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Method for Evaluating Metabolic Functions of Drugs in Bioartificial Liver

Yueng Guen Park[1,2], Hiroo Iwata[2], Seiji Satoh[3], Takehiko Uesugi[3], and Hwa-Won Ryu[4,5]*

[1] Laboratory of Tissue Engineering, Korea Cancer Center Hospital, KAERI, Gongneung-Dong, Nowon-Gu, 215-4, Seoul, Korea
[2] Institute for Frontier Medical Science, Kyoto University, Kyoto 606-8507, Japan
[3] Department of Gastroenterological Surgery, Kyoto University, Kyoto 606-8507, Japan
[4] Faculty of Applied Chemical Engineering, Chonnam National University, Gwangju 500-757, Korea
[5] Institute of Bioindustrial Technology, Chonnam National University, Gwangju 500-757, Korea

Abstract Lidocaine and galactose loading tests were performed on a bioartificial liver (BAL), an extracorporeal medical device incorporating living hepatocytes in a cartridge without a transport barrier across the membranes. The concentration changes were analyzed using pharmacokinetic equations to evaluate the efficacy and limitation of the proposed method. Lidocaine and galactose were found to be suitable drugs for a quantitative evaluation of the BAL functions, as they did not interact with the plasma proteins or blood vessels, making their concentrations easy to determine. The drug concentration changes after drug loading were easily analyzed using pharmacokinetic equations, and the BAL functions quantitatively expressed by pharmacokinetic parameters, such as the clearance (*CL*) and galactose elimination capacity (*GEC*). In addition, these two drugs have already been used in clinical tests to evaluate human liver functions over long periods, and lidocaine *CL* values and *GEC* values reported for a normal human liver. Thus, a comparison of the *CL* and *GEC* values for the *BAL* and a natural liver revealed what proportion of normal liver functions could be replaced by the BAL.

Keywords: bioartificial liver, lidocaine, galactose elimination capacity, clearance, perfusion model

INTRODUCTION

Various research groups have already developed bioartificial livers (BALs) [1-7], however, since there is no universal standard method for analyzing their functions, it ishard to compare the functions of different BALs, and difficult to evaluate what proportion of normal liver functions can be replaced by a BAL in clinical practice. Therefore, the development of methods capable of quantitatively determining the functions of a BAL is very important. Pharmacokinetic equations have already derived that can describe the concentration changes in drugs loaded in a BAL maintained in an *in vitro* circuit, plus pharmacokinetic parameters, such as clearance (*CL*), have been introduced to express BAL functions. The drugs used to evaluate the BAL functions must fulfill several requirements, *i.e.* exert the least adverse effects on the hepatocytes in the BAL, and have a clear metabolic pathway in the hepatocytes, and its concentration be easy to determine. Plus, drugs that have already been used for the evaluation of liver functions in clinical tests are preferable, because the functions of a BAL need to be compared with those of a normal human liver. In the current study, lidocaine and galactose were used as the loaded chemicals to evaluate the functions of a BAL device developed by the present authors. The concentration changes were analyzed using pharmacokinetic equations derived to clarify the efficacy and limitations of methods developed in a previous paper [7].

*Corresponding author
Tel: +82-62-530-1842 Fax: +82-62-530-1849
e-mail: hwryu@chonnam.ac.kr

MATERIALS AND METHODS

Preparation of BAL Cartridge

Institutional guidelines were applied in the care and use of pigs obtained from a local pig breeder. Hepatocytes were harvested from pigs with a body weight of approximately 12 kg using the method reported by Sielaff et al. [8] with modifications [7], as briefly described in the following. After the surgical procedures, an EDTA solution was perfused into the liver through the portal vein at a rate of 800 mL/min at 37°C for 7 min. The solution from the intrahepatic inferior vena cava was discarded to wash out the blood and remove any calcium ions. A collagenase solution (0.5 g/L) was then circulated from the portal vein at a rate of 800 mL/min at 37°C for approximately 20 min until the liver was digested and became

