

TITLE

Study the bioactivity in vitro and pharmacokinetics in vivo of hypoglycemic protein E2HSA

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Objective: This thesis was designed to study the bioactivity in vitro and pharmacokinetics *in vivo* of hypoglycemic protein E2HSA

Methods: The change of cAMP concentration in RINm-5F cells can reflect the vitro bioactivity of Exendin-4 and E2HSA. Meanwhile the cAMP levels be measured by a cAMP immunoassay kit. A chemiluminescent enzyme immunoassay (CLEIA) for analyzing E2HSA and Exendin-4 in blood samples was established and validated. CLEIA was employed to study pharmacokinetics of E2HSA and Exendin-4. Furthermore, ammonium sulfate and CLEIA were applied in studying metabolic conversion and active styles of E2HSA in rhesus monkey.

Results and Conclusions: In response to the GLP-1 molecule and its analogs (e.g. Exendin-4 and E2HSA) binding, GLP-1R directly stimulates adenylyl cyclase, leading to a rise of intracellular cAMP. The change of cAMP concentration in RINm-5F cells reflected the bioactivity of Exendin-4 and E2HSA. The cAMP levels were measured using a cAMP immunoassay kit. E2HSA and Exendin-4 exhibited a dose-dependent stimulation of cAMP accumulation in RINm-5F cells. E2HSA proved to retain only 1% activity of Exendin-4 *in vitro*.

Following a single subcutaneous administration with a dosage 0.3 mg·kg⁻¹ and 15 µg·kg⁻¹ of E2HSA and Exendin-4 to rhesus monkey, the blood samples were collected at different time points. All collected blood samples were centrifuged to obtain serum and the concentration of E2HSA and Exendin-4 in serum were determined by CLEIA method. Pharmacokinetic parameter calculations and pharmacokinetic modeling were carried out using the WinNonlin 5.2 Statistical software. It indicated that the exposure level of E2HSA in serum was increased significantly. There was obviously prolongation of the $t_{1/2}$ for E2HSA comparing with Exendin-4.

Proteins with different molecular weight can be separated by ammonium sulfate precipitation according to their solubility. Results showed that 60% saturated ammonium sulfate can completely precipitate the E2HSA in the serum, while Exendin-4 remained in the supernatant. Ammonium sulfate precipitation with CLEIA can be used to detect the E2HSA metabolism features *in vivo*, which included the concentration of both E2HSA and its metabolite Exendin-4.

Keywords: E2HSA Exendin-4 CLEIA bioactivity