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Evaluation Methods and Design for Bioartificial Liver Based on Perfusion Model

Yueng Guen Park[1]* and Hwa-Won Ryu[2,3]

[1] Radiation Application Research Division, 105, KAERI, Yuseong, Daejeon 305-600, Korea
[2] Faculty of Applied Chemical Engineering, Chonnam National University, Gwangju 500-757, Korea
[3] Institute of Bioindustrial Technology, Chonnam National University, Gwangju 500-757, Korea

Abstract A bioartificial liver (BAL) is a medical device entrapping living hepatocytes or immortalized cells derived from hepatocytes. Many efforts have already been made to maintain the functions of the hepatocytes in a BAL device over a long term. However, there is still some uncertainty as to their efficacy, and their limitations are unclear. Therefore, it is important to quantitatively evaluate the metabolic functions of a BAL. In previous studies on *in vitro* BAL devices, two test methods, an initial bolus loading and constant-rate infusion plus initial bolus loading, were theoretically carried out to obtain physiologic data on drugs. However, in the current study, the same two methods were used as a perfusion model and derived the same clearance characterized by an interrelationship between the perfusate flow rate and intrinsic clearance. The interrelationship indicated that the CL increased with an increasing perfusate flow rate and approached its maximum value, *i.e.* intrinsic clearance. In addition, to set up an *in vivo* BAL system, the toxic plateau levels in the BAL system were calculated for both series and parallel circuit models. The series model had a lower plateau level than the parellel model. The difference in the toxic plateau levels between the parallel and series models increased with an increasing number of BAL cartridges.

Keywords: bioartificial liver, perfusion model, clearance, flow rate, mass transfer

INTRODUCTION

Various types of BAL entrapping living hepatocytes in a cartridge have already been reported [1-4], making BAL one of the most promising approaches for treating patients with severe liver failure. Great efforts have also been made to maintain the hepatocyte functions in a BAL over a long term, while recent advances in BAL technology have allowed its clinical application to support the liver functions of patients as a bridge until an orthotopic liver transplantation is performed [5-7]. In this case, the device should supply a sufficient number of viable and functioning hepatocytes, approximately 10~40% of human hepatocytes [8,9]. However, no BAL has yet been able to successfully support severe liver failure, as the liver combines various metabolic functions, such as synthesizing glucose, lipids, and proteins and detoxifying ammonia, drugs, and their chemicals. As such, it is controversial whether such a small number of hepatocytes can effectively replace the liver functions of patients with severe liver failure. Therefore, to clarify the clinical effectiveness of BALs, a quantitative evaluation of their functions is essential. Several theoretical analyses that have already been proven useful in pharmacokinetic studies [10] could be applicable for such an evaluation [11,12]. A pharmacokinetic analysis investigates the processes, such as absorption, distribution, and elimination, that account for a change in the drug concentration in the body. Therefore, this type of analysis can provide both an evaluation and a quantitative comparison between a BAL and a human liver.

Accordingly, in the current study, two methods, an initial bolus loading and constant-rate infusion plus initial bolus loading using a perfusion model, were theoretically demonstrated to derive the same clearance characterized by an interrelationship between the perfusate flow-rate and intrinsic clearance. In addition, to set up an *in vivo* BAL system, the toxic plateau levels in the BAL system were calculated for both series and parallel circuit models.

***Corresponding author**
Tel: +82-42-868-8079 Fax: +82-42-868-2702
e-mail: parkyg67@hotmail.com

PERFUSION MODEL

In Vitro BAL

The elimination kinetics of drugs in a BAL were investigated using a perfusion model [11]. When a flow-limited drug that obeys a first-order reaction is introduced into the reservoir, the drug is eliminated by hepatocytes within the BAL cartridge that produce a variety of

enzymes characterized by a maximum velocity (V_{max}) and Michaelis-Menten constant (K_m). At a drug concentration below K_m, the rate of drug metabolism within the BAL cartridge can be represented as the rate of disappearance of the drug in the inner space (C_i) of the BAL cartridge.