soft. The two solutions were well oxygenated using a membrane oxygenator (Menox AL-2000, Kuraray, Osaka, Japan), which was set between the reservoir and the portal vein. After digestion, the liver was placed into a sterile basin. Next, the liver capsules were incised on all lobes and the hepatocytes released with gentle agitation into 1,600 mL of a calcium- and magnesium- free Hanks' balanced salt solution (HBSS) at 4°C. The cell suspension was gently filtered through a nylon mesh with a 100 μm opening. The filtrate was then divided into eight 225-mL polypropylene test tubes and gently centrifuged at 50 × g for 3 min. Thereafter, the supernatant was discarded, and the cells resuspended in 1,600 mL of iced HBSS. This washing procedure was repeated two more times to remove the collagenase. The cell number and viability were then assessed by the dye exclusion test, using 0.4% trypan blue in phosphate buffered saline (PBS) and a hemocytometer.

Next, the cell suspension was centrifuged at 50 × g for 3 min, then the cell concentration was adjusted to 1 × 10^8 cells/mL by adding an appropriate amount of HBSS. A hollow fiber cartridge (Duo-Flux M170D, JMS, Hiroshima, Japan), as used for clinical hemo-dialysis, was employed to prepare a BAL cartridge. The hollow fibers in the cartridge were made of cellulose diacetate, and their internal and external diameters were 195 μm and 225 μm, respectively. With a conventional hemo-dialysis procedure, such hollow fibers are able to reject 97% of albumin (MW: 68,000). The total volume of the inner space of the hollow fibers was 99 ml, while the volume of the shell space between the hollow fibers and the cartridge housing was 145 mL. One hundred milliliters of hepatocytes, suspended in HBSS at a final concentration of 1 × 10^8 cells/mL, were immediately inoculated through the arterial end of the cartridge into the inner space of the hollow fibers. Both the blood inlet and outlet of the hollow fiber cartridge were closed using plastic caps.

In vitro Maintenance of BAL Cartridge

To evaluate the BAL cartridge functions, a simple *in vitro* circuit, was set up,consisting of a 1,000 ml glass bottle reservoir, a roller pump (BP-02, JMS, Hiroshima, Japan), dense membrane oxygenator (Menox AL-2000), and BAL cartridge that were all connected by poly (vinyl chloride) tubes. The total volume of the circuit was about 250 mL, excluding the reservoir. The perfusate was a mixture of 800ml of Dulbecco's modified Eagle's medium (DMEM) and 200 mL of human whole blood supplemented with 3.7 g of bicarbonate, 2,500 units of heparin, 40 units of insulin, 10^5 units of penicillin, and 0.1 g of streptomycin. Since a sufficient supply of oxygen to the hepatocytes is of vital importance during the perfusion culture of a BAL, human whole blood containing erythrocytes as an oxygen carrier was added to the perfusate to a final concentration of 20%. The perfusate in the reservoir was well mixed using a magnetic coupled stirring bar. Theereafter, the perfusate, oxygenated using the membrane oxygenator based on a gas mixture of 95% air and 5% carbon dioxide, was circulated between the hollow fibers and the cartridge housing at 200 mL/min. One thousand units of heparin were added to the perfusate by bolus every 24 h.

Bolus Loading test

A lidocaine (Research Biochemicals International, Natick, MA, USA) solution of 10 mg/mL was added by bolus to the perfusate to a final concentration of 12 μg/mL through the reservoir port. Three ml of the perfusate was collected from the same port 0.05, 1, 3, 6, 9, 18, 30, 60, and 90 min after the bolus administration, then the supernatant was collected after centrifugation at 1,800 × g for 7 min and stored at -20°C until assayed. The lidocaine remaining in the perfusate samples was quantified using a TDX analyzer (Dainabot, Tokyo, Japan).

