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Development and *in vivo*/*in vitro* evaluation of novel herpetrione nanosuspension

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ABSTRACT

Herpetrione (HPE), is a new compound extracted from *Herpetospermum caudigerum*, which is proved to be a novel and potent antiviral agent. However, due to poor water solubility, oral bioavailability of the drug was relatively low. To improve the dissolution and absorption of the drug, formulation of HPE as nanosuspension has been performed in this study. HPE nanosuspension were produced by high pressure homogenization and transformed into dry powder by lyophilization. The nanosuspension was then investigated using photon correlation spectroscopy (PCS), zeta potential measurement, SEM and PXRD. To verify the theoretical hypothesis on the benefit of decreased particle size and increased surface area, *in vitro* dissolution characterization and *in vivo* pharmacokinetics were investigated. The inhibitory effect on HBsAg, HBeAg, and HBV-DNA of HPE nanosuspension in 2.2.15 cells was studied. Results showed that a narrow size distributed nanosuspension with a mean particle size of 286 ± 1.3 nm, a polydispersity index of 0.18 ± 0.06 and a zeta potential of -26.9 ± 2.4 mV was obtained. The result of PXRD showed that HPE was amorphous state in both coarse powder and nanosuspension. In the *in vitro* dissolution test, HPE nanosuspension showed an increased dissolution velocity markedly. In the *in vivo* evaluation, compared to coarse HPE, nanosuspension exhibited significant increase in AUC_{0-t}, C_{max} and decrease in T_{max}, MRT. The inhibitory effect of HBsAg, HBeAg, and HBV-DNA of 2.2.15 cells treated by HPE nanosuspension were stronger than those of the HPE. The *in vitro* activity experiments provided evidence for an enhanced efficacy of the HPE nanosuspension formulation compared to HPE coarse suspension. These results revealed that particle size reduction could enhance HPE dissolution rate and absorption in gastrointestinal tract, and nanosuspension might be a good choice for oral delivery of poor bioavailability drug like HPE.

1. Introduction

Herpetospermum caudigerum is a well-known Tibetan medicine in China. It is the dry, mature seed of *H. caudigerum* Wall, which grows widely in the southwest of China, Nepal and the northeast of India. In Tibet it is widely used in traditional medicine for the treatment of liver diseases, cholic diseases, and dyspepsia (Wu, 1985; Chinese Pharmacopoeia Committee, 1995). In the previous investigation, a large amount of lignans have been isolated from *H. caudigerum* (Kaouadji et al., 1978, 1979, 1987; Kaouadji and Favre, 1984a,b) and showed positive activity in the inhibit hepatitis b virus test (Han et al., 2005, 2006). Herpetrione (HPE, Fig. 1), extracted from *H. caudigerum*, is a promising and potent antiviral agent (Yuan et al., 2011). However, pharmacokinetic studies of HPE in rats showed the drug had a less than 12% oral bioavailability, which was due to poor water solubility of drug (47.6 μg/mL). In consideration of further pharmaceutical use of the drug, we must overcome the problem of its poor solubility. Size reduction to nanometer range has been shown to be a very promising approach as it increases surface area and thus leads to enhanced dissolution characteristics and increased system exposure (Hecq et al., 2006). Therefore, the poor solubility of drug may be overcome by particle size reduction using nanoscience approaches (Io et al., 2010).

One of the nanoscience approaches that have quickly gained a proven record in the pharmaceutical field is the nanosuspension (Van Eerdenbrugh et al., 2008). Producing drugs into the form of nanosuspension has been shown to be more cost effective, technically simpler, alternative and particular for poorly soluble compounds (Io et al., 2010). Nanosuspension is a sub-micron colloidal dispersion of pure particles of the drug, which is stabilized by surfactants (Rabinow, 2004). The main advantages of nanosuspension are the small particle size and increased surface area which can lead to an enhanced dissolution rate and improved

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