Mechanism of ammonia removal in treatment of unhepatic dog with bioartificial liver

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INTRODUCTION

The BAL to treat patients with severe liver failure is one promising approach for temporary extracorporeal organ support, and most of the BAL devices contain a huge number of hepatocytes and maintain the functions of the hepatocytes for a long term [1]. This technology has increased the chances of survival of fulminant hepatic failure (FHF). In vivo experiments demonstrated that the metabolic functions in hepatic failure could be replaced, to some degree, by the BAL system. It is generally considered that about 10-40% of normal hepatocytes mass has to sustain life for the normal function of the liver [2]. However, in spite of the development of many types of the BAL systems for the treatment of FHF, satisfactory BAL has not yet been developed and the actual level of the BAL device to support the FHF has not known yet. For this reason, it is difficult to assess and compare the efficacy of various liver assist systems for in vivo, even though difficulties in developing the BAL come from unsuccessful artificial realization of liver functions such as detoxification, synthesis and waste products.

The design and operating parameters of the BAL are critical importance to produce efficient and beneficial solute transport. Although the BAL system has been developed in various stages for clinical use, there are various problems. Among them, the scale-up of the BAL system is very important. In this study, to elucidate the scale-up of the BAL system using the ammonia the function of the BAL was evaluated under in vivo application of the BAL with perifusion model. The actual plateau levels of ammonia in the BAL system were obtained using unhepatic animal as an ideal model before and after the BAL treatment even though it is certainly different from human liver failure.

METHODS AND MATERIALS

Cell harvest: Hepatocytes were harvested from the liver of pig of approximately 12 kg in body weight with the modified method originally reported by Sielaff et al. [3]. Hepatocytes count and viability were assessed by the trypan blue exclusion test.

BAL apparatus: A hollow fiber cartridge (Duo-Flux M170D, JMS, Hiroshima, Japan) which is clinically used as a hemo-dialyzer was employed to prepare the BAL cartridge. The hollow fibers are made of a cellulose acetate membrane with a nominal cut-off molecular weight of 32 kDa. $5x10^9$ cells/ml of the hepatocytes suspension was inoculated through the arterial end of the cartridge into the inner space of the hollow fibers.

In vitro experiment: Ammonium hydrochloride in phosphate buffered solution (PBS) was

added to the perfusate at an ammonia concentration of 1,700 g/dl, and 3 ml of the perfusate was collected at the bolus port in the BAL cartridge as an indicator of metabolic activities of the BAL. The collected samples were centrifuged at 1,800g for 7 min and the supernatants were subjected to determine the ammonia concentration by F-kit Food analysis/Ammonia (Boehringer Mannheim, Germany).

In vivo experiment: In beagle dogs (weighing approximately 12 kg) as liver failure models, a portocaval shunt was made after occlusion of the portal vein (PV) and hepatic artery (HA). Six dogs were connected with and without BAL system after interruption of PV. For access to the BAL, the left internal jugular vein was cannulated with a double-lumen catheter. During the BAL treatment, the blood perfusion flow rate was maintained at 50 ml/min and 200 ml/min between the reservoir and the dog, and the BAL cartridges and the reservoir, respectively. The ammonia concentration was determined using F-kit Food Analysis/Ammonia (Boehringer Mannheim, Germany).

RESULTS AND DISCUSSION

In vitro experiments: The in vitro BAL function was evaluated by an initial bolus loading of ammonia into the perfusate. The ammonia concentration in the reservoir with initial conditions is followed by

$$C_r(t) = AExp[-\alpha t] + BExp[-\beta t] \quad (1)$$

I.C.: $C_r(0) = D/V_r, C_a(0) = 0$

where A and B are constants, and α and β are distribution constant and constant of elimination rate, respectively. Ammonia obeys the first-order reaction (Eq. (1)) and is removed from the perfusate following exponential concentration profile by the BAL cartridge. From the analysis of Eq. (1) with mentioned by Park et al. (3), the values of CL and CL_{int} of ammonia were 15 and 16.5 ml/min, respectively, for Q_a of 200 ml/min.

The effect of extracorporeal circulation rate in the BAL cartridge on CL determined from the concentration-time curve of ammonia after an initial bolus injection. Our main experimental observation is the nonlinear increase in ammonia release rate with the flow rate. The metabolic rate of ammonia is a function of axial flow rate in the luman of the BAL cartridge, and an increasing perfusate flow rate enhanced the function of the BAL device.

In vivo experiments: Ammonia, which is endogenously generated in the body is eliminated by organs including liver with different elimination rates. If the patient is suffering from hepatic failure, ammonia is accumulated in the body, and the accumulated amount can be removed using the BAL system. When the portocaval shunt dog as in vivo model is connected to the BAL device, Fig. 1 shows the systematic representations of ammonia plateau levels without (group A, control) and with (group B) the BAL assist. The plateau level of ammonia of group A increased with time and then approached a steady state, while the plateau level of ammonia of group B was in lower level than that of group A during the observation even though the value with group B showed higher than that of normal dog. After the BAL treatment, the plateau level of ammonia was reduced from 252.9 to 165.9 mol/L.