Galactose dissolved into DMEM was added to the perfusate to a final concentration of 6.25 mg/mL. Thereafter, one mL of the perfusate was collected every 3 h over a 24 h period after the bolus administration, then the supernatant was collected after centrifugation at 1,800 × g for 7 min. The galactose concentration in the perfusate was determined using a K-kit Food analysis Lactose/D-Galactose (Boehringer Mannheim, Germany).

Constant Infusion Test

The lidocaine solution was infused into the perfusate frrom 20 μg/min up to 100 μg/min. When a drug solution is infused into the reservoir at a constant rate without bolus administration, a long tail in the concentration increase in incurred until a plateau is reached. Thereafter, to remove this, a certain amount of lidocaine was added to the perfusate by bolus at the beginning of each infusion study. The amount of lidocaine added is discussed in the Results and Discussion.

During the continuous infusion of the lidocaine solution, 3 mL of the perfusate was collected from the reservoir port every 15 min. The lidocaine remaining in the perfusate samples was then quantified as mentioned above.

RESULTS AND DISCUSSION

Generally, drugs exist freely in blood, either bound to proteins or included in the blood cells. The equations used in the analyses of the drug loading tests are expressed using the drug concentrations in the blood, C_b, yet since most of the drug concentrations determined in the current were the unbound drug concentrations in the plasma, C_u, the values of C_b were determined from the values of C_u based on the following relationship.

$$C_b = \{(1 - H_{ct}) + H_{ct}K\}C_u / f \quad (1)$$

where H_{ct} is the hematocrit, K is the partition ratio of the drug between the blood cells and plasma, and f is the fraction of unbound drug in the plasma. K and f are expressed by:

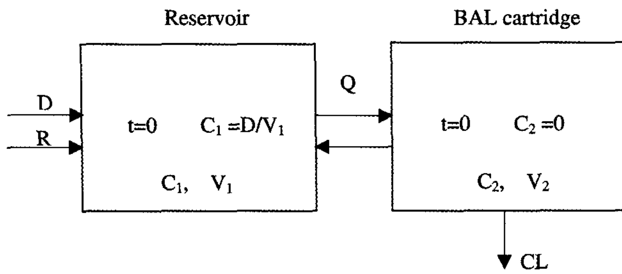

Fig. 1. Schematic representation of *in vitro* system as two-compartment model: The drug solution was infused at a constant-rate (R) immediately following an initial bolus (D).

$$K = \frac{C_c}{C_p} \tag{2}$$

$$f = \frac{C_u}{C_p} \tag{3}$$

where C_c and C_p are the drug concentrations in the blood cells and plasma, respectively.

Great care should be taken when selecting the drugs to evaluate the metabolic functions of a BAL. Rowland *et al.* already carried out extensive studies on lidocaine pharmacokinetic *in vitro* and *in vivo* [9,10], and reported that lidocaine neither binds to plasma proteins nor is entrapped in blood cells. Thus, the values of the above mentioned parameters of K and f are units, and the lidocaine concentrations in the plasma, which can be easily determined experimentally, are equal to those in the blood. The experimental results are also straightforward to analyze. Similarly, galactose has been reported not to interact with plasma proteins or blood cells [11], which is why the current study used these two drugs for the loading tests to evaluate the metabolic functions of the BAL.

Bolus Injection Test of Lidocaine

The elimination kinetics of chemicals in a BAL cartridge can be investigated using a perfusion model [12]. When lidocaine (D) with a constant infusion rate (R) is administered to the BAL to evaluate its functions, the drug is removed by a process that includes adsorption and elimination. The concentration profile of the drug that is removed from the perfusate to the cell compartments is represented as the combination of the metabolic function of the hepatocytes and mass transport of the drug in the perfusate of the BAL without a diffusion barrier between the perfusate and the hepatocytes. For the initial bolus loading test, the models used for the mass transfer mechanism of the drug are shown in Fig. 1. Fig. 2 shows a plot of the concentration decay curve against time after the bolus administration of lidocaine without a constant infusion rate. The initial rapid fall followed by a slower decline can be reproduced by two exponential terms. The pharmacokinetic equation in the reservoir that can describe the concentration decay curve is as follows:

