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Wilforine, the Q-marker and PK-maker of Tripterygium glycosides tablet: Based on preparation quantitative analysis and PK-PD study

Xue Gao\textsuperscript{a}, Xi Du\textsuperscript{a,b}, Lijun An\textsuperscript{a}, Yangyang Wang\textsuperscript{a}, Lili Wang\textsuperscript{a}, Zengguang Wu\textsuperscript{a}, Cong Huang\textsuperscript{a}, Xin He\textsuperscript{a,c,*}

\textsuperscript{a}School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Nankai District, Tianjin, P.R. China, 300193;
\textsuperscript{b}Second Affiliated Hospital of Tianjin University of TCM, Tianjin, China, 300150;
\textsuperscript{c}Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin, China, 300193.

\textsuperscript{*}Corresponding author:

Xin He, PhD

School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine
Nankai District, Tianjin, P.R. China, 300193;
Tel: +86-22-59591635; Fax: +86-22-59596153;
E-mail address: hexintn@163.com
ABSTRACT

Background: The quality standard of Tripterygium glycosides tablet (TGT) by CFDA can not fully reflect the effectiveness and safety. While, Q-marker was proposed to solve the problem of traditional Chinese medicine. PK-marker is mainly used to reflect the material exposure and the influencing factors of Chinese medicine after administration.

Purpose: Based on the study of quantitative analysis, cytotoxicity and pharmacokinetics, this study screened out and confirmed whether wilforine could be served as a potential Q-marker and PK-marker of TGT.

Methods: A sensitive and selective UPLC-MS/MS method was developed and applied to quantitative research of TGT preparation and pharmacokinetics study of TGT. Then, HepG2 cells assay was used to evaluate the cytotoxicity induced by alkaloids in TGT. Then, a PK-PD research was carried out in adjuvant arthritis (AA) rats and control rats after oral administration of TGT, with different dosage and timing. The pharmacokinetic characteristics were determined and calculated by DAS1.0. The pharmacodynamics of TGT was evaluated by the change of paw swelling through one-way ANOVA analysis.
**Results:** The quality of four alkaloids showed significant difference among four manufacturers, and they were abundant component in TGT from three manufacturers of all. HepG2 cells test revealed that wilforine and wilforgine could induce the cytotoxicity obviously. Pharmacodynamics index suggested that TGT had therapeutic effect on adjuvant arthritis. Thus, the four cases of death occurred in the high dose AA rat group had proven the significant toxicity caused by continuous high dose TGT administration. Furthermore, the result of pharmacokinetic study proved that C\text{max}, and AUC\text{(0-inf)} of wilforine have dose-dependent and time-dependent characteristics. But for wilforgine, there was no indication that there was an accumulation phenomenon in-vivo and its plasma concentration showed low exposure. Therefore, it could hardly become the PK-marker of TGT.

**Conclusions:** Wilforine is proposed as a biologically active and toxic component of TGT that can be served both as Q-marker and PK-marker. The quality, clinical safety, and efficacy of TGT should be evaluated by the quality of wilforine.

**Keywords:** Wilforine, Tripterygium glycosides, Alkaloid, Q-marker, PK-marker
Abbreviations: TGT, Tripterygium glycosides tablet; TWHF, Triptergium wilfordii Hook. f.; WFR, wilforine; WFG, wilforgine; WFD, wilfordine; WFT, wilfortrine; TCM, traditional Chinese medicines; AA, adjuvant arthritis.
1. Introduction

*Triptergium wilfordii* Hook. f. (TWHF) has various kinds of biological activities, such as anti-inflammation, anti-fertility, anti-tumor (Ma et al., 2007; Zhao et al., 2016; Reno et al., 2015; Wong et al., 2008). Tripterygium glycosides tablet, a preparation with many active components, is widely used to treat autoimmune diseases such as rheumatoid arthritis, nephrotic syndrome (Li et al., 2015; Xu et al., 2009). Previous studies indicated that TWHF can induce hepatotoxicity (Zhang et al., 2012), nephrotoxicity (Li et al., 2015), and reproductive toxicity (Su et al., 2014; Qu et al., 2015). In principle, the pharmacological and toxic effects of TWHF are dose-dependent. Reduce the toxicity and retain effectiveness are needed, which was required by Chinese medicine prescriptions and multi-criteria quality evaluation system.

