中文摘要

人参皂苷 Rg3 的增溶及其纳米晶颗粒剂药动学研究

人参是中国的传统中药，人参的主要成分包括人参皂苷、人参多糖、蛋白质等。人参皂苷是最重要的活性成分，其中人参皂苷 Rg3(Ginsenoside Rg3)已被证明具有明显的抗肿瘤作用。我国自行开发的第一个在临床应用的抗肿瘤中药一类新药参一胶囊的主要活性成分就是 Rg3。用于抑制肿瘤的新血管生成，常联合手术和化疗治疗原发性肝癌，以改善患者生存质量。有研究表明，Rg3 还具有抗衰老、抗疲劳、抗炎、抗病毒、抗心血管疾病、神经保护和减轻肺损伤的药理作用，其安全性较好。

但 Rg3 水溶性差而导致口服溶出速率慢，吸收差，大鼠灌胃给药 Rg3 的绝对生物利用度仅为 2.63%[1]。因此，开发出稳定性更好，方便给药、生物利用度更高、药效更佳的 Rg3 口服制剂有特别重要的临床意义。目前有研究将 Rg3 制成微乳、脂质体（liposomes）、固体脂质纳米粒、包合物、固体分散体、微球及胶束等以增大其溶解度，提高生物利用度。

目的：

本课题拟通过物理方法和药剂学新技术，筛选增加人参皂苷 Rg3 的溶解度和体外溶出度的最优策略，制备增溶的人参皂苷 Rg3 颗粒剂，促进药物在胃肠道的吸收，提高口服生物利用度。

方法：

1. 使用 HPLC 建立人参皂苷 Rg3 的体外分析方法，考察分析方法的专属性、精密度、稳定性和加样回收率。

2. 提高 Rg3 溶解度。通过潜溶剂的方法增加 Rg3 的溶解度，主要考察潜溶剂中乙醇的最佳百分比；通过包合技术增加 Rg3 的溶解度，比较机械研磨法、手动研磨法和水溶液法的包封率、载药量和溶解度，采用傅里叶红外色谱的方法表征包合物；通过固体分散体技术增加 Rg3 的溶解度，考察制备方法、辅料和药载比。采用傅里叶红外色谱的方法进行表征；通过纳米晶技术增加 Rg3 的溶解度，考察不同稳定剂对 Rg3 的影响。

3. 制备最优增溶效果的人参皂苷 Rg3 颗粒剂。以粒度为指标筛选制备颗粒剂的辅料配比、粘合剂及其浓度、干燥温度和时间。对颗粒剂的粒度、溶化性、流动性和湿度进行评价。以溶出度为指标，筛选最佳提高 Rg3 溶解度的颗粒剂制备工艺。

4. 建立人参皂苷 Rg3 的体内分析方法。

5. 大鼠药动学实验。以市售参一胶囊为对照组与纳米晶 Rg3 颗粒剂比较，检测每个时间点对应的血清和组织中药物浓度，用 PKSolver 软件计算在血清和组织中各组制剂的药时曲线及其药动学参数。

结果：

1. 建立了人参皂苷 Rg3 体外检测方法，回归方程为 y = 4，065968.7212 x - 2559.7241，R^2 =0.9998，表明人参皂苷 Rg3 在 0.0025 ~ 0.16 mg/mL 的范围内线性关系良好，该方法的精密度和稳定性良好，可用于人参皂苷 Rg3 的体外分析。

2. 增加 Rg3 的溶解度的潜溶剂乙醇的最佳百分比为 75%；制备包合物的最佳方法为机械研磨法，红外色谱证明包合物形成。该法制备的包合物载药量为 5.66%，溶解度为 6.6 μg/mL，包封率为 89%；制备固体分散体的最佳方法是熔融溶剂法，辅料为 F68，药载比为 1:5。红外色谱证明固体分散体形成，该法制备的固体分散体载药量为 8.61%，溶解度为 12.3 μg/mL；制备纳米晶的最佳稳定剂为 F68，制备 Rg3 纳米晶。该法制备的纳米晶溶解度为 0.33 mg/mL，载药量为 12.3%，粒径为 200 nm，电位是-7.52 mV。

3. Rg3 纳米晶颗粒剂的最佳制备工艺为以 80%乙醇作为粘合剂，制备软材，制粒，室温干燥 30 min。过筛，整粒可得颗粒剂。粒度、溶化性、流动性和湿度试验表明颗粒剂质量合格。Rg3 纳米晶颗粒剂内 Rg3 的含量为 0.3%以上。Rg3 纳米晶颗粒剂、包合物颗粒剂、固体分散体的溶出实验对比结果为纳米晶颗粒剂组 10 min 内，溶出率达 80%。固体分散体颗粒剂组和包合物颗粒剂组相较参一胶囊组也可以在 45 min 内达到各自的最大溶出。