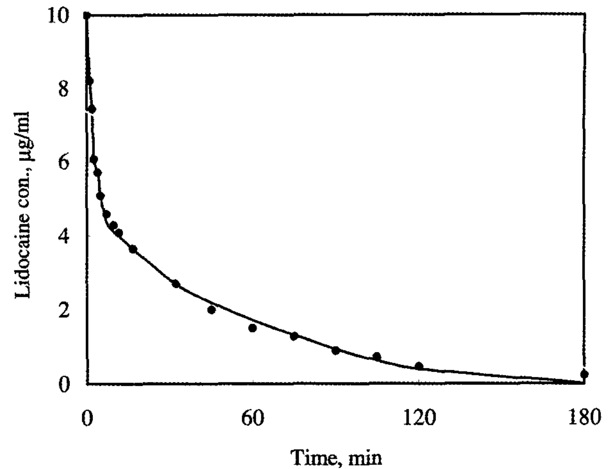

Fig. 2. Lidocaine concentration profiles for *in vitro* perfusate system after initial bolus loading of 10 μg of lidocaine.

$$C_1(t) = AExp[-\alpha t] + BExp[-\beta t] \tag{4}$$

$$A = \frac{D\alpha(1/V_1 - \beta/CL)}{\alpha - \beta} \tag{5}$$

$$B = \frac{D\beta(\alpha/CL - 1/V_1)}{\alpha - \beta} \tag{6}$$

$$\alpha\beta = \frac{CLQ}{V_1V_2} \tag{7}$$

$$\alpha + \beta = \frac{Q(V_1 + V_2)}{V_1V_2} \tag{8}$$

where C_1 is the lidocaine concentration in the reservoir, V_1 is the total volume of the reservoir and oxygenator, V_2 is the volume of the BAL cartridge, Q is the circulating rate of the perfusate, and A and B are constants. In addition, the pair of parameters, α and β, are expressed by:

$$\alpha, \beta = 1/2[(Q/V_1 + Q/V_2) \pm \{(Q/V_1 + Q/V_2)^2 - \frac{4CLQ}{V_1V_2}\}^{1/2}]\alpha \geq \beta \tag{9}$$

Since it is quite tedious to evaluate the CL value from the experimental data shown in Fig. 2 using these equations, the simple relationships between the set of parameters, α and β, and A and B, with an another set of parameters, D and CL were deduced:

$$CL = \frac{D}{\int C_1 dt} = \frac{D}{A/\alpha + B/\beta} \tag{10}$$

Various computer software is now available for determining the parameters, A and B, and α and β based on fitting Eq. (4) to the experimental data. The CL value can then

Table 1. Metabolic functions of BAL and natural liver

	CL, mL/min	Cl_{int}, mL/min	*GEC*, mg/min
BAL	36.1	46.0	1.34
Human liver[a)]	720	-	432
Porcine liver[b)]	-	-	124
Isolated porcine liver[c)]	-	-	93

a); Ref. 10, b); Ref. 14, c); Ref. 15

be easily determined from the values of these parameters using Eq. (10). The lidocaine *CL* values for the BAL are listed in Table 1, along with those for whole human liver.

Constant Infusion Test Using Lidocaine

When lidocaine was infused into the perfusate at a constant rate, *R*, its concentration monotonously increased to a plateau value. The pharmacokinetic equation that can describes the concentration increase curve was derived as:

$$C_1(t) = \frac{QR}{V_1 V \alpha \beta_2} + \frac{(D\alpha - R)(Q - \alpha V_2)}{V_1 V_2 \alpha(\beta - \alpha)} Exp[-\alpha t] + \frac{(D\beta - R)(Q - \beta V_2)}{V_1 V_2 \beta(\alpha - \beta)} Exp[-\beta t] \quad (11)$$

When a drug solution is infused for an infinite time, the lidocaine concentration in the reservoir continues to increase to a plateau value expressed by:

$$C_{1,t=\infty} = \frac{QR}{V_1 V_2 \alpha\beta} = \frac{R}{CL} \quad (12)$$

Thus, the clearance value can easily be determined from the constant infusion rate, *R*, and plateau concentration, $C_{1,t=\infty}$.

