System 4000 (Ciphergen Biosystems, Yokohama, JAPAN)[3]. Sixteen samples were obtained from four patients with fulminant hepatitis who underwent living donor LT (LDLT) at four time points ; pre-operative, one post-operative day (1POD), 3POD, and 7POD. All four patients recovered their consciousness at 1POD after LDLT. We used three chips made by the Biomek2000 robot ; a weak cation-exchange chip (CM 10 ; 100 mM sodium acetate, pH4), a second weak cation-exchange chip (CM 10 ; 50 mM HEPES, pH7), and a cupper-binding chip (IMAC 30 ; 100 mM sodium phosphate, pH7 + 0.5 M NaCl). Samples and chips were incubated (30 min), irrigated (5 min×3), desalinized using Milli-Q water, added with energy absorption molecules, and measured. Data collection was optimized 6500 m/z (up to 100000 m/z) and 20000 m/z (up to 200000 m/z). All duplicated samples were assayed and analyzed using the CiphergenExpress™ data manager. Data were analyzed using the pared-t test at each point, and p-values of less than 0.05 were considered to indicate statistical significance.

Results

The typical pattern of change in the intensity of the identified peak in our analyses is demonstrated in Fig. 1. The pre-operative intensity of this identified peak (IMAC 30, Fraction 1, 2743 m/z) was significantly higher compared to those at 1-, 3-, and 7POD in each four patient

Table 1 Patterns of peri-operative changes of identified peaks of protein

Patterns	Typical graphs	The number of peaks
#1. Changes in 1-, 3-, and 7POD		207
#2. Changes in day 1POD		117
#3. Changes in 3POD		101
#4. Changes in 7POD		117
#5. Changes in 1POD and 7POD		82
#6. Changes in 3POD and 7POD		82
#7. Changes in 1, 3, and 7POD		49

(p=0.0209). We divided the peri-operative changes in the intensity of identified peaks into seven patterns, and summarized our results in Table 1. The number of peaks whose intensity shows significant changes peri-operatively reached 755. With regard to identifying the causative agent for HE, we considered pattern #1 (significant changes at 1POD, 3POD, and 7POD) to be the most important ; however, only in this pattern, the number of identified peaks reached 207.

Discusion

Our trials conducted to assess the peri-operative changes in serum protein in patients with ALF who underwent LDLT using a protein chip array is the first of its kind ; however, as many as 755 candidates for causative agents of HE were identified. Of course, it is difficult to determine each structure in all 755 peaks ; therefore, we should narrow down the candidates for causative agents of HE in further studies. One of the reasons that too many candidates were identified is the administration of a large amount of fresh frozen plasma and many drugs peri-operatively. Therefore, to exclude the influence of surgery and patients' peri-operative management, one possible method for narrowing our peaks is to compare our results to those of patients with another etiology such as liver cirrhosis or hepatocellular carcinoma who underwent LT. Even by this method, the additional analyses would require a great deal of labor, have high research costs, and would be time-consuming. On the other hand, more small molecules would be important concerning of passages of blood brain barrier. From this point of view, metabolomics analysis should be the potent method.

Trials intended to determine the causative agent of HE are important, because positive results could lead to the development of new liver support devices which can absorb this agent[4]. However, our own results suggest that many difficulties lie ahead in determining the causative agent of HE.

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（和文抄録）

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肝性脳症の原因物質はまだ同定されていない．我々は，肝移植を受けた急性肝不全患者の意識回復が時に劇的である事から，このような患者において肝性脳症の原因物質が周術期に劇的に変化していると考えた．そこで，肝性脳症の原因物質を同定するために，プロテインチップシステム4000®（サイファージェンバイオシステムズ，横浜）を用いて，患者血清中のバイオマーカーを検討した．

生体肝移植を受けた急性肝不全患者4名より，周術期4ポイント（手術前，術後1日，術後3日，術後7日）で血清を採取して，合計16のサンプルを得た．今回は，Biomek2000ロボットにより作製された3つのチップを使用した．測定はサンプル毎に2回行い，結果はCiphergenExpress™データマネージャーを用いて分析した．周術期における発現ピークの変動パターンを7パターンに分割し，周術期に有意に変動した発現ピークとして755個を同定した．

もちろん，755個の発現ピーク全ての構造を決定することは困難である．従って，我々は更に検討を重ね，肝性脳症の原因物質の候補を絞り込む必要がある．我々の今回の検討結果は，肝性脳症の原因物質を決定するには多くの困難が待ち受けていることを示唆している．