4. 建立了 Rg3 的体内高效液相色谱-紫外检测法，血清的线性方程为 y = 1,100,888.64 x+3,793.59，R^2=0.9982；肺的线性方程为 y = 752,131.21 x+2,409.98，R^2=0.9979；在范围 0.0025~0.16 mg/mL 线性良好。血清药动学参数结果为参一胶囊的 C_{max} 为 0.049±0.017 mg/mL，T_{max} 为 8 h，AUC_{0-t} 为 0.208±0.021 mg·h/ mL。

颗粒剂组的 C_{max} 为 0.072±0.075 mg/mL，T_{max} 为 2 h，AUC_{0-t} 为 0.319±0.048 mg·h/ mL。肺药动学参数结果为参一胶囊的 C_{max} 为 0.011±0.221 mg/mL，T_{max} 为 1 h，AUC_{0-t} 为 0.055±0.041 mg·h/ mL。颗粒剂组的 C_{max} 为 0.032±0.137 mg/mL，T_{max} 为 0.167 h，AUC_{0-t} 为 0.083±0.076 mg·h/ mL。颗粒剂组 T_{max} 提前，C_{max} 变大，生物利用度明显提高。

结论：

1. 75%乙醇作为潜溶剂可以增加 Rg3 的溶解度。

2. Rg3 的包合物、固体分散体和纳米晶较原料药，溶解度均有提高。其制备的颗粒剂溶出表明，纳米晶颗粒剂组可以最快最先溶出。体内药动学表明，纳米晶颗粒剂组优于纳米晶组优于参一组。为开发人参皂苷 Rg3 新剂型和新的给药途径提供了参考。

创新点：

1. 研究发现了乙醇水作为潜溶剂，可以增加 Rg3 的溶解度，并为 Rg3 包合物和固体分散体的制备，提供了有效的溶剂。

2. 首次采用纳米晶制备了 Rg3 颗粒剂，药动学研究结果表明，生物利用度得到提高。

关键词：

人参皂苷 Rg3，溶解度，包合物，固体分散体，纳米晶，药动学

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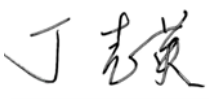
作者签名：  　　指导教师签名： 

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Abstract

Solubilization of Ginsenoside Rg3 and Pharmacokinetic Study of Nanocrystalline Granules

Ginseng is a traditional Chinese medicine. The main components of ginseng include ginsenosides, ginseng polysaccharides and proteins. Ginsenosides are the most important active ingredients, among which Ginsenoside Rg3 (Ginsenoside Rg3) has been proven to have significant anti-tumour effects. It is used to inhibit cardiovascular production of tumours and is often combined with surgery and chemotherapy to treat primary liver cancer in order to improve the quality of life of patients. Some studies have shown that Rg3 also has anti-aging, anti-fatigue, anti-inflammatory, anti-viral, anti-cardiovascular disease, neuroprotective and lung injury reducing pharmacological effects, Rg3 is relatively safe.

However, the poor water solubility of Rg3 results in slow oral dissolution rate and poor absorption, and the absolute bioavailability of Rg3 administered by gavage to rats is only 2.63%. Therefore, it is of particular clinical importance to develop Rg3 formulations that are more stable, easier to administer and more effective. Currently, studies have been carried out to make Rg3 into microemulsions, liposomes, solid lipid nanoparticles, inclusions, solid dispersions, microspheres and micelles in order to increase the solubility of the drug and improve its bioavailability in vivo.

Objective:

This project proposes to screen the optimal strategy to increase the solubility and in vitro dissolution of ginsenoside Rg3 by physical methods and new pharmacological techniques to prepare oral ginsenoside Rg3 granules to promote drug absorption in the gastrointestinal tract and improve oral bioavailability.

Methods:

1. An in vitro analytical method for ginsenoside Rg3 was established using HPLC to investigate the precision, stability and recovery of the analytical method.

2. To increase the solubility of Rg3. The solubility of Rg3 was increased by latent solvent method, mainly examining the optimal percentage of ethanol in the latent solvent; the solubility of Rg3 was increased by encapsulation technique, comparing the encapsulation rate, drug loading and solubility of mechanical grinding method, manual grinding method and aqueous solution method, and characterizing the encapsulation by Fourier infrared chromatography; the solubility of Rg3 was increased by solid dispersion technique, examining the preparation method, excipients and drug loading ratio. The inclusion complexes were characterized by Fourier infrared chromatography; the effect of different stabilizers on Rg3 was investigated by increasing the solubility of Rg3 through nanocrystal technique.

3. Preparation of ginsenoside Rg3 granules. The particle size was used as an indicator to screen the ratio of excipients, binder concentration, drying temperature and time for the preparation of granules. The granules were evaluated for particle size, solubility, flowability and moisture content. Screening the best granules preparation process to improve the solubility of Rg3 using solubility as an indicator.

4. To establish an in vivo analytical method for ginsenoside Rg3.

5. Pharmacokinetic experiments in rats. Commercially available ginsenyi capsules were used as a control group for comparison with Rg3 granules. The concentrations of the drug in serum and tissues at various time points were determined, and the pharmacokinetic curves and pharmacokinetic parameters of each formulation in serum and tissues were calculated using PKSolver pharmacokinetic software.