The evaluation method described above is simple, however, the experiment is time-consuming, as there can be a long delay between the start of the infusion and the establishment of the plateau concentration. The concentration change exhibits biexponential disposition characteristics, as expressed by Eq. (11). The half-lives of the second and third terms of Eq. (11) are given by:

$$t_{\alpha,1/2} = 0.693/\alpha \quad (13)$$

$$t_{\beta,1/2} = 0.693/\beta \quad (14)$$

Since β is assumed to be smaller than α, the long tail of the concentration increase is mainly caused by the third term on the right side of Eq. (11). If a bolus amount of lidocaine:

$$D = \frac{R}{\beta} \quad (15)$$

is added at the beginning of the experiment followed by a

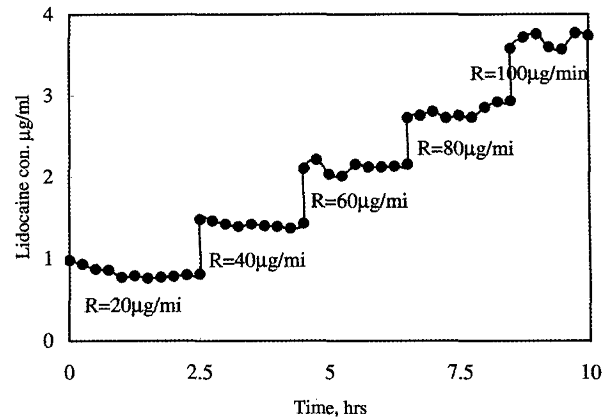

Fig. 3. Lidocaine concentration changes in *in vitro* circulation system during constant infusion at rates indicated in figure.

constant infusion rate, under such conditions, Eq. (11) is reduced to

$$C_1(t) = \frac{QR}{V_1 V \alpha \beta_2} - \frac{R(Q/V_2 - \alpha)}{V_1 \alpha\beta} Exp[-\alpha t] = \frac{R}{CL} - \frac{R(Q/V_2 - \alpha)}{V_1 \alpha\beta} Exp[-\alpha t] \quad (16)$$

thereby removing the long tail. Fig. 3 shows the results of the constant rate infusion tests. The plateau level, which is given by

$$C_{1,t=\infty} = \frac{R}{CL} \quad (17)$$

can be rapidly attained by a combination of the bolus administration of R/β and a constant infusion at *R*. After the plateau lidocaine concentration was achieved, the next infusion test was initiated by continuous administration at an increased infusion rate in combination with the bolus administration:

$$\Delta D = \frac{\Delta R}{\beta} \quad (18)$$

Based on a series of infusion tests, stepwise increases in the lidocaine concentration were observed, as shown in Fig. 3. The steady state concentrations were plotted against the infusion rates as shown in Fig. 4. As expected from Eq. (18), this produced a linear relationship between the plateau concentrations and the infusion rates. The clearance value for the BAL cartridge was estimated to be 15 mL/min from the slope using Eq. (18).

When the lidocaine was administrated with R/β as the initial bolus loading, the result is shown in Fig. 5. The initial bolus amount, 4.35 mg under R = 40 µg/min, was determined from the initial bolus loading in the previous experiment. The concentration of lidocaine dropped, then rapidly reached a plateau level. As such, the initial bolus drug amount determined from the previous experiment