Till now, there are more than 100 chemical compositions were separated, including alkaloids, diterpenoids, triterpenes (Qu et al., 2015). Triptolide is the most active and representative compound of diterpenoids, and it is the main toxic component, which could cause many adverse reactions (Lu et al., 2017; Xue et al., 2011; Zhou et al., 2014). The quality standard of TGT is focus on triptolide and wilforlide A (The regulation WS3-B-3350-98-2011 was set by CFDA in which the quality of triptolide should less than 10 μg and wilforlide A more than 10 μg per tablet). Nevertheless, the components of *Tripterygium Wilfordii* and its preparations are so complicated, and the quality of only two chemical components does not fully reflect the quality of *Tripterygium Wilfordii* and its preparations. It has been reported that there were enormous
differences between TGT from different pharmaceutical manufactures both in the quality and compositions of components (Qu et al., 2015; Su et al., 2015). Clinical observation found that the adverse reactions were different when patients taking TGT produced by different manufacturers. Therefore, it is necessary to establish multiple indexes quality control method. In this case, more constituents that concerned with effect and toxicity in TWHF and its preparations should be brought to the attention and monitored.

As is well known, the quality of traditional Chinese medicines (TCM) is closely related to the active ingredients’ qualities, which is important for the quality evaluation and quality control. Q-marker, a new model of quality research, could better characterize this traditional Chinese medicine (Zhang et al., 2016a; Liu et al., 2016). Component, efficacy and toxicity are the primary means of Q-marker. The relationship between “property-response-component” was explored, and the method was established about discovering bioactive constituents, studying integrative mechanism and formulating Q-marker of TCM (Zhang et al., 2016b). In another study, a research on reasonable identification and scientific control of Q-marker was defined based on efficacy-toxicity correlation (Sun et al., 2016). The quality markers of *Leonurus japonicus* and *Penthorum chinense* were discriminated from five aspects: effective material basis, specificity of the isolated components, chemical structure and bioactivity, measurability, and fingerprint spectrum (Xiong et al., 2016).
The PK markers, active compounds presented in TCM complex preparation or their metabolite, could be determined in biological sample (such as blood, urine) and should be related with pharmacological activities in therapeutics (Xiao et al., 2008; Liu et al., 2016). PK-marker could to reflect the material exposure, the influencing factors and he metabolism of the medicine in vivo after administration. The methods of research on PK-marker mainly include in vivo PK properties, in vitro and in silico assessments of permeability and solubility (Lu et al., 2008). Plasma and urinary tanshinol were considered to be PK markers for cardiotonic pills with these methods. The pharmacokinetics of PK-markers in Huanglianjiedu Decoction to cerebral ischemia reperfusion model mice was determined by HPLC method. The results showed that the variation of concentrations of PK-markers in plasma can reflect the variation of concentrations in brain tissue (Xiao et al., 2008).

With a brief summary, the definition and properties of Q-marker and PK-marker were suggested from these aspects: Q-markers: (1) they are the intrinsic chemical components in TCM materials and products, or processing/preparation-resultant; (2) they are functional properties-associated, with definite chemical structures; (3) they can be qualitatively characterized and quantitatively determined; (4) for the formulae, the representative substances of the Monarch are firstly considered, and those from the Minister, Assistant, and Guide, should be considered as well, following the compatibility theory (Yang et al., 2017). While for the PK-markers: (1) they must be associated with efficacy; (2) they exist in biological sample and can be
determined by analytic method; (3) they should reflect the relationship between concentration and time (Li et al., 2015). Even though Q-marker take less consider of the metabolites and process on bioactive mechanisms in-vivo, focus more on intrinsic chemical components in TCM, but if the innate components are provided with both characteristics of Q-marker and PK-marker, they must be the very objects for the quality control standard from raw materials to in-vivo bioactivities of TCM continuously, and should be hunted and brought to the forefront.

Q-marker and PK-marker of TGT should be the main quality of the active component in the preparations. It has been reported that there were enormous differences between TGT from different pharmaceutical manufactures both in the quality and compositions of components (Qu et al., 2015). Clinical observation found that the adverse reactions were different when patients taking TGT produced by different manufacturers. In this case, more constituents that concerned with effect and toxicity in TWHF and its preparations should be brought to the attention and monitored.

It had reported that total alkaloids of TWHF could inhibit the symptom of collagen-induced arthritis rats significantly by inhibiting the production and the expression of IL-6, IL-8 (Zhang et al., 2013). While, alkaloids are the main components in TGT preparations. In another hand, according to literature reports liver injury was induced by TGT. The toxicity of Hunan Xieli (HNXL) TGT on the liver and kidney was more serious than that of Huangshi Feiyun (HSFY) TGT, the alkaloids in HSFY TGT was 38.2%. In contrast, in
HNXL TGT, the alkaloids take up the most contents with 44.0% (Qu et al., 2015; Zhang et al., 2012; Zhao et al., 2015). Therefore, alkaloids in TGT may be the effective and toxic components.