Perifusion model: To display the behavior of ammonia in the body, the perifusion model used in in vitro can be applied to in vivo model. Fig. 2 shows the perifusion model between

the body and two BAL cartridges in parellel for in vivo application with reservoir. If the toxin is accumulated in the body, the differential equations with reservoir under well-stirred condition are:

$$V_r \frac{dC_r}{dt} = Q_b(C_b - C_r) + Q_a(C_a - C_r) \tag{2}$$

$$V_b \frac{dC_b}{dt} = Q_b(C_r - C_b) + P - CL_{\text{int}\,b} C_b \tag{3}$$

$$2V_a \frac{dC_a}{dt} = Q_a(C_r - C_a) - 2CL_{\text{int}\,a} C_a \tag{4}$$

where Q_b is the blood flow rate between the body and the BAL cartridge or the reservoir, P is the production rate of toxin in the body and subscripts r, b and a represent the reservoir, the body and the BAL cartridges, respectively. Under the steady state, the accumulated toxin in the body, from Eqs. (2) to (4), becomes as

$$C_b / P = \frac{Q_b^{\,2}(Q_a + 2CL_{\text{int}\,a})}{(Q_b + Q_a)(Q_b + CL_{\text{int}\,b})(Q_a + 2CL_{\text{int}\,a}) - Q_b^{\,2}(Q_a + 2CL_{\text{int}\,a}) - Q_a^{\,2}(Q_b + CL_{\text{int}\,b})} + \frac{1}{Q_b + CL_{\text{int}\,b}} \tag{5}$$

To analyze experimental data in Fig. 1 with perifusion model, Table 1 lists the values of plateau level and clearance in the body before and after connecting the BAL cartridges to the portocaval shunt dog, along with those of normal dog liver (base line). The production rate ($P=C_bCL$) of ammonia in unhepatic dog using the plateau level and clearance obtained from normal dog could be calculated roughly and used to obtain the CL values of ammonia for group A and group B. For Q_b =50 ml/min, Q_a =200 ml/min, CL_{intb} =240 ml/min and CL_{inta} =15 ml/min, the clearance in the body after the BAL treatment was obtained using Eq. (5) and represented as ammonia metabolic rate of the whole body for the brain, skeletal muscle, and livers in the BAL and the body. CL_{inta} was obtained from in vitro system before the BAL was connected to portocaval shunt dog, and CL_{intb} was obtained from in vivo system (group A) based on perifusion model.

The actual removal mechanism of ammonia in animal model after the BAL treatment is not yet to known. The amount of toxin which is cleared by the BAL is critical to analyze unhepatic dog model and compare it with human liver. To investigate the maximum removal rate of ammonia in the unhepatic dog model, the values of C_b/C_n (calculated ratio of the plateau level in the unhepatic dog to that in the normal dog) were calculated for CL_{intb} =250 ml/min, Q_a =200 ml/min and P =60 μg/min/kg which are obtained from our experimental data. After the BAL treatment, the values of C_b/C_n of our system were improved from 4.8 to 3.1 and 4.6 for experimental and model values, respectively. The difference of 1.5 between experimental and model values of plateau index means that the function of the unhepatic dog was improved by the BAL substantially. In fact, the clearance of the unhepatic dog after the BAL treatment increased from 19.6 to 29.8 ml/min/kg.

Under the same assumptions like those for unhepatic dog model, the perifusion model could be applied to human patient, which is treated by the BAL. C_b (base line) and CL_{intb} of ammonia from normal human obtained were 97.6 μmol/L and 100 ml/min/kg, respectively, and these were used to calculate the value of P. The values of P thus obtained was used to calculate CL_{intb} of ammonia before and after the BAL treatment. The value of CL_{intb} obtained using Eq. (5) before the BAL treatment of the patient was 4,200 ml/min for $C_{b\infty}$ = 97.6 μmol/L, P =680 μmol/min and Q_a =200 ml/min. The calculated and experimental

indexes for Demetrious BAL system [5] with our perifusion model were improved from 1.70 to 1.65 and to 1.02, respectively, after the BAL treatment. The difference between calculated and experimental values in plateau index represents the improvement of ammonia removal rate, which is likely to be related to the external factors. Therefore, the value of CL_{intb} of ammonia for Demetrious BAL system was improved from 57 to 96 ml/min/kg, which is 96% of normal human clearance.

The BAL system can give significantly beneficial effects on animals with severe liver failure and may contribute to the development of remarkable reduction in ammonia concentration without the burden on other organs. And also, it will be advantageous to develop the BAL system to provide adequate metabolic support for the remaining functional liver mass to recover from disease or to cover the time until normal liver is available for transplantation.

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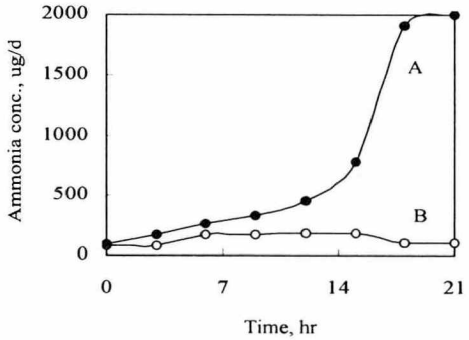

Figure 1. Plateau levels of ammonia for portocaval shunt dog with and without the BAL cartridges.[○;with the BAL, ●; without the BAL]

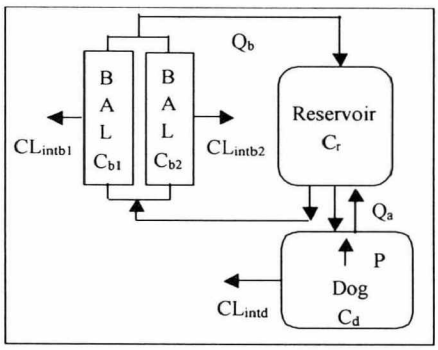

Figure 2. Flow diagram of hybrid artificial system with in vivo BAL cartridges.

Table 1. The plateau levels and clearances before and after the BAL treatment in unhepatic dog model (P =5 μ g/min/kg)

	C_∞, μ g/dl	CL, ml/min/kg
Base line	53	94
No treatment	253	20
Treatment	166	30