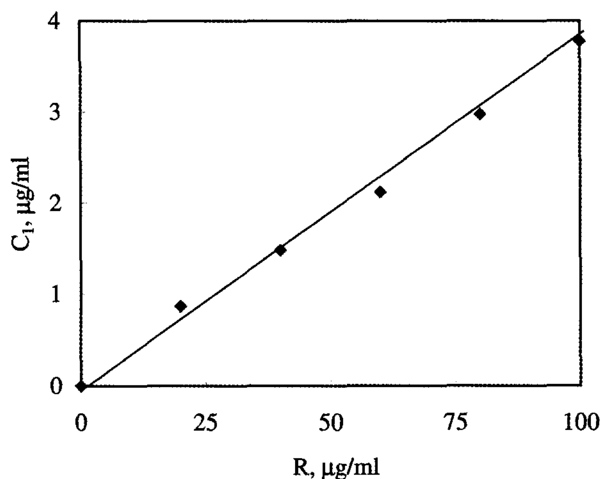

Fig. 4. Linear relationship between plateau concentrations and infusion rates observed in constant lidocaine infusion tests.

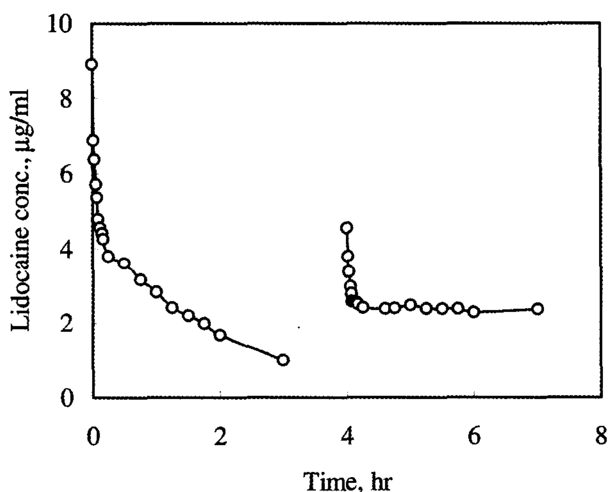

Fig. 5. Lidocaine concentration changes with constant-rate infusion plus initial bolus loading.

was important to rapidly achieve a plateau level with C-I-B, and had to be administrated at the level of R/β.

As seen in the series of lidocaine loaded tests shown in Figs. 3 and 5, the *CL* value was determined much more precisely in the constant infusion test than in the bolus loaded test. However, there is an inherent contradiction in the method mentioned above for determining the *CL* value, as the *CL* value should be known beforehand to calculate the amount of bolus administration. Therefore, this method can only be applied to confirm the *CL* value obtained by other methods.

Intrinsic Clearance

As can be found from the experimental procedure to determine the *CL* value for the BAL, the *CL* value depends on various experimental setups, such as the interaction of the drugs with the blood components, hematocrit values and plasma protein concentration in the perfusate, flow pattern in the BAL cartridge, and circulation rate of the perfusate. The *CL* value does not directly show the intrinsic metabolic functions of a BAL, as it can only express the metabolic function of a BAL under certain experimental conditions. As such, the intrinsic clearance (CL_{int}), which represents the intrinsic metabolic capacity of a BAL cartridge, must be introduced. Detailed discussions related to this subject can be found elsewhere [12].

Various models, such as the well-stirred model and parallel tube model, are used in pharmacokinetic. The current study used the well-stirred model, as the resulting derived equations are simple and give clear images of the dependence of the *CL* value on various factors, plus Rowland *et al.* reported that it effectively expresses the *in vivo* pharmacokinetic of lidocaine [13]. The well-stirred model also provides a relationship between *CL* and CL_{int}:

$$CL = \frac{QCL_{int}f}{Q + CL_{int}f} \tag{19}$$

When the circulation rate was low, the drug metabolic rate was mainly determined by the amount of drug administered into the BAL within a time unit by the perfusion flow. The *CL* value increased with an increase in the circulation rate, then reached a plateau maximum at an infinite circulation rate. Table 1 also lists the average lidocaine *CL* and CL_{int} values for 8 BAL cartridges determined during the initial 24-h circulation, along with those for the whole human [10].

