Improved modeling of non-hepatic cellular uptake and degradation of low density lipoprotein

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ABSTRACT

An improved mathematical/kinetic model is proposed to describe receptor-mediated uptake and its degradation of LDL on human fibroblasts. The hierarchy of kinetic models is presented, which leads to the model introducing the parameter of degree of preferential insertion of recycled receptors to the surface of cell membrane. The results of its prediction were presented in various types of experiments and in various LDL concentrations. Its ability to predict Brown and Goldstein's ample experimental data was excellent.

INTRODUCTION

Robeneck and Hezs[1] suggested that insertion was restricted to regions plaques where new coated pits are going to form on the contrary to what Goldstein assumed[2]. In their experiments, receptor clusters on the cell surface were observed and were called plaque. Preferential insertion was modeled by Wolfsy et al.[3] considering that receptors were replaced uniformly within annular regions surrounding coated pits called plaques. It was concluded that insertion in plaques dramatically reduces the mean capture time determined by diffusion and uniform insertion for the LDL receptors. In this paper, the processes involved in LDL uptake and degradation are modeled under the hypothesis of non-random insertion of recycled receptor according to the results of the experiments conducted by Brown and Goldstein on human fibroblasts, as a model for the behaviour of human arterial endothelial cells. .

HIERARCHY

The Boston model is described as Eq. (1):

$$L + R^{p} \rightarrow L^{p} \rightarrow L^{i} + R^{p} \quad (1)$$

It is notable that this mechanism conserves the total number of receptors given by $R^{p}+L^{p}$ and requires them to essentially always be on the cell's surface.

In model (2) L^{a} and L^{p} are distinguished as well as R and R^{p} are. Analogous to $L^{a} \rightarrow L^{p}$, R can bind to a coated pit to give R^{p}. The model is described as:

$$
\begin{array}{l}
L+R^{p} \rightarrow L^{p} \rightarrow L^{i}+R \\
\quad\uparrow \\
L+R \rightarrow L^{a} \\
L^{i} \rightarrow L^{d} \\
R \quad R^{p}
\end{array}
\qquad (2)
$$

Mechanism (2) conserves the total number of receptors, i.e., $L^a+L^p+R+R^p$, and requires all receptors to essentially, like model (1), always remain on the cell's surface.

It may be assumed that the process $L^a \rightarrow L^p$ is much slower than $R \rightarrow R^p$, which was estimated and confirmed in the previous work.[4] In contrast to the ligand-bound receptors, the transformation of a non LDL-bound receptor R to one bound to a coated pit R^p cannot be slow since the steady distribution of LDL receptors in the absence of LDL shows 50 to 80 percent receptors bound to coated pits.[5]

According to the experimental evidence of preferential insertion of recycled receptors to the areas of plaques surrounding coated pits[1], mean capture time was calculated and the insertion in plaques was turned out to reduce dramatically the mean capture time determined by diffusion and uniform insertion for the LDL receptors.[3] Therefore model (2) is modified as below to incorporate the effect of preferential insertion in the model.

$$
\begin{array}{l}
L+R^{p} \rightarrow L^{p} \rightarrow L^{i}+\alpha R+(1-\alpha) R^{l} \\
\quad\uparrow \\
L+R \rightarrow L^{a} \\
L^{i} \rightarrow L^{d} \\
R \quad R^{p} \\
R^{p} \rightarrow (1-\alpha) R^{p}+\alpha R
\end{array}
\qquad (3)
$$

The parameter of α is introduced in the model to indicate the degree of preferential insertion contradictory to the experimental evidence of preferential insertion[1] of recycled receptors. The conserved quantity is identical to that of model (2).

RESULTS AND DISCUSSION

Figures 1a[6] and 1b[6] are prototypical experimental results (corrected for non-specific binding by subtracting the amount of non-specific binding in FH cells) for the three types of experiments discussed above. The figures also contain, for comparison, the model-generated curves based on the parameters in Table 1.

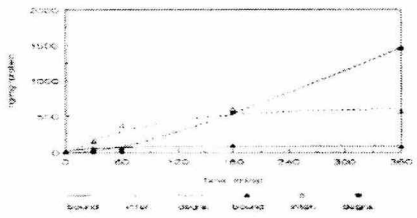

Fig. 1a. Unsteady state Kinetics (Model(2))

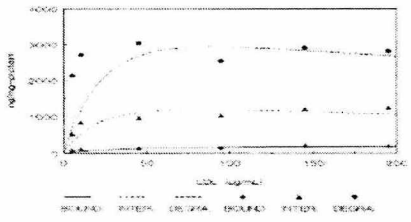

Fig. 1b. Steady State Kinetics (Model (2))

It is worth noting that although the types of experiments were quite different from one another, possibly resulting in slightly different actions of the regulatory processes ignored in the current treatment, the values determined for each of the parameters are generally in good agreement with one another.