In this current study, a UPLC-MS/MS approach was applied for the quantitative analysis of four alkaloids (wilforine, wilforgine, wilfirtrine, wilfordine, the structures have been showed in Fig 1.) in TGT produced by four pharmaceutical manufacturers with different batches, and it showed a significant quality difference among different manufacturers. Then, the cytotoxicity research had been carried out that two alkaloids were the toxic ingredients, which might induce liver injury. Furthermore, the pharmacokinetics of them in TGT with adjuvant arthritis (AA) rats and normal rats had been evaluated with different dosage and timing of administration, the concentration-time curves and pharmacodynamics indexes suggested that WFR is a PK-maker. Overall, the efficaciy and toxicity of WFR were systematically evaluated in-vitro and in-vivo, and the relationship of “quality-efficacy-toxicity” was established too, indicated WFR was a potential Q-marker and PK-maker of TGT.

2. Experimental

2.1. Reagents and materials

Wilfortrine (NO. 151128), wilforine (NO. 151218), wilforgine (NO. 150922), and wilfordine (NO. 160127) reference substances (purity >98%) were all purchased from Sichuan Weikeqi Biological Technology Co., Ltd., tripterygium glycosides tablet samples were purchased from local pharmaceutical
stores (Tianjin, China), which were produced by four manufactures (De-en De (DND); Huangshi Feiyun (HSFY); Shanghai Fudan Fuhua (FDFH); Hunan Xieli (HNXL)), Complete Freund adjuvant (CFA) were purchased from sigma (Lot#SLBP0432V). Irbesartan (HPLC purity 99.8%) obtained from National Institute for Food and Drug Control (Beijing, China) was used as an internal standard (IS); acetonitrile (Merck, Germany); water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA); methanol (Fisher, USA); formic acid (Tedia, USA); Discovery DV215CD Lac part analytical balance (USA, OHAUS); tape; Electronic Balance BT-600 (Shanghai yousheng company); YLS-7B Plethysmometer (Volume meter) (Ji'nan Yi Yan Technology Development Co., Ltd.)

2.2. Animals

Male Sprague–Dawley (SD) rats (200 ± 20 g) were purchased from Beijing Huafukang Company (Beijing, China, SCXK (Jing) 2014-0004). All the rats were conventional raised for a week and kept under specific pathogen-free conditions: 25 ± 5 °C, 60 ± 20% humidity, 12/12 h light/dark cycle, and free access to food. The rats were housed in the Animal Lab Center Building of Tianjin University of Traditional Chinese Medicine.
2.3. Chromatographic and Mass spectrometry condition

ACQUITY UPLC BEH C18 (2.1*100mm, 1.7 μm) was used as an analytical column. The flow rate was set at 0.3 mL/min. 10mM ammonium acetate (0.1% formic acid) and acetonitrile (0.1% formic acid) were set as mobile phases A and B, respectively. The column temperature was set at 25 °C; the Gradient elution conditions of UPLC were as follows: from 0-2 min, 80% phase A; 2.2-5.8 min, 45% phase A; 6-8.5 min, 15% phase A; 8.5-11 min, 80% phase A.

A Waters ACQUITY UPLC - tandem AB Triple Quad 5500 with electrospray ionization (ESI) source (Waters Co., US; AB Sciex Co., USA) was used for the analysis. The injection volume was 6μL. The mass spectrometric parameters were set as follows: CUR: 35.0 psi; CAD: 7 psi; Ion spraying voltage (IS): 5500 V; TEM: 450 °C; GS1: 50 psi; GS2: 50 psi. MRM Method parameter was set as Table 1.

2.4. Method validation

The specificity, linearity, precision, stability and repeatability were well validated. The methodological evaluation was according to the guidelines for the validation and verification formulated by FDA. All the samples were analyzed by the UPLC-MS/MS method (n = 6).

2.5. The preparation of TGT sample and standard solution

The TGT (5 tablets) were weighed, and then were ground into powder in a mortar, 0.1000g were accurately stated, 1mL methanol was added. The sample was extracted by ultrasonication at room
temperature for 30 min, and then centrifuged at 14000 rpm for 10 min. After passing through 0.22 μm filter, the samples were injected in the UPLC-MS/MS system.

The standards of WFR, WFG, WFT, WFD were weighed, and 1mL methanol was added for preparing standard solution, respectively. The standard of Irbesartan (IS) was weighed, methanol was added to 1 mg/ml. The solution was then diluted with methanol.

2.6. The study of cytotoxicity of HepG2

The human hepatoma HepG2 cells were obtained from the Chinese Academy of Medical Sciences and tumor cell library. The well-grown cells of the third passage (passage 11) were harvested and seeded into plates at a density of 5 × 10^4 cells/ml for experiment. WFR, WFG, WFT, WFD were diluted to 300, 100, 30 μM in DMEM with 1% FBS and 0.1% S-P. Diclofenac sodium as positive control was diluted to 300 μM, 100 μM, 30 μM, to evaluate the feasibility of the method. HepG2 cells were seeded into 96-well plates and cultivated for two days and then 100 μl WFR, WFG, WFT, WFD and diclofenac sodium were added and incubated for 20 h at 37 °C with 5% CO₂ conditions (n = 6).

