Distribution Model for the Intact Urokinase and Urokinases Modified by Soluble Macromolecules in Rat and Mouse Bodies

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The activity of intact urokinase (UK) and urokinases modified by soluble macromolecules (dextran and dextran sulfate sodium) in a mouse body was traced after injection of the 125I-labelled enzymes. The residual fraction of the enzyme in blood can be correlated with the time (t) as follows:

\[ X_t = Ae^{-at} + Be^{-bt} + Ce^{-ct} \]

Since \( a > b > c \), the residual fraction of the enzyme in blood chiefly depends on the magnitude of parameter C. The constant C for modified urokinase was larger than that for urokinase, showing the relative residual in blood was increased by modification of the enzyme. The apparent utilization in 50 min was 13.8% for intact UK, 28.1% for the dextran-UK and 25.2% for the sulfate dextran-UK. Therefore, the apparent utilization of UK in blood was approximately doubled by the modification. Since the half-lives of UK and modified UK in kidney and liver were not long, there were no unacceptable accumulation of the enzymes.

Key Words: urokinase-iodine-125, dextran-urokinase-iodine-125, dextran sulfate sodium-urokinase-iodine-125, urokinase model in blood

1. Introduction

Urokinase (UK) is a proteinase that has been widely used for the treatment of thrombosis. The circulatory half-life of UK in blood, however, is too short (less than 20 min in human blood), which limits the usefulness of UK. To increase the stability of UK in the blood, it was chemically modified with soluble macromolecules such as dextran and dextran sulfate sodium. The modified UK was more resistant to other proteinases and inhibitors in blood, and could be used more effectively for the treatment of thrombosis9.

In this work, a kinetic model for UK and modified UK in animal bodies was investigated and the modification effect on the medical treatment of thrombosis was theoretically estimated.

2. Materials and Methods

2.1 Materials

Urokinase was obtained from Dandong Victory Chemical Plant of China: specific activity, 52,000 IU/mg pr.; M.W., 54,700 daltons. The mice (weight, 18±1 g) were obtained from Bethune Medical University. The radioisotope, Na125I, was obtained from The Nuclear Institute of China's Academy of Science. The equipment used for the measurement of radioactivity was model FH-408.

2.2 Radiiodination of urokinase

The urokinase was radiiodinated by the method of Greenwood3. After preparation of...
UK-tagged-\(^{129}\text{I}\), the UK-\(^{129}\text{I}\) was divided into three parts: one of them was used in mice experiments, others were used for the chemical modification of UK.

2.3 Preparation of chemically modified UK
Modified UK was obtained by the dialehdex method of Fleming\(^3\). Dialehdex dextran and dextran sulfate sodium were prepared by NaI\(_2\) oxidation, and the UK-\(^{129}\text{I}\) was covalently bound to the dialehdex dextran and dextran sulfate sodium to yield the dextran-tagged-UK-\(^{129}\text{I}\) (D-UK-\(^{129}\text{I}\)) and dextran sulfate sodium-tagged-UK-\(^{129}\text{I}\) (S-UK-\(^{129}\text{I}\)).

2.4 Experiment in animal body
In order to reduce the amount of iodine adsorbed onto the thyroid gland, drinking water containing 0.05% of KI was given to the mice two days before the experiment. The mice were divided into three groups: the first was used in experiments of UK-\(^{129}\text{I}\), the second was used in experiments of D-UK-\(^{129}\text{I}\) and the third group was used in experiments of S-UK-\(^{129}\text{I}\). Each group was also divided into subgroups depending on the duration time of experiment, and five mice were used in each subgroup. The UK-\(^{129}\text{I}\) and modified UK-\(^{129}\text{I}\) were injected into mice through the tail vein in a dose of \(1.2 \times 10^8\) cpm/0.2 ml (about 2,000 units of urokinase). The mice were dissected at intervals of defined time and the blood (0.2 ml) and objective tissues were isolated. The objective tissues were liver, kidney, heart, lung and spleen. The radioactivity of each sample was assayed to determine the amount of enzyme contained in the tissues.