Bolus Loading of Large Amount of Chemical

A galactose loading test, which is clinically used for patients with hepatic failure to determine the functional liver cell mass, was also applied to evaluate the BAL metabolic function. When the concentration of galactose loaded in the BAL was as high as that employed in a clinical test, a linear decline in the galactose concentration was observed, as seen in Fig. 6. The galactose elimination capacity (*GEC*) instead of the galactose *CL* was

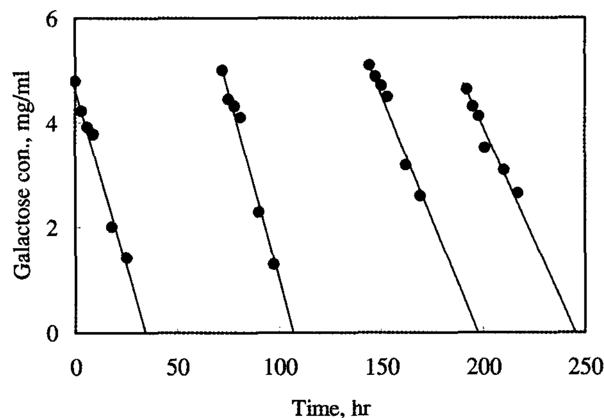

Fig. 6. Galactose concentration changes in reservoir after loading with 5 mg/mL galactose.

used to express the metabolic ability of the BAL, as employed in the representation of the metabolic capability of a natural liver in clinical tests. The *GEC* value was easily determined from the slope of the linear plot of the galactose concentration in the perfusate, C_1, relative to the perfusion time using the following equation [9]:

$$C_1 - C_{1,t=0} = -\frac{tV_{max}}{V_1 + V_2} = -\frac{tGEC}{V_1 V_2} \quad (20)$$

The average *GEC* values for 8 BAL cartridges determined during the initial 24-h circulation are listed in Table 1, along with those for whole human and porcine livers.

As mentioned above, the BAL cartridges exhibited 1/13 of the lidocaine metabolic function of a whole human liver, yet the *GEC* of the BAL device was only 1/322 of that of a human liver. The human liver can eliminate galactose 3 times more efficiently than a porcine liver, as seen in Table 1. The *GEC* of a porcine liver *in situ* is 50% higher than that of an isolated perfused liver [14,15]. Thus it would seem that the small *GEC* value for the current BAL reflected two effects.

CONCLUSION

Lidocaine and galactose are suitable drugs for the quantitative evaluation of BAL functions without a transport barrier across the membranes. They are free from interaction with the plasma proteins and erythrocytes, making their concentrations easy to determine. The results of drug loading tests can then be easily analyzed using pharmacokinetic equations, plus BAL functions can be quantitatively expressed by pharmacokinetic parameters, such as the *CL* and *GEC*. In addition, these two drugs have already been used in clinical tests to evaluate liver functions over long periods. Accordingly, a comparison of the *CL* values for a BAL and natural liver can reveal the proportion of normal liver functions that can be replaced by a BAL.

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Nomenclature

A and B	Constant [μg/min]
C	Concentration of chemical [μg/mL]
CL	Clearance [mL/min]
CL_{int}	Intrinsic clearance [mL/min]
D	Drug administration [mg]
f	Fraction of unbound drug in plasma
GEC	Galactose elimination capacity [mg/min]
H_{ct}	Hematocrit [-]
K	Partition ratio of drug between blood cells and plasma [-]
Q	Perfusate flow rate [mL/min] or oxygen consumption rate [mmHg/min]
R	Constant-rate infusion [μg/min]
V	Volume [mL]
V_{max}	Maximum elimination rate [mg/min]

Greek letters

α	Distribution constant [min^{-1}]
β	Constant of elimination rate [min^{-1}]

Subscripts

1	Total volume of reservoir
2	Total volume of BAL cartridge
c	Blood cell
u	Plasma
p	Plasma for unbound drug

REFERENCES

[1] Sussman, N. L., G. T. Gislason, and J. H. Kelly (1994) Extracorporeal liver support application to fulminant hepatic failure. *J. Clin. Gastroenterol.* 18: 320-324.