CCK-8 was diluted to 10% in DMEM with 1% FBS and 0.1% S-P. And then 100 μl was added into 96-well plates and cultivated for 1.5 h. Relative viability was determined by PerkinElmer.
2.7. Preparation of TGT suspension

The TGT produced by HNXL were used to investigate the PK-PD research, which were widely used in clinical. 2, 6, 18 tablets were weighed, respectively. And then ground into powder in a mortar, and transferred to a conical flask, respectively. 0.5% CMC-Na was added to reach a volume of 20 ml. The suspension were extracted by ultrasonication at room temperature for 30 min, and stored at 4 °C.

2.8. Preparation of AA model and evaluation of pharmacodynamics

AA model was induced by subcutaneous injection with 0.1 ml complete Freund’s Adjuvant in right hind footpad. The equivalent dose was injected again after day 7. The degree of arthritis was evaluated by the change of paw swelling. Till day 19, paw swelling of model group showed statistical significance change compared with control group (with same procedure as modeling group but the injection was 0.1 ml saline instead of complete Freund’s Adjuvant), indicated AA rats successful modeling. After modeling, for administration of TGT, intervention groups were intragastric administration TGT suspension with 10 mg/kg, 30 mg/kg, 90 mg/kg continuously for 21 days in AA rats; the control group and model group were given 10 mg/kg saline respectively also for 21 days. The degree of paw swelling were measured at day 0, 7, 14 and 21 and make a comparation among control, model and intervention groups to illustrate pharmacodynamics of TGT.
2.9. Single and multiple dose of TGT pharmacokinetics study

The single dose pharmacokinetics study were performed after the rats acclimated for 1 week without modeled. 18 rats were randomly divided into 3 groups with 6 rats per group, and given TGT suspension once respectively with dose of 10 mg/kg·day$^{-1}$, 30 mg/kg·day$^{-1}$, 90 mg/kg·day$^{-1}$. The dosage of 10 mg·kg$^{-1}$·day$^{-1}$, which was in accordance with clinical therapeutic dose for human beings.

Multiple dose pharmacokinetics study were performed on AA rats. Because after the whole procedure of blood sample collection, rats might die, with consider of that, 36 AA rats were divided into 6 groups randomly and given TGT suspension continuous in 14 days and 21 days, respectively: AA-14 day groups (10 mg/kg·day$^{-1}$, 30 mg/kg·day$^{-1}$, 90 mg/kg·day$^{-1}$) and AA-21 day groups (10 mg/kg·day$^{-1}$, 30 mg/kg·day$^{-1}$, 90 mg/kg·day$^{-1}$).

Blood samples were collected from orbital venous sinus into 2 ml heparinized capillary EP tubes at 0 and 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h after the last administration. Plasma pharmacokinetics of WFR and WFG were determined by UPLC–MS/MS.

Plasma samples 50 μl was spiked with 5 μl 2 ng/ml IS solution in 2 ml EP tube. Then 300 μl ethyl acetate was added and vortex-mixed for 3 min. The tubes were centrifuged at 14000 rpm for 10 min. The supernatant was taken into a new EP tube. And then 200 μl ethyl acetate was added into the primary EP and
vortex-mixed for 3 min. 200 μl supernatant was taken after centrifuging at 14000 rpm for 10 min and all the 500 μl supernatant was dried with nitrogen. The residues were reconstituted in 50 μl 20% acetonitrile (0.1% formic acid), and 6 μl supernatant was injected into the column for the UPLC–MS/MS analysis.

2.10. Statistical analysis

The degree of paw swelling were calculated by GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA). The pharmacokinetic parameters, including V/F, T_{max}, C_{max}, AUC(0-t), T_{1/2α}, MRT(0-t), were calculated by the computer program ‘Drug and Statistics 1.0’ (DAS 1.0; Medical College of Wan nan, China). The one-way ANOVA analysis were used for statistical comparisons in SPSS 17.0 (SPSS Inc., Chicago, IL). P < 0.05 was considered as statistical significance.

3. Results

3.1. UPLC/MS-MS method validation for preparation quantitative analysis

There are good linearity in the scale of 0.5-100ng/ml concentration of these four alkaloids with all the "R" value more than 0.99. The relative standard deviations (RSDs) of WFG, WFR, WFD and WFT were in the range of 2.44–6.39% for intra-day precision, and 3.69–6.26% for inter-day precision, respectively, which indicated that the method was accurate and reproducible for the determination of 4 active ingredients. The stability (RSDs) of 4 alkaloids were in the range of 1.83-7.11%. The results of recovery was in the range of
1.79–5.85%. All the data of validation met the acceptance criteria. Specificity for 4 alkaloids in TGT (n = 6) has been showed in Fig 2. The results of method validation indicated that the method was feasible for the determination of 4 alkaloids.