$$\text{Rate of disappearance of drug in the BAL cartridge} = \frac{V_{max} f' C_i}{K_m + f' C_i} = CL_{int} f' C_i \quad (1)$$

where f' is the fraction of unbound drug in the blood ($f'=1$) and CL_{int} is the intrinsic drug clearance in the BAL cartridge, which describes the ability of the liver to eliminate the drug in the absence of a flow rate limitation. As such, CL_{int} is the enzymatic activity, which is independent of the perfusate flow rate and drug binding within the BAL, when the flow rate through the membranes is not rate limited.

After a chemical is loaded by bolus, D, at time 0 and continuously infused at a constant rate, R, to the reservoir (V_r) of a BAL, the changes in the chemical concentrations in the reservoir (C_r), and shell space (C_s) and inner space of the BAL cartridge using a three-compartment model under complete mixing conditions are:

$$V_r \frac{dC_r}{dt} = Q_a(C_s - C_r) + R \quad (2)$$

$$V_s \frac{dC_s}{dt} = Q_a(C_r - C_s) + KA(C_i - C_s) \quad (3)$$

$$V_i \frac{dC_i}{dt} = KA(C_s - C_i) - CL_{int} C_i \quad (4)$$

where a BAL cartridge is composed of two different areas, a shell space of hollow fibers with a volume (V_s) and inner space of hollow fibers containing hepatocytes with a volume (V_i), K is the mass transfer coefficient through the membranes in the BAL cartridge, A is the contact area of the hollow fiber surface, and Q_a is the perfusate flow rate between the reservoir and the BAL cartridge. For the drug removal rate from the perfusate in the BAL, Eq. (4) under $CL_{int}C_i = CLC_s$ can be replaced by Eq. (5).

$$V_i \frac{dC_i}{dt} = KA(C_s - C_i) - CLC_s \quad (5)$$

where CL (mL/min) is the clearance of the BAL, defined as the volume of the inflow to the BAL cartridge from which the drug can be entirely removed within the time unit. In the case of $R=0$, the initial conditions for Eqs. (2), (3), and (5) are

$$C_r = C_{r0} = D/V_r, \quad C_s = C_i = 0 \quad (6)$$

The differential equations can be solved numerically by the RKG method. However, the three-compartment model can be simplified to a two-compartment model under a quasi-steady state condition. The dependency of KA on CL under the conditions of a CL_{int} of 20 mL/min and Q_a of 200 mL/min is shown in Fig. 1. CL increases rapidly with KA and eventually reaches a maximum value that corresponds to the perfusate flow rate. Therefore, if there is no transport barrier across the membranes, the BAL is assumed to be under a steady state (or quasi-steady state) condition, and Eqs. (2), (3), and (5) can be reduced to Eqs. (7) and (8).

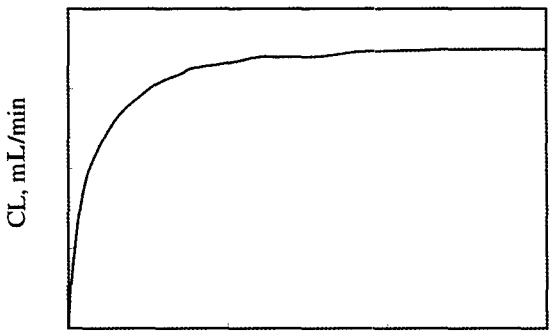

Fig. 1. Effect of mass transfer of drug through membranes in BAL cartridge on clearance [CL_{int} of 20 mL/min and Q_a of 200 mL/min].