[2] Nyberg, S. L., R. A. Shatford, M. V. Peshwa, J. G. White, F. B. Cerra, and W. S. Hu (1993) Evaluation of a hepatocyte-entrapment hollow fiber bioreactor: A potential bioartificial liver. *Biotechnol. Bioeng.* 41: 194-203.

[3] Tilles, A. W., H. Baskaran, P. Roy, M. L. Yarmush, and M. Toner (2001) Effect of oxygenation and flow on the viability and function of rat hepatocytes cocultured in a microchannel flat-plate bioreactor. *Biotechnol. Bioeng.* 73: 379-389.

[4] De Bartolo, L., G. Jarosch-Von Schweder, A. Haverich, and A. Bader (2000) A novel full-scale flat membrane bioreactor utilizing porcine hepatocytes: cell viability and tissue-specific functions. *Biotechnol. Prog.* 16: 102-108

[5]Kawada, M., S. Nagamori, H. Aizaki, K. Fukaya, M. Niiya, T. Matsuura, and H. Sujino (1998) Massive culture of humane liver cancer cells in a newly developed radial flow bioreactor system: ultrafine structure of functionally enhanced hepatocarcinoma cell lines. *In Vitro Cell Dev. Biol. Anim.* 34: 109-115.

[6] Gion, T., M. Shimada, K. Shirabe, K. Nakazawa, H. Ijima, T. Matsushita, and K. Funatsu (1999) Evaluation of a hybrid artificial liver using a polyurethane foam packed-bed culture system in dogs. *J. Surg. Res.* 82: 131-136.

[7] Iwata, H., T. Sajiki, H. Maeda, Y. G. Park, B. Zhu, S. Satoh, T. Uesugi, I. Ikai, Y. Yamaoka, and Y. Ikada (1999) In Vitro evaluation of metabolic functions of a bioartificial liver *ASAIO J.* 45: 299-306.

[8] Sielaff, T. D., W. S. Hu, and F. B. Cerra (1995) A technique for porcine hepatocytes harvest and description of differentiated metabolic functions in state culture. *Transplantation* 59: 1459-1463.

[9] Pang, K. S. and M. Rowland (1977) Hepatic clearance of drugs: II. Experimental evidence for acceptance of the "Well-stirred" model over the "Parallel tube" model using lidocaine in the perfused rat liver *in situ* preparation. *J. Pharmacokinet. Biopharm.* 5: 655-680.

[10] Rowland, M., P. D. Thomson, A. Guichard, and K. L.

Melmon (1971) Disposition of lidocaine in normals subjects. *Ann N. Y. Acad. Sci.*179: 383-398.
[11] Fabbri, A., G. Bianchi, E. Motta, M. Brizi, M. Zoli, and G. Marchesini (1996) The galactose elimination capacity test: A study of the technique based on the analysis of 868 measurements. *Am. J. Gastroenterol.* 91: 991-996.
[12] Rowland, M. and T. N. Tozer (1989) *Clinical Pharmacokinetics Concepts and Applications*. Lea & Febiger, Philadelphia, PA, USA.
[13] Pang, K. S. and M. Rowland (1977) Hepatic clearance of drugs: I. Theoretical considerations of a "Well-stirred" model and a "Parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J. Pharmacokinet. Biopharm.* 5: 625-653.
[14] Keiding, S., S. Johansen, and K. Winkler (1982) Hepatic galactose elimination kinetics in the intact pig. *Scand, J. Clin. Lab. Invest.* 42: 253-259.
[15] Keiding, S., S. Jihansen, K. Winkler, K. Tonnesen, and N. Tygstrup (1976) Michaelis-Menten kinetics of galactose elimination by the isolated perfusaed pig liver. *Am. J. Physiol.* 230: 1302-1313.

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