3.2. Quantitative research of TGT from four different manufacturers

The result of quantitative research about 4 alkaloids has been showed in Table 2. TGT samples were purchased from local pharmaceutical stores, therefore, the numbers of batches were different. WFR was the abundant component in TGT preparations produced by 3 pharmaceutical manufacturers, except DND. And the quality of 4 alkaloids in TGT was obviously different between different manufacturers, respectively.

3.3. Cytotoxicity study of 4 alkaloids by HepG2 test

Compared with control group, the relative viability of HepG2 was significantly different with diclofenac sodium, which indicated that the method is feasible. The relative viability of HepG2 was markedly different (**P< 0.01) in WFR and WFG groups, which indicated that WFR and WFG might cause cytotoxicity. There was no significant difference in WFT and WFD groups. Therefore, we investigated the pharmacokinetic of WFR and WFG in TGT. The result has been showed in Fig 3.

3.4. Pharmacodynamics study of TGT in AA rats

The changes of paw swelling in the control group, model group and intervention groups were shown in Table 3, from the time of successful modeling as day 0. Compared with control group, paw swelling was
significantly increased after injected Freund's adjuvant (***P < 0.01). The result indicated that AA model established was successful and stable in this study.

Compared with model group, the paw swelling of intervention groups (ΔΔP < 0.05 and ΔΔΔP < 0.01) decreased significantly at day 7 and more in day 14 and day 21. The result suggest that TGT can relieve the symptoms of rheumatoid arthritis, and it may be related with the quality of alkaloids.

3.5. Single and multiple dose Pharmacokinetics study of WFR and WFG

The rats were divided randomly and administrated TGT suspension respectively as mentioned before. While, two cases of death happened in AA-14 day and AA-21 day groups, respectively. Therefore, the value of n is set as 4 in the results of pharmacokinetics study. Blood samples were collected at 0 and 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h after the last administration in PK control, AA-14 day and AA-21 day groups.

The UPLC-MS/MS method was successfully used for the pharmacokinetics study after intragastric administration. The pharmacokinetic parameters of active components were calculated by Drug and Statistics (DAS) software 1.0. The mean plasma concentration–time curves of WFR and WFG in rats after given TGT suspension are presented in Fig 4-5. The main pharmacokinetic parameters are shown in Table 4-5.
The time-concentration curve and pharmacokinetic parameters $AUC_{(0-tn)}$ of WFR showed a tendency of time-dependent and dose-dependent. The present findings confirmed obvious hepatotoxicity and nephrotoxicity in rats treated by tripterygium glycosides. In this study, totally four cases of death happened, two cases happened at day 5, day 8 in AA-14 day group and the other two occurred in day 11, day 18 in AA-21 day group, respectively, all at 90 mg/kg dosage. The result suggest that the toxicity may be related with high dose exposure. While, the $AUC_{(0-tn)}$ of WFG were all shown at low level and with no obvious regular.

4. Discussion

It is reported that total alkaloids in THWF are the effective components. Same as report mentioned before (Zhang et al., 2013), the quantification analysis of four alkaloids confirmed that the quality was several hundred times difference between DND and other three manufacturers, revealed the large discrepancy effective material basic in-vitro. Then, the results of cytotoxicity of HepG2 suggested that WFR and WFG could induce hepatotoxicity at high concentration.

Therefore, we investigated and established the pharmacodynamic and pharmacokinetic characteristics with different dose and different time of WFG and WFR in TGT by AA rats. WFG presented a low level exposure either in control group nor intervention group. Moreover, there was no indication that WFG had an accumulation phenomenon in-vivo, so WFG hardly become the Q-maker nor PK-maker in TGT. Meanwhile,
the pharmacokinetic parameters of $\text{AUC}_{(0-tn)}$ was different between intervention group and PK control groups and the degree of arthritis may relate to $\text{AUC}_{(0-tn)}$, which indicated that WFR could be a biologically active substance in TGT. Furthermore, $\text{AUC}_{(0-tn)}$ revealed the feature of dose-dependent and time-dependent. At 90 mg/kg group during the test, four cases of death happened, that demonstrated TGT might cause serious toxicity. WFR was the suspect that might induce liver damage and other grievous side-effect when using TGT continuously or at high doses. Therefore, WFR should be served as PK marker of TGT.

