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HYDRODYNAMIC-BASED TRANSFECTION OF NAKED PLASMID DNA ENCODING HUMAN HEPATOCYTE GROWTH FACTOR (HGF): IS IT AVAILABLE IN THE RAT BLADDER?

Aims of Study

Hepatocyte growth factor (HGF) is a multi-functional cytokine, and is known to promote tissue protection and repair. In addition, HGF has been demonstrated to have a therapeutic potential in tissue fibrosis and dysfunction in chronic diseases (e.g. liver cirrhosis, chronic allograft nephropathy and cardiac infarction etc.), suggesting that “low compliance bladder” could be a candidate for HGF treatment. In this study, we examined whether hydrodynamic-based transfection of human HGF plasmid, safer procedure than those using viral vectors, is available in the rat bladder.

Methods

Female S-D rats, weighing 100-150 grams, were used. Under inhalation anesthesia, human HGF plasmid was injected by a hydrodynamic-based gene transfection procedure. Different amount of human HGF plasmid (50, 100, 200 μg) was diluted in 5 or 10 ml of saline, and injected via the tail vein into the circulation within 15 seconds. This human HGF cDNA (2.2 kb) was inserted into the EcoRI and NotI sites of the pUC-SRa expression vector plasmid. In this plasmid, transcription of the HGF cDNA is under the SRa promoter control. As a control, saline was alone administered. Animals’ blood was sampled on day 1, 2, 3, 5 and 7 following the injection. In addition, the bladder, kidney and liver were removed and homogenized on day 7. The amount of human plasma and tissue HGF protein level was measured using the enzyme-linked immunosorbent assay (Immunis HGF-EIA kit, Institute of Immunology, Tokyo, Japan). This kit is specific to human HGF and detects no rat HGF, thus HGF expression here was delivered by the naked plasmid injection. Total protein levels in each tissue extract were determined using a bicinconinic-acid protein assay kit (PIERCE, IL, USA).

Results

The level of HGF gene expression was dependent on the amount of injected plasmid. In rats that received 200 μg plasmid in 10 ml saline, the level of human HGF in the circulation peaked on day 1 following the intravenous injection (0.33 ng/ml), and thereafter soon declined to the concentration less than 0.03 ng/ml until day 7. Plasma human HGF levels in rats with 50 and 100μg plasmid were much lower than those in the 200 μg plasmid-injected rats. Furthermore, 5 ml of solution volume was not enough for optimal expression of exogenous gene by this delivery approach. Tissue levels of human HGF protein in the bladder, kidney and liver were 0.6, 9.7 and 2.9 ng/μg protein, respectively.

Conclusions

In our rats, similar to mice (Yang-J et al., Hepatology, 33:848-859, 2001), the plasma level of human HGF expression peaked within 24 hours following the injection. However, the reached plasma level of human HGF was lower than expected. The kidney and liver contained richer human HGF than the bladder. This may be come from: 1) bolus injection of plasmid produced a high hydrostatic pressure in the inferior vena cava (IVC), 2) the high pressure forced DNA into the organs, and thus the kidney and liver, directly connected to IVC, might be transfected more efficiently. We conclude that 1) a hydrodynamic-based naked plasmid transfer procedure may be available in rats, but 2) the optimal transfection is restricted to the organs around IVC, such as the kidney and liver.