Brief Report

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# Evaluation of *in vivo* pharmacokinetic study of the anti-cancer drug imatinib using silkworms as an animal model

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**SUMMARY** Imatinib is an oral molecular targeted therapy that acts as a tyrosine kinase inhibitor. Silkworms present a promising experimental model for elucidating the pharmacokinetic and toxicity profiles of various compounds. This study aimed to establish an experimental paradigm for investigating the pharmacokinetics of imatinib in silkworms. A comparative analysis of imatinib pharmacokinetic parameters across silkworms, humans, mice, and rats revealed similarities in time to maximum concentration ($T_{max}$) and apparent clearance values between silkworms and humans. However, differences in elimination half-life ($t_{1/2}$) and apparent volume of distribution between silkworms and humans remained within 5- and 4-fold ranges, respectively. Importantly, mice demonstrated pharmacokinetic parameters closer to those of humans than rats during imatinib studies. Additionally, silkworms and mice exhibit similar $T_{max}$ and $t_{1/2}$ values. This study highlights the potential of silkworms as valuable tools for investigating imatinib metabolism in pharmacokinetic studies. Furthermore, it underscores the applicability of silkworms in elucidating the pharmacokinetic parameters of various molecular-targeted drugs, thus facilitating advancements in drug development and evaluation.

***Keywords*** Tyrosine kinase inhibitor, comparative analysis, *Bombyx mori*

## 1. Introduction

The tyrosine kinase inhibitor imatinib was the first oral molecular targeted drug developed to target a specific protein kinase and is currently approved as standard care for patients with BCR-ABL-positive chronic myeloid leukemia and gastrointestinal stromal tumors (*1*). Imatinib interacts with several metabolic enzymes that are major sites of drug–drug interactions (DDIs). It is primarily metabolized by cytochrome P450 (CYP) 3A4. Co-administration of imatinib with CYP3A4 and P-glycoprotein modulators alters the pharmacokinetic profile of imatinib (*2*). Intra- and inter-individual variabilities in drug exposure have been extensively documented (*3*). Thus, imatinib is a drug for which therapeutic drug monitoring is recommended due to its exposure–response and exposure–safety relationships (*4*). The feasibility of therapeutic drug monitoring-guided dosing to achieve a minimum blood plasma imatinib concentration of 750-1,500 ng/mL was demonstrated in a prospective randomized controlled trial (*5*).

The silkworm *Bombyx mori* is a valuable experimental animal for evaluating the pharmacokinetic and toxicity of compounds (*6*). Compared to mammals, silkworms offer several advantages, including lower breeding costs, suitability for rearing in smaller spaces, fewer ethical concerns, and easier quantification of injected sample solutions (*7*). Moreover, drug pharmacokinetic and toxicity in silkworms have been studied (*6,8*). Compound absorption from the silkworm intestinal tract is similar to that of mammals (*9,10*). The total clearance, volume of distribution, and half-life values of antimicrobial agents such as chloramphenicol, tetracycline, vancomycin, rifampicin, micafungin, and fluconazole are also comparable in silkworms and mammals (*11*). Therefore, silkworms are suitable experimental animals for evaluating the pharmacokinetic of imatinib. However, the pharmacokinetic of imatinib in silkworms has not yet been studied. Our study aimed to develop an experimental model for studying the pharmacokinetic of imatinib in silkworms.

## 2. Materials and Methods

### 2.1. Reagents

Imatinib (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was dissolved in methanol (Wako, Osaka, Japan),

and stored as a stock solution (10 mg/mL) at −60°C until use. For silkworm injections, imatinib was diluted with physiological saline (0.9% w/v NaCl). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol (Kanto Chemical Co., Inc., Tokyo, Japan) and $KH_2PO_4$ (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were utilized as HPLC mobile phases.

2.2. Silkworm rearing

Silkworm rearing followed established procedures (*12*). Silkworm eggs, acquired from Ehime-Sanshu Co., Ltd. (Ehime, Japan), were disinfected and incubated at 25-27°C. Silkworms were nourished with an artificial diet, Silkmate 2S, supplemented with antibiotics from Ehime-Sanshu Co., Ltd. Fifth-instar larvae were employed for infection experiments.