Previous studies found that triptolide (Shen et al., 2014; Zhang et al., 2016) and celstrol (Jin et al., 2015) of TGT exhibited the inhibition on CYP3A4. For alkaloids, by cocktail probe assay in-vitro, WFT exhibited moderate inhibition on CYP3A4 at 50 μM and WFG presented inhibition at 5μM, while WFR and WFD showed negligible inhibitory effect on CYP3A4 but partly metabolized via it (Wang, et al., 2017). Thus, the metabolize of alkaloids may be reduced by inhibiting the activity of CYP3A4. Furthermore, Triptolide is the substance of CYPs, and CYP3A4 was the primary isoform responsible for hydroxylation of triptolide (Lu et al., 2017; Xue et al., 2011; Shen et al., 2014). Triptolide may induce hepatotoxicity via inhibition of CYP450s by alkaloids and celstrol of TGT. Competitive inhibition may also turn up between WFR and triptolide / celstrol, which can cause more exposure and will lead to efficacy even toxicity. Therefore, further research is needed to confirm the relationship between “component-quality-efficacy-toxicity”.
The quality control for TGT preparation is obviously not adequately to assess the clinical safety of this drug, which should be improved and needed much more precautions. In CFDA, as triptolide need to be controlled less than 10 μg and wilforlide A more than 10 μg per tablet, we should control the content of WFR to improve the safety and effectiveness. The quality control for WFR need more evidence for further confirmation. Previous studies found that the patient's discomfort was different with TGT produced by different manufacturers, which may be related with the difference of the quality of WFR. From both in-vitro and in-vivo test in this current study, WFR serve as a Q-marker is very necessary, which could adjust the relationship between efficacy and toxicity of TGT.

5. Conclusion

The result has been showed that WFR was abundant component in TGT preparations produced by three pharmaceutical manufacturers, except DND. While, the quality was obviously different among different manufacturers. However, the standard promulgated by CFDA cannot fully reflect the quality and effect of TGT. We investigated the relationship between “quality-efficacy-toxicity” of WFR in TGT. The results indicated WFR could reflect the quality and pharmacodynamics of TGT, and the AUC\(_{(0-t)}\) showed time-dependent and dose-dependent tendency, that WFR should be served as Q-marker and PK-marker of TGT. However, further research about the Q-maker of TGT is still needed.
Conflict of interest

The authors declare that there is not any conflict of interest.

Acknowledgments

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References


(Danshen) can be used as pharmacokinetic markers for cardiotonic pills, a cardiovascular herbal medicine. Drug Metabolism & Disposition 36, 1578-1586.


Table legends

Table 1. Parameter of four alkaloids in TGT and Irbesartan (IS)

Table 2. The quality of four alkaloids in TGT (μg/tablet) n = 3

Table 3. The change of paw swelling (n = 4).

Table 4. Pharmacokinetic parameters of wilforine in rats (n = 4) after oral administration tripterygium glycosides tablet

Table 5. Pharmacokinetic parameters of wilfogine in rats (n = 4) after oral administration tripterygium glycosides tablet

Figure legends

Fig. 1. Structures of four main alkaloids in tripterygium glycosides tablet.
Fig. 2. Selectivity for 4 alkaloids of TGT (A: blank solvent; B: blank solvent spiked with 0.2 ng/ml ingredients and IS; C: sample)
Fig. 3. The relative viability in HepG2 (n = 6)
Fig. 4. The plasma concentration–time curves of wilforine (n = 4)
Fig. 5. The plasma concentration–time curves of wilforgine (n = 4)
Table 1

Parameter of four alkaloids in TGT and Irbesartan (IS)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>DP (V)</th>
<th>CE (V)</th>
<th>CXP (V)</th>
<th>RT (min)</th>
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<tr>
<td>wilforgine</td>
<td>858.1</td>
<td>206.2</td>
<td>85</td>
<td>45</td>
<td>12</td>
<td>4.92</td>
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<tr>
<td>wilforine</td>
<td>868.3</td>
<td>206.2</td>
<td>70</td>
<td>45</td>
<td>11</td>
<td>5.79</td>
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<tr>
<td>wilfordine</td>
<td>884.3</td>
<td>856.4</td>
<td>75</td>
<td>34</td>
<td>18</td>
<td>4.76</td>
</tr>
<tr>
<td>wilfortrine</td>
<td>875.2</td>
<td>847.4</td>
<td>65</td>
<td>32</td>
<td>17</td>
<td>4.24</td>
</tr>
<tr>
<td>Irbesartan(IS)</td>
<td>429.0</td>
<td>207.0</td>
<td>80</td>
<td>18</td>
<td>17</td>
<td>3.88</td>
</tr>
</tbody>
</table>
Table 2