$$V_r \frac{dC_r}{dt} = Q_a(C_a - C_r) + R \quad (7)$$

$$V_a \frac{dC_a}{dt} = Q_a(C_r - C_a) - C_r CL \quad (8)$$

$[C_a = C_s = C_i,\ CL_{int}C_a = CLC_r]$

where V_a is composed of the shell (V_s) and inner (V_i) spaces of hollow fibers. With the initial conditions ($C_r(t=0)=D/V_r$, $C_a(t=0)=0$), Eqs. (7) and (8) can be solved analytically as follows:

$$C_r(t) = \frac{Q_a R}{V_r V_a \alpha\beta} + A\text{Exp}[-\alpha t] + B\text{Exp}[-\beta t] \quad (9)$$

where A and B are constants, and α (distribution term) is larger than β (elimination term). These equations are expressed as involving an instantaneous bolus loading to the reservoir combined with a constant-rate infusion. Thus, to evaluate the BAL functions, two test methods, an initial bolus loading and constant-rate infusion plus initial bolus loading, can be carried out to obtain physiologic data on a flow-limited drug.

Constant-rate Infusion without or with Initial Bolus Loading of Drug

Although the function of a BAL can be easily tested using an initial bolus loading method, the plateau level of a drug with a constant-rate infusion is required for clinical

use. Therefore, when a constant-rate infusion is carried out, the drug concentration, obtained from Eq. (9) without a bolus loading of the drug (D=0), can be written as

$$C_r(t) = \frac{R}{CL} - \frac{R(1/V_r - \beta/CL)}{\alpha - \beta}\mathrm{Exp}[-\alpha t] + \frac{R(1/V_r - \alpha/CL)}{\alpha - \beta}\mathrm{Exp}[-\beta t] \quad (10)$$

The toxicity of a drug depends on a constant drug level in the blood to maintain the desired clinical response. As such, when a drug is administered at a constant-rate over a long enough period, the plateau concentration of the drug is achieved and maintained when the rate of elimination matches the rate of infusion. The drug amount and perfusate concentration are then considered to have reached a steady state. Under a steady state condition (ss), Eq. (10) becomes as follows:

$$C_{r,ss} = \frac{R}{CL} \quad (11)$$

where CL can be determined from the constant-rate infusion and concentration in a steady state.

Relationship Between Clearances at Initial Bolus Loading and in Steady State

In the case of R=0, an initial bolus loading test, solving Eqs. (7) and (8) with CL_{int}, produces

$$C_r(t) = \frac{D/V_r(\alpha - Q_a/V_a - CL_{int}/V_a)}{\alpha - \beta}\mathrm{Exp}[-\alpha t] + \frac{D/V_r(Q_a/V_a + CL_{int}/V_a - \beta)}{\alpha - \beta}\mathrm{Exp}[-\beta t] \quad (12)$$

CL can then be determined by integrating the area under the concentration-time curve (AUC). After the addition of Eqs. (7) and (8) based on R=0, and the integration of the equation with time from zero to infinity, Eq. (13) is obtained:

$$CL = -\frac{\int d(V_r C_r + V_a C_a)}{\int C_r dt} \quad (13)$$

Substituting Eq. (12) into Eqs. (13) and (14) can be obtained for CL

$$CL = \frac{Q_a CL_{int}}{Q_a + CL_{int}} \quad (14)$$

Conversely, if the drug for a constant-rate infusion test (D=0) is infused at a constant rate (R), eventually a steady state condition will be achieved when the infusion rate is balanced by the drug loss across the BAL cartridge. At this time, the incoming ($C_{r,ss}$) and outgoing ($C_{a,ss}$) drug concentrations remain constant, and Eq. (15) can be obtained from Eqs. (7) and (8) with CL_{int}

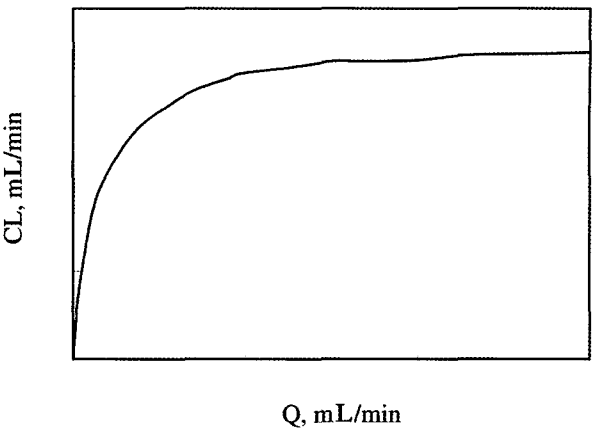

Fig. 2. Effect of perfusate flow rate on clearance in BAL [CL_{int} of 20 mL/min].

