DEVELOPMENT OF BRAIN-TARGETED LIPOSOMES WITH ANTIVIRAL DRUGS FOR TREATING LETHAL VIRAL ENCEPHALITIS

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CONCLUSIONS

- GSH-PEG liposomes can be safely used for drug delivery to the brain.
- Ribavirin, as a proof-of-concept drug, incorporated in GSH-PEG liposomes is able to exert an antiviral effect after brain delivery.

BACKGROUND

Viral encephalitis
- Up to 30% mortality (4-10 days after admission)
- Survivors left with severe neurological damage
- No therapy available
- Viral replication in vascular endothelium
- Cerebral edema
- Perivascular inflammation
- Necrotic foci associated with blood vessels

Ribavirin GSH-PEG liposomes for treating lethal viral encephalitis
- Increase plasma pharmacokinetic profile, without inducing peripheral toxicity.
- Increase brain delivery to reach and maintain therapeutic concentrations in brain.

RESULTS

Safety
- 12 daily intra-cardiac injections of ribavirin GSH-PEG liposomes were well tolerated: no weight loss and no signs of CNS involvement in the control group.
- Compared to PEG liposomes, GSH-PEG liposomes showed less accumulation of the rhodamine-PE label in lung, kidney and liver, while the brain uptake was higher at day 15.

Pharmacokinetics in rats with microdialysis
- Dose-dependent steady-state plasma levels after repeat administrations were found in control hamsters.
- Antiviral concentrations were reached in the perfused brain homogenates with 50 mg/kg/day ribavirin GSH-PEG liposomes.

METHODS

Experimental design
- Inoculation of immune competent hamsters with MODOC virus (MDOV) provides an excellent preclinical model for validation of clinical endpoints for e.g., Japanese Encephalitis, West Nile, or Tick-borne Encephalitis virus.
- Free ribavirin (50 mg/kg/day) and ribavirin PEG liposomes (50 mg/kg/day) slightly increased mortality in hamsters inoculated with MODV as observed in several pilot studies (data not shown).

Bathing schedule
- Day 0: immune competent hamsters received an i.p. injection of MODV (LD90) or PBS (control and safety study).
- Days 1-12: daily microdialysis injections with ribavirin GSH-PEG liposomes (0.2% GSH, 10, 25, or 50 mg/kg ribavirin) or PBS (n=4-6 per group).
- Days 1, 4, 7, 15: plasma samples for ribavirin analysis (day 15 also organs).

Efficacy in hamsters with viral encephalitis
- Survivors left with severe neurological damage
- 12% mortality in hamsters inoculated with MODV as observed in several studies.
- Free ribavirin GSH liposomes were effective in rodents, mice, rabbits, and non-human primates, which are all affected by viral encephalitis.

Effects of GSH vs. no GSH

Liposomes
- Liposomes of DPPC (55%), cholesterol (41%), rhodamine-PE (0.04%) mPEG-DSPPE (4.4%) and GSH-PEG-DSPPE (0.02%) were prepared by a post-insertion of GSH-PEG-DSPPE into preformed liposomes containing ribavirin. Ribavirin GSH-PEG liposomes were 90 nm and contained 10 mg/ml ribavirin.

Figure 1: Ribavirin GSH-PEG liposomes. Ribavirin (green) is entrapped in the liposomes, while the tripeptide GSH (red-white-blue) is present on the tips of the liposomes.

Figure 2: Left: Hamsters with viral encephalitis receiving ribavirin GSH-PEG liposomes (50 mg/kg/day) for 12 days showed significantly less weight loss (P<0.05) compared to the animals in the control (PBS) group.

Right: injections with ribavirin GSH-PEG liposomes (50 mg/kg/day) for 12 days resulted in an increased survival of hamsters with viral encephalitis compared to the control group (P<0.05).

Figure 3: Brain uptake is increasing with higher amounts of GSH, even though the plasma AUCGSH is similar for all groups. Average half-life was 19 hours for all liposomes.

Middle: Schematic representation of the microdialysis sampling technique in the brain behind the BBB.

Right: Higher concentrations of free ribavirin are measured in the brain after i.v. injection with liposomes coated with increasing amounts of GSH.

Figure 4: Pharmacokinetics in hamsters.
- Dose-dependent steady-state plasma levels after repeat administrations were found in control hamsters.
- Antiviral concentrations were reached in the perfused brain homogenates with 50 mg/kg/day ribavirin GSH-PEG liposomes.

Ribavirin for viral encephalitis
- Marketed broad spectrum antiviral agent including: Hepatitis C, respiratory syncytial virus (RSV), West Nile, St.Louis, Japanese and Tick-borne Encephalitis Virus, Rabies, Ebola, Marburg, Hantaan, Lassa, Dengue, La Crosse, Yellow Fever.
- Intravenous ribavirin administration is effective in rodent encephalitis models, but intravenous at maximum tolerated daily dose (50 mg/kg) usually is not.

Glutathione for enhanced brain drug delivery
- The glutathione transporter has a preferential expression in CNS and BBB and is present from mice to man.
- Glutathione (GSH) is a safe targeting ligand:
  - Endogenous tripeptide
  - Functional food ingredient and antioxidant
  - Supportive therapy in cancer and HIV treatments
  - Excipient in parenteral formulations

Efficacy of GSH as targeting ligand on liposomes coated with polyethylene glycol (PEG) has been validated by bio-imaging and pain models (the Industrial Technology Research Institute (ITRI) of Taiwan).