3. Theoretical Method
After the injection of intact or modified UK, the radioactivity in blood fell rapidly and 20–30% radioactivity of the initial dose was found in the livers and kidneys after 3 min. The distribution of radioactivity in the heart, lung and spleen was less than 1% of the administered radioactivity in our experiment. Within 24 h, the 63% and 1/4.1% of the administered radioactivity were recovered in the urine and feces\(^9\). From those phenomena, a three compartment model for the behaviour of UK-\(^{129}\text{I}\) and modified UK-\(^{129}\text{I}\) in animal bodies was proposed as follows:

![Injection of sample diagram]

For simplicity, it is assumed that radioactivity in other tissues, i.e., heart, lung and spleen, was negligible and the uptake rates of radioactivity follow first order kinetics. Then the reduction rates of radioactivity in blood, liver and kidney are written as follows:

\[
\frac{dC_b}{dt} = K_{-1} C_b + C_k - (K_1 + K_k) C_b \\
\frac{dC_l}{dt} = K_1 C_b - (K_1 + K_k) C_l \\
\frac{dC_k}{dt} = K_k C_b - (K_k + K_l) C_k
\]

where \(C_b\): concentration in blood
\(C_l\): concentration in liver
\(C_k\): concentration in kidney.

The equations are solved by the Laplace transform, then the residual radioactivity in blood is given as follows:

\[
C_b = C_b(0) \left[ \frac{e^{-at} - (K_1 + K_k + K_k + K_k) a + (K_1 + K_k) (K_b + L_a)}{(a-c)(a-b)} \right] + \frac{b - (K_1 + K_k + K_k + K_k) b + (K_1 + K_k) (K_b + L_a)}{(b-c)(b-a)} e^{-at} + \frac{c - (K_1 + K_k + K_k + K_k) c + (K_1 + K_k) (K_b + L_a)}{(c-a)(c-b)} e^{-at}
\]

The concentration in liver:

\[
C_l = C_b(0) K_1 \left[ \frac{K_1 + K_k - a}{(a-b)(a-c)} e^{-at} + \frac{K_1 + K_k - c}{(a-b)(a-b)} e^{-at} \right]
\]

The concentration in kidney:

\[
C_k = C_b(0) K_k \left[ \frac{K_1 + K_k - a}{(a-b)(a-c)} e^{-at} + \frac{K_1 + K_k - c}{(a-b)(a-b)} e^{-at} \right]
\]
where

\[ t: \text{time after injection} \]
\[ C_b(0): \text{initial concentration in blood} \]

The constants \( a, b \) and \( c \) are defined as

\[
\begin{align*}
    a+b+c &= K_1+K_2+K_3+K_4+K_5+K_6, \\
    ab+bc+ac &= (K_1+K_2)(K_3+K_4) \\
    &+ (K_2+K_3)(K_4+K_5) \\
    &+ (K_3+K_4)(K_5+K_6) \\
    &- K_1K_2-K_3K_4, \\
    abc &= (K_1+K_2)(K_1+K_3)(K_2+K_4) \\
    &- K_1K_2(K_1+K_3) - K_3K_4(K_1+K_2).
\end{align*}
\]

The percent concentrations \((X_b, X_1, X_k)\) of UK and modified UK which are proportional to the radioactivity concentrations \((C_b, C_1, C_k)\) can be written as follows, respectively:

\[
\begin{align*}
    X_b &= Ae^{-at} + Be^{-bt} + Ce^{-ct} \quad (10) \\
    X_1 &= A'e^{-at} + B'e^{-bt} + C'e^{-ct} \quad (11) \\
    X_k &= A''e^{-at} + B''e^{-bt} + C''e^{-ct} \quad (12)
\end{align*}
\]

When \( A, B \) and \( C \) are constant, these equations can predict the distribution of the UK concentration in blood, liver and kidney.

### 4. Results and Treatment of Data

It was experimentally found that most of the UK-\(^{125}\text{I}\) and modified UK-\(^{125}\text{I}\) were distributed in blood, liver and kidney after injection, while the distribution in heart, lung and spleen was less than 1% of the injection dose. The changes in distribution of the urokinases in blood, liver and kidney are shown in Tables 2, 3, and 4.

#### 4.1 Urokinases in blood

Based on the experiment of Woodard\(^{3}\), the behaviour of UK-\(^{125}\text{I}\) in the blood of rat are shown in Table 1. Using the experimental data, Eq. (10) was solved by the method of regression and the constants were obtained as

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>2000 units</th>
<th>200 units</th>
<th>20 units</th>
<th>Calculation data from Eq. (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% of inj. dose)</td>
<td>(% of inj. dose)</td>
<td>(% of inj. dose)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>58.9</td>
<td>57.6</td>
<td>62.3</td>
<td>67.3</td>
</tr>
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<td>53.6</td>
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</tr>
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<td>40.3</td>
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</tr>
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<td>22.1</td>
<td>20.0</td>
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</tr>
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<td>18.7</td>
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<td>16.5</td>
</tr>
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<td>14.8</td>
<td>12.5</td>
</tr>
<tr>
<td>240</td>
<td>5.4</td>
<td>7.2</td>
<td>12.8</td>
<td>7.4</td>
</tr>
</tbody>
</table>