$$R = CL_{int}C_a = \frac{Q_a}{C_{1,ss} - C_{2,ss}} \quad (15)$$

The clearance value in a steady state, obtained by measurements across the BAL cartridge, is given by

$$CL_{ss} = \frac{Q_a(C_{r,ss} - C_{a,ss})}{C_{r,ss}} \quad (16)$$

Substituting Eq. (15) into Eq. (16), yields Eq. (17).

$$CL_{ss} = \frac{Q_a CL_{int}}{Q_a + CL_{int}} \quad (17)$$

which is identical to Eq. (14). Hence, if the model is appropriate, the mean clearance, obtained by dividing the dose by the area under the concentration-time curve, is equal to the clearance in a steady state.

Eq. (17) notes that CL increases with an increasing perfusate flow rate and approaches its maximum value, that is, intrinsic clearance (Fig. 2). The metabolic capacity of a BAL depends on the intrinsic metabolic capacity, perfusate flow rate, and drug binding to plasma proteins. The binding of a drug to plasma proteins and blood cells determines whether the drug will cross cellular-membranes or enter hepatocytes, as only an unbound drug can transverse across cellular membranes and be metabolized by hepatocytes. The extent of binding determines how much of a drug is available in a form that can be cleared. However, the CL value, which can be evaluated by the methods described above, only reflects the metabolic capacity of a BAL under certain experimental conditions. Thus, a parameter is needed that can represent the intrinsic metabolic capacity of a BAL cartridge for a quantitative comparison of the metabolic functions with those of a human liver or other BAL cartridges.

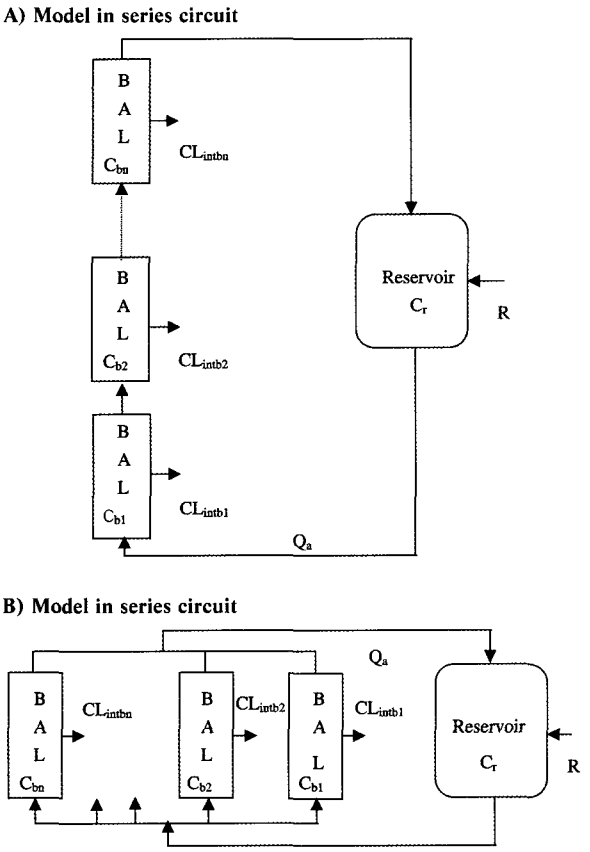

Fig. 3. Flow diagrams of BAL cartridges in series and parallel models.