2.3. Pharmacokinetic study

To measure the pharmacokinetic parameters of imatinib in the silkworm model, hemolymph samples were collected at 0.1, 1, 2, 4, 8, and 12 h post- imatinib injection (10 mg/kg). Fifth instar silkworm larvae were fasted overnight on Silkmate 2S diet. Imatinib solution (50 µL, 10 mg/kg) was administered into the midgut using a 1 mL tuberculin syringe (Terumo Medical Corporation, Tokyo, Japan). Hemolymph collection followed a previously established method (*13*). Hemolymph was obtained by severing the first leg and centrifuging at 8,000 rpm for 3 min (MX-100; Tomy Seiko Co., Ltd., Tokyo, Japan). The supernatant (50 µL) was mixed with 450 µL of methanol and centrifuged at 15,000 rpm for 15 min. The resulting supernatant was subjected to HPLC analysis.

2.4. HPLC conditions for detecting imatinib

The HPLC system used for detecting imatinib in silkworm hemolymph comprised a pump (PU-4180, Jasco, Tokyo, Japan), UV detector (UV-4075, Jasco, Tokyo, Japan), and autosampler (AS-4550, Jasco, Tokyo, Japan). An octadecylsilyl column (Capcell Pack C18 MG II, 250 mm × 4.6 mm i.d., 5 µm; Osaka Soda, Tokyo, Japan) with a guard column (Capcell Pack C18 MG II guard column, 10 mm × 4.0 mm; Osaka Soda, Tokyo, Japan) served as the analytical column at 25°C (room temperature). Detection wavelength was set at 250 nm. The mobile phase consisted of acetonitrile and 0.5% $KH_2PO_4$ (pH 4.4; 32:68, v/v), with a flow rate of 1.0 mL/min. A 10 µL sample of silkworm hemolymph, prepared as previously described, was injected. Calibration concentrations for imatinib ranged from 0.25 to 12.5 µg/mL. The retention time for imatinib was 6.0 min. A linear six-point standard calibration curve was established over the concentration range of 0.25-12.5 µg/mL.

2.5. Pharmacokinetic analysis

HPLC was used to measure imatinib concentration in silkworm hemolymph ($n$ = 3 silkworms). Non-compartmental pharmacokinetic analysis of imatinib was conducted using Phoenix WinNonlin 8.3 (Certara, Princeton, NJ, USA).

**3. Results and Discussion**

The time course of imatinib concentration in silkworm hemolymph following injection of 10 mg/kg imatinib into the midgut is illustrated in Figure 1. The maximum concentration and time to maximum concentration ($T_{max}$) were 6.5 ± 0.8 µg/mL and one hour, respectively. The elimination half-life ($t_{1/2}$) was 2.9 hours. The apparent volume of distribution (Vz/$F$) and apparent clearance (CLz/$F$) were calculated as 1,315 mL/kg and 319 mL/h/kg, respectively.

Table 1 presents a comparison of imatinib pharmacokinetic parameters in silkworms, humans, mice, and rats (*14-17*). The $T_{max}$ and CLz/$F$ values of imatinib showed similarities between silkworms and humans. The differences in $t_{1/2}$ and Vz/$F$ between silkworms and humans were within 5-fold and 4-fold ranges, respectively. Notably, the Vz/$F$ and CLz/$F$ ratios were lowest in rat models. In mice and humans, the Vz/$F$ was within a 2-fold range, while the CLz/$F$ was approximately 10-fold greater in mice than in humans. Interestingly, the imatinib pharmacokinetic parameters of mice exhibited a closer resemblance to those of humans than those of rats. Additionally, both $T_{max}$ and $t_{1/2}$ were comparable between silkworms and mice. Thus, our findings suggest that silkworms hold promise for pharmacokinetic studies aimed at evaluating imatinib metabolism. The results of this study imply the potential for clarifying pharmacokinetic parameters of other molecular targeted drugs using silkworms.

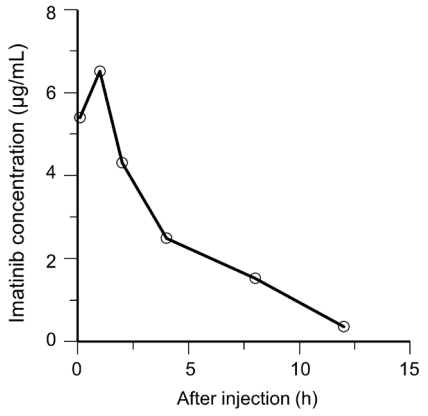


**Figure 1. Time course of imatinib concentration changes in silkworm hemolymph.** Imatinib injected into the silkworm midgut, followed by hemolymph harvesting at 0.1, 1, 2, 4, 8, and 12 h post-injection. $n$ = 3/group.

