# Pharmacokinetics, Safety, and Tolerability of Amygdalin and Paeoniflorin After Single and Multiple Intravenous Infusions of Huoxue-Tongluo Lyophilized Powder for Injection in Healthy Chinese Volunteers

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## ABSTRACT

**Purpose:** Huoxue-Tongluo lyophilized powder for injection (HTLPI), a traditional Chinese medicine preparation, is a compound of Persicae semen and Paeoniae Radix Rubra that is used mainly for treating blood-stasis obstruction syndrome in the acute stage of cerebral ischemic stroke. Amygdalin (AD) and paeoniflorin (PF) are 2 typical bioactive components in HTLPI and were selected as indicators for this pharmacokinetic study of HTLPI. The objective of this study was to investigate the safety profile, tolerability, and pharmacokinetic properties of AD and PF after single and multiple intravenous infusions of HTLPI in healthy Chinese volunteers.

**Methods:** Twenty-one healthy Chinese subjects were recruited for this open-label, single ascending-dose (3, 6, and 9 g) and multiple-dose (6 g, once daily) study. Safety profile was assessed by adverse events and physical examination throughout the study. Serial plasma and urine samples were analyzed by HPLC-MS/MS. Pharmacokinetic parameters of AD and PF were calculated using noncompartmental analysis.

**Findings:** In the single-dose phase of the study, the mean maximum plasma concentration and the mean area under the plasma concentration−time curve of AD and PF increased proportionally with each dose escalation. In the multiple-dose phase, the steady state was achieved by day 4 after multiple-dose administration of 6 g HTLPI. Mean pharmacokinetic parameters achieved on day 1 were similar to those on day 7. No significant accumulation was observed after repeat doses of 6 g HTLPI. Approximately 79.6% of the administered AD and 48.4% of the administered PF were excreted unchanged in urine within 24 hours. No serious adverse events were observed during the entire study.

**Implications:** The pharmacokinetic properties of AD and PF were linear after a single intravenous infusion of HTLPI in the dose range of 3−9 g. No systemic accumulation was observed with repeat doses of HTLPI. Sex had no significant effect on the pharmacokinetic properties of AD and PF. Intravenous infusion of HTLPI was well tolerated in healthy Chinese subjects. (*Clin Ther.* 2016;38:327–337)


**Key words:** amygdalin, clinical trial, LC-MS/MS, paeoniflorin, pharmacokinetic properties.

## INTRODUCTION

Chinese medicine injections, as an innovative dosage form, combine modern pharmaceutical technology with traditional Chinese medicine and represent a big step forward for both the Chinese pharmaceutical industry and the modernization of traditional Chinese medicine. Huoxue-Tongluo lyophilized powder for injection (HTLPI) is a simplified Chinese medicine preparation derived from the well-known traditional Chinese medicine prescription Tao-Hong-Si-Wu-Tang.

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It is composed primarily of the extracts of Persicae semen and Paeoniae Radix Rubra. The principal indications of HTLPI were used to treat blood-stasis obstruction syndrome in the acute stage of cerebral ischemic stroke.

Persicae semen, the dried mature seed of the rose *Prunus persica* (L.) Batsch or *Prunus davidiana* (Carr.) Franch, acts as the "Emperor" herb in HTLPI, according to traditional Chinese medicine formulation theory. Amygdalin (AD) (Figure 1A), a phytochemical marker for the quality control of Persicae semen in Chinese Pharmacopoeia,[1] is considered the major physiologically active constituent in Persicae semen.[2] It has been reported that AD could promote blood circulation to dissipate blood stasis by prolonging thromboplastin time, decreasing plasma viscosity and fibrinogen content, reducing platelet aggregation, and protecting vascular endothelial cells.[3]

Paeoniae Radix Rubra is the dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch. It plays an auxiliary role and acts as the "Minister" herb in HTLPI, according to traditional Chinese medicine formulation theory. The primary bioactive constituent in Paeoniae Radix Rubra is paeoniflorin (PF) (Figure 1B).[4-6] PF is a phytochemical marker for the quality control of Paeoniae Radix Rubra in Chinese Pharmacopoeia[1] and has been reported to produce a delayed protective effect on cerebral ischemia injury in rats via inhibition of the peripheral and cerebral inflammatory response.[7,8]

In recent years, many pharmacokinetic studies of AD[9,10] or PF[11-15] have been performed in animals. However, no clinical pharmacokinetic studies of AD or PF have been conducted in humans. This is the first study in which AD and PF were administered to humans by intravenous infusion of HTLPI. The primary objective of the present study was to evaluate the pharmacokinetic profiles of AD and PF after single and multiple intravenous infusions of HTLPI in healthy Chinese volunteers, as well as to assess the safety profile and tolerability of HTLPI.

## METHODS

### Chemicals and Reagents

HTLPI (3 g/vial containing 13.2 mg AD and 18.3 mg PF, 6 g/vial containing 25.3 mg AD and 35.8 mg PF) were supplied by Jiangsu Kanion Pharmaceutical Co., Ltd (Lianyungang, China). AD (purity 93.6%), PF (purity 96.5%), and geniposide (IS; purity 99.7%) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Ammonium acetate and formic acid (analytical grade) were purchased from Nanjing Chemical Reagents Co., Ltd. (Nanjing, China). Methanol and acetonitrile were HPLC grade and purchased from Merck KGaA (Darmstadt, Germany). Water was prepared with double distillation.

### Subjects

Healthy Chinese male and female volunteers aged 18–45 years with a body mass index of 19–24 kg/m$^2$ were eligible for the study. All subjects were free of significant cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal, and hematologic disease, as determined by medical history, physical examination, and routine laboratory tests (ie, hematology, blood biochemistry, urinalysis, and liver and kidney functions). Subjects were instructed to abstain from using any drug for 2 weeks before and during the study

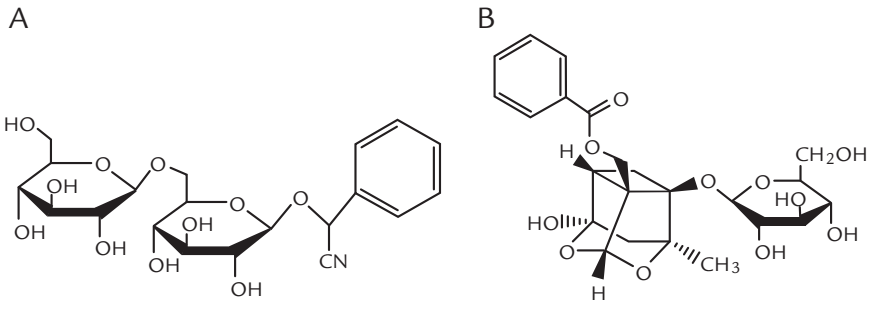


Figure 1. Chemical structures of (A) amygdalin and (B) paeoniflorin.