In Vivo BAL

Some BALs include a BAL cartridge, plus a perfusion cartridge and/or charcoal cartridge. Although the function of a BAL is mainly determined by the BAL cartridge, it can also be modified by other appendices. To avoid encountering difficulties in the quantitative functional evaluation of a BAL, the BAL circuit should be as simple as possible. To display the behavior of ammonia in the body, the perfusion model used in the *in vitro* system can be applied to an *in vivo* model. Thus, to set up an *in vivo* BAL system, the plateau levels in the reservoir are calculated for both series and parallel circuit models as below:

Series Model

Fig. 3 shows flow diagrams of series and parallel circuit models of hepatic elimination for an *in vitro* perfusion model. When the number of BAL cartridges in a series circuit is n, the equations for the reservoir and BAL cartridges are:

$$\frac{V_r dC_r}{dt} = Q_a(C_{an} - C_r) + R \tag{18}$$

$$\frac{V_{a1} dC_{a1}}{dt} = Q_a(C_r - C_{a1}) - CL_{int\,a1} C_{a1} \tag{19}$$

$$\frac{V_{a2} dC_{a2}}{dt} = Q_a(C_{a1} - C_{a2}) - CL_{int\,a2} C_{a2} \tag{20}$$

$$\frac{V_{an} dC_{an}}{dt} = Q_a(C_{a(n-1)} - C_{an}) - CL_{int\,an} C_{an} \tag{21}$$

After a plateau value is achieved, and if the functions and volumes of each BAL cartridge are assumed to be the same, Eqs. (18) to (21) become

$$C_{rn}/R = \frac{(Q_a + CL_{int})^n}{Q_a(Q_a + CL_{int})^n - Q_a^{n+1}} \tag{22}$$

$$C_{a1}/R = \frac{Q_a C_{r1}}{Q_a + CL_{int}} \tag{23}$$

$$C_{a2}/R = \frac{Q_a^2 C_{r2}}{(Q_a + CL_{int})^2} \tag{24}$$

$$C_{an}/R = \frac{Q_a^n C_{rn}}{(Q_a + CL_{int})^n} \tag{25}$$

$[V_a = V_{a1} = V_{a2} = V_{an},\ CL_{int} = CL_{int\,a1} = CL_{int\,a2} = CL_{int\,an}]$

Parallel Model

In the case of BAL cartridges in a parallel model, under complete mixing conditions, where R is the constant-rate infusion in the reservoir, the mass balance in the reservoir and BAL cartridges can be expressed as,

$$\frac{V_r dC_r}{dt} = (1/n)Q_a(C_{a1} - C_r) + (1/n)Q_a(C_{a2} - C_r) + (1/n)Q_a(C_{an} - C_r) + R \tag{26}$$

$$\frac{V_{a1} dC_{a1}}{dt} + \frac{V_{a2} dC_{a2}}{dt} + \frac{V_{an} dC_{an}}{dt} = (1/n)Q_a(C_r - C_{a1}) + (1/n)Q_a(C_{an1} - C_{a2}) \; (1/n)Q_a(C_{a2} - C_{a3}) + (1/n)Q_a(C_{a(n-1)} - C_{an}) - CL_{int\,a1} C_{a1} - CL_{inab2} C_{a2} - CL_{int\,an} C_{an} \tag{27}$$

$[V_a = V_{a1} = V_{a2} = V_{an}, C_a = C_{a1} = C_{a2} = C_{an}, CL_{int} = CL_{int\,a1} = CL_{int\,a2} = CL_{int\,an}]$