**Table 1. Imatinib pharmacokinetic parameters in silkworm and mammals**

| | Silkworm | Human (*14*) | Mice (*15*) | Rat (*16,17*) |
|---|---|---|---|---|
| $T_{max}$ (h) | 1 | 1.3 | 0.66 | 2.4, 4.8 |
| $t_{1/2}$ (h) | 2.9 | 13.5 | 2.4 | 3.9, 6.2 |
| V*z/F* (mL/kg) | 1,315 | 4,900 | 8,100 | 0.0023, 0.0061 |
| CL*z/F* (mL/h/kg) | 319 | 251 | 2,310 | 0.0003, 0.0006 |

$T_{max}$, time to maximum concentration; $t_{1/2}$, elimination half-time; V*z/F,* apparent volume of distribution; CL*z/F,* apparent clearance.

The $t_{1/2}$ of imatinib in silkworms was shorter compared to that in humans. Imatinib primarily undergoes metabolism *via* CYP3A4. In silkworms, a total of 79 genes encoding cytochrome P450 have been identified using whole-genome sequencing (*11*). Furthermore, the administration of carbon tetrachloride ($CCl_4$), a substrate activated by human CYP3A4, to silkworms leads to cytotoxicity. Interestingly, pre-administration of cimetidine, a CYP3A4 inhibitor, significantly attenuates $CCl_4$-induced cytotoxicity (*18*). These findings suggest the presence of a metabolic mechanism akin to CYP3A4 in humans within silkworms. Hamamoto *et al*. reported that microsomal fractions from the silkworm midgut exhibit metabolic capacities comparable to those of mammals, with a majority of cytochrome P450 enzymes being present in the silkworm midgut (*11*). Consequently, the metabolism of imatinib in silkworms is presumed to be expedited relative to humans, primarily due to the first-pass effect occurring in the silkworm midgut. It is hypothesized that drugs metabolized by CYP3A4 may undergo faster metabolism in silkworms compared to humans; however, future research aims to evaluate the pharmacokinetic parameters of CYP3A substrate drugs, given that a substantial proportion of drugs fall under this category.

The bioavailability of imatinib in humans exceeds 98% (*2*). To examine imatinib absorption in silkworms, the $AUC_{(0–12)}$ of imatinib administered into the hemolymph and midgut was evaluated. The $AUC_{(0–12)}$ of imatinib was 30.95 μg/hr/L when administered into the midgut and 27.44 μg/hr/L when administered into the hemolymph. Thus, in silkworms, as in humans, imatinib was well absorbed and the bioavailability of imatinib was 113%. The absorption of the compound from the intestinal tract of silkworms was similar to that in mammals.

We previously assessed the pharmacokinetic of voriconazole in a silkworm model infected with *Candida*, revealing alterations akin to those observed in human infections (*19*). In this study, we discovered that silkworm pharmacokinetic parameters more closely resemble those of humans compared to other experimental animals, marking a novel observation.

Imatinib is a substrate of various biological pathways, including CYP3A, organic cation transporter 1, organic anion-transporting polypeptide (OATP) 1A2, OATP1B3, breast cancer resistance protein, and P-glycoprotein (*20*). Given that patients on imatinib often use multiple concomitant medications, there is a heightened susceptibility to DDIs. Furthermore, patients with cancer frequently turn to herbal products to ameliorate treatment side effects and enhance quality of life. However, the cumulative impact of these co-administrations on the pharmacokinetic of imatinib remains inadequately explored. Therefore, future investigations are warranted to assess the pharmacokinetic profile of imatinib in the context of DDIs, shedding light on potential interactions with commonly co-prescribed medications in clinical practice. In conducting those studies, we found it useful to examine the use of silkworms as experimental animals.

In conclusion, our study demonstrates the utility of silkworms as an alternative animal model for investigating the single-dose pharmacokinetics of imatinib during the clearance phase.

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