The quality of four alkaloids in TGT (μg/tablet) n = 3

<table>
<thead>
<tr>
<th>Manufacturers</th>
<th>Batches</th>
<th>Wilforgine</th>
<th>Wilforine</th>
<th>Wilfordine</th>
<th>wilfortrine</th>
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</thead>
<tbody>
<tr>
<td>DND</td>
<td>1412108B</td>
<td>1.23 ± 0.11</td>
<td>2.76 ± 0.21</td>
<td>25.47 ± 0.87</td>
<td>9.34 ± 0.56</td>
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<tr>
<td></td>
<td>1507120B</td>
<td>2.14 ± 0.52</td>
<td>4.63 ± 1.19</td>
<td>17.63 ± 4.36</td>
<td>7.82 ± 1.91</td>
</tr>
<tr>
<td></td>
<td>1508101B</td>
<td>1.82 ± 0.10</td>
<td>3.97 ± 0.18</td>
<td>15.34 ± 0.98</td>
<td>6.58 ± 0.63</td>
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<tr>
<td>FDFH</td>
<td>1510115B</td>
<td>0.91 ± 0.22</td>
<td>2.10 ± 0.60</td>
<td>18.41 ± 3.51</td>
<td>8.56 ± 1.46</td>
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<td></td>
<td>1511111B</td>
<td>1.19 ± 0.03</td>
<td>2.36 ± 0.34</td>
<td>19.73 ± 1.64</td>
<td>7.47 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>1603134B</td>
<td>1.28 ± 0.11</td>
<td>2.86 ± 0.34</td>
<td>28.22 ± 2.41</td>
<td>11.79 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>1605120B</td>
<td>0.88 ± 0.05</td>
<td>1.80 ± 0.28</td>
<td>16.04 ± 2.58</td>
<td>6.98 ± 0.92</td>
</tr>
<tr>
<td>HSFY</td>
<td>141201</td>
<td>261.68 ± 14.23</td>
<td>680.09 ± 25.48</td>
<td>443.78 ± 23.73</td>
<td>134.71 ± 6.54</td>
</tr>
<tr>
<td></td>
<td>150201</td>
<td>244.60 ± 9.12</td>
<td>649.59 ± 17.98</td>
<td>413.81 ± 9.23</td>
<td>121.72 ± 4.65</td>
</tr>
<tr>
<td></td>
<td>150502</td>
<td>276.25 ± 50.84</td>
<td>686.09 ± 75.97</td>
<td>457.38 ± 88.85</td>
<td>136.18 ± 26.30</td>
</tr>
<tr>
<td>QJXL</td>
<td>20140601</td>
<td>202.02 ± 18.06</td>
<td>517.83 ± 39.61</td>
<td>298.54 ± 32.17</td>
<td>76.11 ± 7.90</td>
</tr>
<tr>
<td></td>
<td>20140903</td>
<td>177.86 ± 82.31</td>
<td>463.49 ± 236.31</td>
<td>398.42 ± 174.94</td>
<td>102.67 ± 43.86</td>
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<tr>
<td></td>
<td>20141003</td>
<td>177.94 ± 25.33</td>
<td>300.30 ± 38.95</td>
<td>303.94 ± 41.01</td>
<td>115.28 ± 15.59</td>
</tr>
<tr>
<td></td>
<td>20150302</td>
<td>156.21 ± 4.87</td>
<td>439.57 ± 13.84</td>
<td>392.97 ± 8.97</td>
<td>101.96 ± 2.80</td>
</tr>
</tbody>
</table>
Table 3

The change of paw swelling (n = 4)

<table>
<thead>
<tr>
<th></th>
<th>control group</th>
<th>model group</th>
<th>intervention groups: TGT suspension (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>day 0</td>
<td>1.88 ± 0.18</td>
<td>3.73 ± 0.58***</td>
<td>3.83 ± 0.40***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.37 ± 0.98***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.77 ± 0.74***</td>
</tr>
<tr>
<td>day 7</td>
<td>2.08 ± 0.18</td>
<td>3.92 ± 0.45</td>
<td>3.60 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.41 ± 0.32△△</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.11 ± 0.37△△△</td>
</tr>
<tr>
<td>day 14</td>
<td>2.02 ± 0.20</td>
<td>4.00 ± 0.55</td>
<td>3.36 ± 0.28△△</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.27 ± 0.16△△△</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.08 ± 0.29△△△</td>
</tr>
<tr>
<td>day 21</td>
<td>2.15 ± 0.16</td>
<td>4.08 ± 0.51</td>
<td>3.42 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.20 ± 0.22△△△</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.20 ± 0.26△△△</td>
</tr>
</tbody>
</table>

(Compared with control group, ***P < 0.01 indicated that AA model established was successful and stable. Compared with model group, △△ P < 0.05 and △△△ P < 0.01 suggested that TGT can relieve the symptoms of rheumatoid arthritis)
Table 4

Pharmacokinetic parameters of wilforine in rats (n = 4) after oral administration tripterygium glycosides tablet