Results:

1. An in vitro assay for ginsenoside Rg3 was established with a regression equation of y = 4,065968.7212 x - 2559.7241，R^2 =0.9998, indicating that the linearity of ginsenoside Rg3 was good in the range of 0.0025 ~ 0.16 mg·mL^{-1} and that the method has good precision and stability for the in vitro analysis of ginsenoside Rg3.

2. The optimum percent of ethanol, the latent solvent for increasing the solubility of Rg3, was 75%; the best method for preparing the inclusion complexes was mechanical grinding and infrared liquid chromatography proved inclusion complex

formation. Infrared chromatography showed the formation of solid dispersions with 8.61% drug loading and 12.3 μg/mL solubility; the best method for the preparation of nanocrystals was the molten solvent method with F68 as the excipient and a drug loading ratio of 1:5. The best stabilizer was F68 for the preparation of Rg3 nanocrystals. The solubility of the nanocrystals prepared by this method was 0.33 mg/mL with a drug loading of 12.3%. The particle size was 200 nm and the potential was -7.52 mV.

3. The optimum preparation process for the granules was 80% ethanol as a binder, preparation of soft material, granulation, drying at room temperature for 30 min. sieving and whole granules were obtained. The content of Rg3 in Rg3 nanocrystalline granules was more than 0.3%.The dissolution test of Rg3 nanocrystalline granules, clathrate granules and solid dispersions showed that the dissolution rate of nanocrystalline granules reached 80% within 10 min. Compared with the Shenyi group, both the solid dispersion granule group and the inclusion compound granule group could reach their respective maximum dissolution within 45 min.

4. An in vivo high performance liquid chromatography-UV detection method was developed for Rg3. The linear equation for serum was y = 1,100,888.64 x+3,793.59, R^2 =0.9982; the linear equation for lung was y = 752,131.21 x+2,409.98, R^2 =0.9979, with good linearity in the range of 0.0025~0.16 mg/mL . Serum pharmacokinetic parameters resulted in a C_{max} of 0.049±0.017 mg/mL, T_{max} of 8 h and AUC_{0-t} of 0.208±0.021 mg·h /mL for the Shenyi capsule. The C_{max} for the granule group was 0.072±0.075 mg/mL, T_{max} of 2 h and AUC_{0-t} of 0.319±0.048mg·h/ mL. The results of the pulmonary pharmacokinetic parameters were 0.011±0.221mg/mL, T_{max} of 1 h and AUC_{0-t} of 0.055±0.041 mg·h/mL for the Ginsenyi capsules 0.083±0.076 mg·h/mL. T_{max} is advanced, C_{max} become larger and bioavailability is improved in the granule group.

Conclusion:

1. 75% ethanol as a latent solvent can increase the solubility of Rg3.

2. The solubility of the inclusion, solid dispersion and nanocrystals of Rg3 was increased compared to the API. In vivo pharmacokinetics showed that the gavage granule group was superior to the nanocrystal group superior to the ginseng group. It

provides a reference for the development of new dosage forms and new routes of administration of ginsenoside Rg3.

Points of innovation:

1. It is found that ethanolic water as a latent solvent can increase the solubility of Rg3 and provide an effective solvent for the preparation of Rg3 inclusion complexes and solid dispersions.

2. For the first time, Rg3 granules were prepared using nanocrystals.The dissolution of nanocrystalline granules showed that the nanocrystalline granules could be dissolved first, efficiently and quickly. And the results of pharmacokinetic studies showed that the bioavailability was improved.

Keywords:

Ginsenoside Rg3,Solubility,Inclusion complex,Solid dispersion,Nanocrystalline, Pharmacokinetics

符号说明

英文缩写	英文全称	中文全称
Rg3	Ginsenoside Rg3	人参皂苷 Rg3
AUC	Area under the concentration-time curve	药时-曲线下面积
NCs	Nanocrystalline particulates	纳米晶
C_{max}	Maximum concentration	达峰浓度
CL	Clearance	清除率
HP-β-CD	Hydroxypropy-β-Cyclodextrin	羟丙基-β-环糊精
SD	Solid Dispersion	固体分散体
PEG	Polyethylene glycol	聚乙二醇
PVP	Polyvinyl pyrrolidone	聚乙烯吡咯烷酮
FT-IR	Fourier transform infrared spectroscopy	红外分光光度法
HPLC	High-performance liquid chromatography	高效液相色谱法
PEG4000	Polyethylene glycol 4000	聚乙二醇 4000
PEG6000	Polyethylene glycol 6000	聚乙二醇 6000
F127	Poloxamer407	泊洛沙姆 407
F68	Poloxamer188	泊洛沙姆 188
SDS	Sodium Dodecyl Suphfate	十二烷基硫酸钠
API	Active Pharmaceutical Ingredient	药物活性成分

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