In a steady state, Eqs. (26) and (27) become:

$$C_{a1}/R = C_{a2}/R = C_{an}/R = \frac{1}{nCL_{int}} \tag{28}$$

$$C_r/R = \frac{Q_a + nCL_{int}}{nQ_a CL_{int}} \tag{29}$$

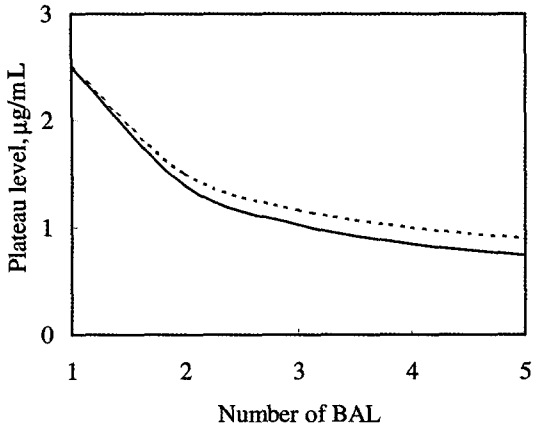

Fig. 4. Dependence of toxic plateau level on type of model with increasing number of BAL cartridges. [— ; series circuit, ---- ; parallel circuit], [R =100 μg/min, CL_{intb} =50 mL/min, Q_a = 200 mL/min].

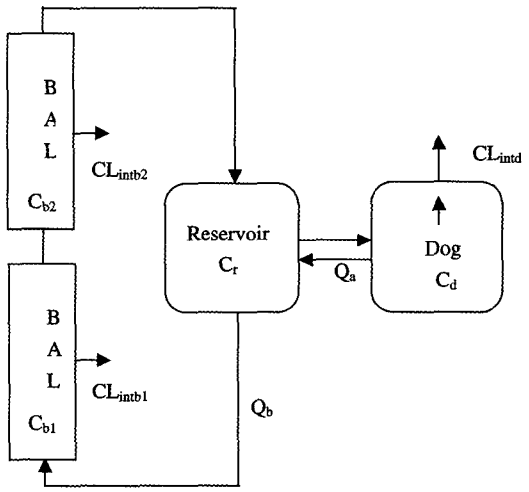

Fig. 5. Model of *in vivo* BAL assistance system with two BAL cartridges in series.

A comparison of the toxin plateau levels in the reservoir for the series and parallel circuit models is shown in Fig. 4. A model simulation was carried out for R =100 μg/min, Q_a =200 mL/min, and CL_{int} =50 mL/min. Although the plateau levels in both models decreased sharply and then slowly with an increasing number of BAL cartridges, the series model had a lower plateau level than the parallel model. The difference in the toxic plateau levels between the parallel and series models increased with an increasing number of BAL cartridges.

Thus, a two-BAL-cartridge series model can be set up as an *in vivo* perfusion model. Also, several research groups [13,14] have used plasmapheresis to raise the survival rate of a BAL cartridge before it is connected to a patient. For such a system, Fig. 5 shows a perfusion model between the body and two BAL cartridges in series for *in vivo* application with a reservoir. The changes in the toxic concentrations in the reservoir, body (C_b), and two BAL cartridges can be expressed as:

$$V_r \frac{dC_r}{dt} = Q_b(C_b - C_r) + Q_a(C_{a2} - C_r) \tag{30}$$

$$V_b \frac{dC_b}{dt} = Q_b(C_r - C_b) + P - CL_{int\,b} C_b \tag{31}$$

$$V_a \frac{dC_{a1}}{dt} = Q_a(C_r - C_{a1}) - CL_{int\,a} C_{a1} \tag{32}$$

$$V_a \frac{dC_{a2}}{dt} = Q_a(C_{a1} - C_{a2}) - CL_{int\,a} C_{a2} \tag{33}$$

where Q_b is the blood flow rate between the body (dog or patient) and the reservoir, P is the toxin production rate in the body, V_b is the volume of the body, and CL_{intb} is the intrinsic clearance of toxin in the body. Whole blood perfusion is a relatively creeping flow condition that must balance the fluid flow rate, the effect of shear force on the blood cellular components, the risk for coagulation, hemolysis, and thrombocytopenia. Therefore, the liver support system is used to perfuse with plasma. As such, the reservoir is usually inserted between the BAL cartridge and the patient to effectively separate the plasma by plasmapheresis. The charcoal hemoperfusion term was considered in the current analysis due to its insignificant effect on detoxifying metabolic and hemodynamic supports compared to a BAL [15]. Eqs. (30) to (33) can be solved numerically by the RKG method. In a steady state, from Eqs. (30) to (33), the accumulated toxin concentration in the body becomes

$$C_r / P = \frac{Q_b(Q_a + CL_{int\,a})}{(Q_b + Q_a)(Q_b + CL_{int\,b})(Q_a + CL_{int\,a}) - Q_b^{\,2}(Q_a + CL_{int\,a}) - Q_a^{\,3}(Q_b + CL_{int\,b})} \tag{34}$$