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Group</th>
<th>V/F (L)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>AUC&lt;sub&gt;(0-tn)&lt;/sub&gt; (ng/ml*h)</th>
<th>MRT&lt;sub&gt;(0-tn)&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>PK-control</td>
<td>0.10 ± 0.04</td>
<td>7.29 ± 8.66</td>
<td>32.57 ± 7.89</td>
<td>295.04 ± 97.11</td>
<td>7.03 ± 1.08</td>
</tr>
<tr>
<td></td>
<td>AA-14 day</td>
<td>0.03 ± 0.01</td>
<td>2.25 ± 1.64</td>
<td>52.76 ± 2.72</td>
<td>331.08 ± 98.30</td>
<td>4.39 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>AA-21 day</td>
<td>0.02 ± 0.02</td>
<td>2.33 ± 1.53</td>
<td>55.28 ± 11.20</td>
<td>415.79 ± 33.74</td>
<td>5.69 ± 1.14</td>
</tr>
<tr>
<td>30</td>
<td>PK-control</td>
<td>0.04 ± 0.01</td>
<td>4.17 ± 2.23</td>
<td>52.50 ± 6.18</td>
<td>671.01 ± 128.12</td>
<td>8.40 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>AA-14 day</td>
<td>0.03 ± 0.01</td>
<td>4.67 ± 3.06</td>
<td>62.22 ± 23.78</td>
<td>678.61 ± 164.10</td>
<td>7.18 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>AA-21 day</td>
<td>0.01 ± 0.01</td>
<td>2.00 ± 0.00</td>
<td>139.90 ± 21.88</td>
<td>1469.08 ± 415.47</td>
<td>5.69 ± 1.96</td>
</tr>
<tr>
<td>90</td>
<td>PK-control</td>
<td>0.02 ± 0.01</td>
<td>6.33 ± 3.44</td>
<td>97.47 ± 16.80</td>
<td>1311.48 ± 208.70</td>
<td>9.16 ± 1.04</td>
</tr>
<tr>
<td></td>
<td>AA-14 day</td>
<td>0.01 ± 0.01</td>
<td>6.67 ± 4.62</td>
<td>165.91 ± 33.41</td>
<td>2087.41 ± 616.14</td>
<td>7.91 ± 1.70</td>
</tr>
<tr>
<td></td>
<td>AA-21 day</td>
<td>0.01 ± 0.01</td>
<td>5.00 ± 3.61</td>
<td>226.00 ± 73.43</td>
<td>3188.16 ± 538.73</td>
<td>9.84 ± 1.10</td>
</tr>
</tbody>
</table>
Table 5

Pharmacokinetic parameters of wilfogine in rats (n = 4) after oral administration tripterygium glycosides tablet

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Group</th>
<th>V/F (L)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>AUC&lt;sub&gt;(0-tn)&lt;/sub&gt; (ng/ml*h)</th>
<th>MRT&lt;sub&gt;(0-tn)&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PK-control</td>
<td>1.51 ± 1.04</td>
<td>0.63 ± 0.26</td>
<td>2.15 ± 1.01</td>
<td>5.86 ± 2.28</td>
<td>5.98 ± 2.72</td>
</tr>
<tr>
<td>10</td>
<td>AA-14 day</td>
<td>0.09 ± 0.10</td>
<td>1.00 ± 0.00</td>
<td>4.02 ± 3.35</td>
<td>8.41 ± 5.17</td>
<td>1.99 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>AA-21 day</td>
<td>0.79 ± 0.47</td>
<td>0.88 ± 0.14</td>
<td>1.51 ± 0.48</td>
<td>4.95 ± 2.60</td>
<td>1.71 ± 0.66</td>
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<tr>
<td></td>
<td>PK-control</td>
<td>2.42 ± 0.73</td>
<td>1.08 ± 0.47</td>
<td>1.22 ± 0.33</td>
<td>5.70 ± 1.86</td>
<td>6.24 ± 0.79</td>
</tr>
<tr>
<td>30</td>
<td>AA-14 day</td>
<td>0.05 ± 0.04</td>
<td>1.67 ± 0.58</td>
<td>4.09 ± 3.24</td>
<td>15.37 ± 11.34</td>
<td>2.86 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>AA-21 day</td>
<td>0.53 ± 0.00</td>
<td>0.81 ± 0.13</td>
<td>11.17 ± 2.47</td>
<td>25.76 ± 8.90</td>
<td>2.00 ± 0.44</td>
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<td>PK-control</td>
<td>0.41 ± 0.38</td>
<td>1.29 ± 0.56</td>
<td>6.21 ± 3.05</td>
<td>29.11 ± 20.26</td>
<td>4.45 ± 0.75</td>
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<tr>
<td>90</td>
<td>AA-14 day</td>
<td>0.24 ± 0.39</td>
<td>1.50 ± 0.87</td>
<td>10.92 ± 7.48</td>
<td>38.40 ± 35.07</td>
<td>2.77 ± 0.16</td>
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<td></td>
<td>AA-21 day</td>
<td>0.26 ± 0.06</td>
<td>0.75 ± 0.35</td>
<td>10.88 ± 1.65</td>
<td>48.86 ± 3.82</td>
<td>4.29 ± 0.58</td>
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</tbody>
</table>
Graphical Abstract