Table 1: Best-Fit Parameter Values of model (2)

Process	Rate Constant	Pulse Unsteady	Step Unsteady	Step Steady
Binding of $L+R^{(I)}$	k_1	n/a	0.0038(ml/μg/min) (±0.0005)	0.0038(ml/μg/min) (given)
Internalization	k_2	0.2752/min (±0.0472)	0.27/min (min.)	0.28/min (given)
Degradation	k_3	0.01372/min (±0.0013)	0.009/min (min.)	0.01/min (given)
Migration of L^{II}	k_0	0.00743/min (±0.00398)	0.0129/min (±0.0011)	0.03/min (max.)
Migration of R	k_0'	0.28 1.12/min	1.6/min (max.)	1.574/min (±0.242)
	R^I (total) *	n/a	236.4μg/mg (±24.5)	207.5μg/mg (±9.572)
	R^{II} (t=0) *	n/a	max.(.8R^I)	max.(.8R^I)
	L^I (t=0) *	15.94ng/mg (±2.340)	n/a	n/a
	L^{II} (t=0) *	9.88ng/mg (±1.961)	n/a	n/a
	L^{II} (t=0) *	34.07ng/mg) (±2.16)	n/a	n/a

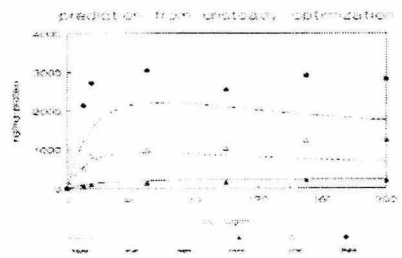

Fig. 2. Steady State Prediction (Model (2))

Table 2: Best-Fit Parameter Values of model (3)

Process	Rate Constant	Step Steady
Binding of $L+R^{(I)}$	k_1	0.0056(ml/μg/min)
Internalization	k_2	0.32/min
Degradation	k_3	0.01/min
Migration of L^I	k_0	0.03/min
Migration of R	k_0'	0.3/min
The degree of random insertion	α	0.41
	R^I (total)	200μg/mg
	R^{II}(t=0)/R(t=0)	160/40

Figure 2 shows the steady state-prediction of model (2) with the set of optimized parameters from unsteady state experimental data. Table 2 shows the set of rate constants based on model (3),

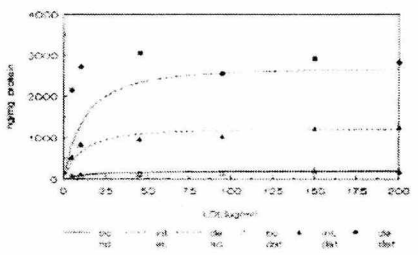

Fig. 3. Steady State Kinetics (Model (3))

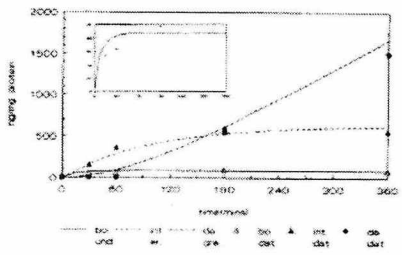

Fig. 4. Unsteady State Prediction (Model (3))

determined from steady-state experiments (Fig. 3) and its prediction for unsteady experiments is shown in Fig. 4.

CONCLUSIONS

Model (1) is too efficient mechanism to satisfy the experimental data with human fibroblasts unlike bovine smooth muscle cells. It is mainly due to the reinsertion of internalized receptor directly to coated pits. Considering random insertion of recycled receptors to membrane surface Model (2) was devised, in which receptors have to travel through membrane surface to reach coated pits. However actual phenomena supports preferential insertion of recycled receptors, which was the motivation of model (3). In fact model (3) lies between model (1) and model (2). When the value of α is zero model (3) becomes identical to model (1). In the meantime model (3) becomes identical to model (2) with the value of α of unity. It is noticeable that as in Fig. 3 and 4, model (3)-prediction of the set of parameters from one type of experiments to the other is excellent while model (2)-prediction was fair and it even showed the optimum LDL-concentration in the medium.

요 약

인간 fibroblasts의 receptor를 통한 LDL의 섭취와 분해에 대하여 보다 개선되어진 수학적/동역학적 모델을 제시하였다. 관련된 동역학적 모델의 hierarchy를 통하여 세포 멤브레인 표면으로 recycle되는 receptor의 선택적 insertion 정도를 나타내는 파라미터, α를 가지는 모델을 제안하였다. 여러 가지의 LDL 농도의 미디움과 여러 가지의 실험조건에서 모델예측을 수행하였는데, Brown과 Goldstein의 많은 실험데이터에 잘 일치하였다.

NOMENCLATURE

L	LDL concentration in the medium (μg/ml)
L^p	LDL-bound receptor bound to coated pit (ng/mg protein)
L^a	LDL-bound receptor not yet bound to coated pit (ng/mg protein)
L^i	Internalized LDL (ng/mg protein)
L^d	Degraded LDL (ng/mg protein)
R	Free receptor not yet bound to coated pit (ng/mg protein)
R^p	Free receptor bound to coated pit (ng/mg protein)
k_1	Binding rate constant of L to R(P) (ml/μg/min)
k_2	Internalization rate constant of coated pits (/min)
k_3	Degradation rate constant of internalized LDL (/min)
k_0	Migration rate constant of La to coated pits (/min)
k_0'	Migration rate constant of R to coated pits (/min)
α	The degree of random insertion

REFERENCES

1. H. Robeneck and A. Hezs(1983), *European J. Cell. Biol.*, **31**, 275
2. B. Goldstein, C. Wolfsy and H. Echavarria -Heras(1988), *Biophys. J.*, **53**, 405
3. C. Wolfsy, H. H. Echavarria and B. Goldstein (1985), *Cell Biophys.*, **7**, 197
4. K.-H. Lim(1997), *Korean J. Biotechnol. Bioeng.*, **12**(2), 236
5. M. S. Brown and J. L. Goldstein(1979), *Proc. Natl. Acad. Sci. USA.*, **76**, 3330.
6. M. S. Brown and J.L. Goldstein(1976), *Cell*, **9**, 663.