The important parameters characterizing the plateau level in the body are the blood flow rates (Q_b and Q_a) and metabolic rates (CL_{inta} and CL_{intb}).

A number of hollow fiber bioreactors have been proposed for the mass culture of mammalian cells [16] and a bioartificial pancreas [17]. The problem associated with these applications is limiting the mass transfer, especially the oxygen transfer. Axial and radial oxygen depletions are believed to be critical scale-limiting factors in the design of cell culture hollow fiber bioreactors. A limited oxygen supply to the hepatocytes in the hollow fibers can cause necrosis of the hepatocytes. Sardonini and DiBiasio studied the growth profiles of hybridoma cells in the extracapillary space of a single hollow fiber [18]. The medium was equilibrated with 20% oxygen and passed through the inside of single hollow fibers. Cells located within 370 μm from the surface of the hollow fibers remained viable and formed a continuous cell mass, whereas cells further away did not grow because of hypoxia, suggesting that a sufficient amount of oxygen was supplied to the hepatocytes located even in the central

part of the internal hollow fibers. Therefore, for an *in vivo* perfusion model, although a series model is better than a parallel one as regards lowering the plateau level in the body, the oxygen transfer across the membranes within the BAL cartridge should also be considered. A series model has a larger axial oxygen depletion than a parallel model. As such, the mass transfer of chemicals within a BAL cartridge, including flow rate-limited and enzyme-limited chemicals involving oxygen, is required to determine the optimal operation conditions of a BAL, which is currently under investigation.

CONCLUSION

A new perfusion model for BAL functions was developed to describe the absorption, distribution, and elimination of administered drugs. The model also provides various useful concepts for quantitatively evaluating the metabolic capacities of a BAL. In this study, equations were derived to quantitatively evaluate the metabolic functions of *in vitro* and *in vivo* BALs. For an *in vitro* BAL device, two methods, an initial bolus loading and constant-rate infusion plus initial bolus loading, were demonstrated to derive the same clearance characterized by an interrelationship between the perfusate flow-rate and intrinsic clearance. The interrelationship indicated that the *CL* increased with an increasing perfusate flow rate and approached its maximum value, *i.e.* intrinsic clearance. In addition, to set up an *in vivo* BAL system, the toxic plateau levels in the BAL system were calculated for both series and parallel circuit models. The series model had a lower plateau level than the parallel model.

NOMENCLATURE

A and B	Constant [μg/min]
C	Concentration of drug [μg/mL]
CL	Clearance [mL/min]
CL_{int}	Intrinsic clearance [mL/min]
D	Drug administration [mg]
f	Fraction of free drug in plasma [-]
K	Mass transfer coefficient [cm/min]
KA	Overall mass transfer coefficient [mL/min]
K_m	Michaelis-Menten constant [-]
n	Number of BAL cartridges
P	Production rate of toxin in body [μg/min]
Q_a	Perfusate flow rate between BAL cartridge and reservoir [mL/min]
Q_b	Perfusate flow rate between reservoir and body [mL/min]
R	Constant-rate infusion [μg/min]
V	Volume [mL]
V_{max}	Maximum elimination rate [mg/min]
α	Distribution constant [min^{-1}]
β	Constant of elimination rate [min^{-1}]

Subscripts

a	BAL cartridge
b	Body (dog or patient)
f	Free
i	Inner space of hollow fiber
r	Reservoir
s	Shell space of hollow fiber
ss	Steady state

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