The experimental data are from Ref. 4)

#### Table 1 Disappearance of \(^{125}\text{I}\)-tagged urokinase in blood of rats

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>UK-tagged-(^{125}\text{I})</th>
<th>D-UK-tagged-(^{125}\text{I})</th>
<th>S-UK-tagged-(^{125}\text{I})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental data</td>
<td>Calculation data from Eq. (14)</td>
<td>Calculation data from Eq. (15)</td>
</tr>
<tr>
<td>1</td>
<td>43.2</td>
<td>47.4</td>
<td>63.1</td>
</tr>
<tr>
<td>3</td>
<td>22.7</td>
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</tr>
<tr>
<td>50</td>
<td>10.3</td>
<td>10.6</td>
<td>17.0</td>
</tr>
<tr>
<td>180</td>
<td>8.4</td>
<td>7.0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Blood volume of mice was assumed six percent of the total body weight
The theoretical curve of Eq. (13) is shown in Fig. 1 together with points of experimental data. It is clear that the theoretical curve is in good accordance with the experimental data.

The experimental data of Table 2 are written in Eq. (10) by the method of regression as follows: The concentration (percent of injection dose) of UK-125I in the blood of mice is

\[ X_b = 69.3 e^{-0.193t} + 15.8 e^{-0.149t} + 14.9 e^{-0.00992t}. \]

(13)

The experimental curve of Eq. (13) is shown in Fig. 1 together with points of experimental data. It is clear that the theoretical curve is in good accordance with the experimental data.

The experimental data of Table 2 are written in Eq. (10) by the method of regression as follows: The concentration (percent of injection dose) of UK-125I in the blood of mice is

\[ X_b = 79.5 e^{-0.831t} + 8.0 e^{-0.204t} + 12.5 e^{-0.0031t}. \]

(14)

That of D-UK125I is

\[ X_b = 43.0 e^{-0.131t} + 29.0 e^{-0.119t} + 28.0 e^{-0.00996t}. \]

(15)

That of S-UK125I is

\[ X_b = 56.7 e^{-0.131t} + 16.3 e^{-0.243t} + 27.0 e^{-0.00994t}. \]

(16)

The theoretical curves given by Eqs. (14), (15) and (16) are shown in Fig. 2 together with experimental results. It is shown that the theoretical curves are in good accordance with the experimental data.

The apparent utilization (relative residual of

---

Table 3 Radioactivity in liver of mice (% of injected dose)

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>UK-tagged-125I Experimental data</th>
<th>Calculation data from Eq.(18)</th>
<th>D-UK-tagged-125I Experimental data</th>
<th>Calculation data from Eq.(19)</th>
<th>S-UK-tagged-125I Experimental data</th>
<th>Calculation data from Eq.(20)</th>
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<tr>
<td>1</td>
<td>—</td>
<td>15.5</td>
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<td>19.5</td>
</tr>
<tr>
<td>3</td>
<td>20.4</td>
<td>19.4</td>
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<td>26.0</td>
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<tr>
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<td>11.6</td>
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<tr>
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<td>3.0</td>
<td>5.2</td>
<td>5.2</td>
<td>5.2</td>
<td>5.2</td>
</tr>
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</table>

Table 4 Radioactivity in kidney of mice (% of injected dose)

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>UK-tagged-125I Experimental data</th>
<th>Calculation data from Eq.(21)</th>
<th>D-UK-tagged-125I Experimental data</th>
<th>Calculation data from Eq.(22)</th>
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<th>Calculation data from Eq.(23)</th>
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<td>0.58</td>
<td>0.58</td>
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</table>
4.2 Urokinases in liver

The experimental data in Table 3 are expressed by use of Eq. (11) as follows.

For UK-125I:

\[ X_t = -23.7 e^{-1.23t} + 17.5 e^{-0.0077t} + 6.2 e^{-0.00408t} \]  (18)

For D-UK-125I:

\[ X_t = -34.2 e^{-0.55t} + 20.2 e^{-0.0545t} + 14.0 e^{-0.00541t} \]  (19)

For S-UK-125I:

\[ X_t = -31.1 e^{-1.11t} + 16.1 e^{-0.0543t} + 15.0 e^{-0.00502t} \]  (20)

The theoretical data and curves are shown in Fig. 3.

4.3 Urokinases in kidney

The experimental data shown in Table 4 are expressed by using Eq. (12). The percent of initial dose for UK-125I is:

\[ X_t = -17.7 e^{-0.497t} + 12.0 e^{-0.0919t} + 5.7 e^{-0.00375t} \]  (21)

For D-UK-125I:

The theoretical curves are shown in Fig. 4.

5. Discussion

(1) The kinetic three-compartment model proposed can well explain the experimental data. Therefore, all of the Eqs. from (1) to (23) can be used to express the behaviours of UK-125I, D-UK-125I and S-UK-125I in mice or rats. This is a theoretical assessment of the stability or pharmaceutical effect of urokinase in the animal body. We did not find any other report related to the kinetic model for the fate of urokinase and modified urokinase in an animal body.

(2) The general equation expressing the behaviour of UK and modified UK in the blood of rats or mice is:

\[ X_t = A e^{-at} + B e^{-bt} + C e^{-ct} \]
where
\[ a > b > c, \quad A + B + C = X_b(0) = 100\% \]

(injection dose)

Because \( a > b > c \), when the time \( t \) is less than a certain limit (about 50 min), the time change of \( C \cdot \exp(-ct) \) is very small. Therefore, the amount of residual enzyme in blood depends on the constant \( C \). The bigger constant \( C \) is, the more residual enzyme in blood is found.

Making a comparison with each other using Eqs. (14), (15) and (16), the constants \( C \) of the two modified enzymes were nearly double as large as that of the intact enzyme, and the apparent utilization (relative remains in blood) of the modified enzyme in 50 min was higher than that of the intact enzyme. Therefore, the effect of modified enzymes for the treatment of thrombosis may be expected higher than that of the intact enzyme.

(3) Since \( a > b > c \), when the time \( t \) is long enough in Eqs. (18), (19) and (20):
\[
C' \exp(-ct) \\
C' \exp(-ct) \\
\]
Therefore, these equations can be written approximately as
\[
X_1 = C' \exp(-ct)
\]
The half-life of enzyme in liver is
\[
t_h = 0.693/c
\]
The half-life expressed the level of accumulation in liver. The longer circulatory half-life is, the more accumulation in liver is found. The half-life was 170 min for UK-\(^{38}\)I, 125 min for D-UK-\(^{131}\)I and 117 min for S-UK-\(^{125}\)I. All the half-lives were not so long, indicating there were no much accumulation of urokinases in the liver of mice.

(4) Similarly, the half-life of urokinases in kidney was 120 min for UK-\(^{38}\)I, 74.5 min for D-UK-\(^{131}\)I and 120 min for S-UK-\(^{125}\)I, showing that the urokinases did not much accumulate in the kidney of mice. The constant \( B'' \) for two modified enzymes is zero.

(5) The kinetic constants \( K_1, K_{-1}, K_3, K_{-3}, K_4 \) and \( K_t \) do not necessarily express the true process of the passage of urokinases through the biological membranes. This problem needs further investigation.

In summary, the remains of enzyme in blood are found to depend on the numerical value of constant \( C \). As the constant \( C \) for the modified enzyme is larger than that for the intact enzyme, the residual fraction of modified urokinases in the blood of mice is larger than that of the intact enzyme. Moreover, since the half-life of the enzymes in liver and kidney is not long, substantially there is no accumulation which is unacceptable. These features are favourable for the pharmaceutical use of modified enzymes in the medical treatment of thrombosis.

Nomenclature

\[ A, B, C \] constants in equations for blood
\[ A', B', C' \] constants in equations for liver
\[ A'', B'', C'' \] constants in equations for kidney
\[ a, b, c \] exponential constants
\[ C_b \] concentration in blood
\[ C_b(0) \] initial concentration in blood
\[ C_k \] concentration in kidney
\[ C_1 \] concentration in liver
\[ K_1, K_{-1}, K_3, K_{-3}, K_4, K_t \] rate constants of uptake for radioactivity
\[ t \] time (min)
\[ t_h \] circulatory half-life
\[ X_b \] percent of injected dose in blood
\[ X_b(0) \] initial percent of injected dose in blood (100%)
\[ X_k \] percent of injected dose in kidney
\[ X_1 \] percent of injected dose in liver

We acknowledge the help of Ma Xueyan, Li Xin, Kang Xixong and Cheng Binzu in some of animal experiments.

References

マウスにおけるウロキナーゼおよびデキストラン結合ウロキナーゼの動態

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*中国吉林大学分子生物学系

ウロキナーゼおよびデキストランあるいは硫酸デキストランに結合したウロキナーゼのマウスにおける動態をisotope$^{125}$Iの標識法で比較した。これらのウロキナーゼの血中動態はつぎのモデルで表現された。

$$X_0 = Ae^{-at} + Be^{-bt} + Ce^{-ct}$$

このモデルの中で、$a > b > c$であるので定数$C$はウロキナーゼの血中での滞留期の大きさを決定している。デキストランウロキナーゼは定数$C$が大きいので滞留期も長かった。

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