

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

Pieter J. Gaillard<sup>1</sup>, Pieter Leyssen<sup>2</sup>, Chantal Appeldoorn<sup>1</sup>, Rick Dorland<sup>1</sup>, Joan van Kregten<sup>1</sup>, Maria Francesca Manca<sup>1</sup>, and Johan Neyts<sup>2</sup>

## 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.

### 致死性のウイルス性脳炎治療に、脳標的化リポソームと抗ウイルス薬の開発。

- GSH-PEGリポソームは、安全に脳までドラッグデリバリーするために使用できる。
- リバビリン(概念実証薬)はGSH-PEGリポソームに合併されることにより、抗ウイルス薬が脳へ達成後に効力を現す。

## 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.



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 PEG on the outside of the 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.
- Intraventricular 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

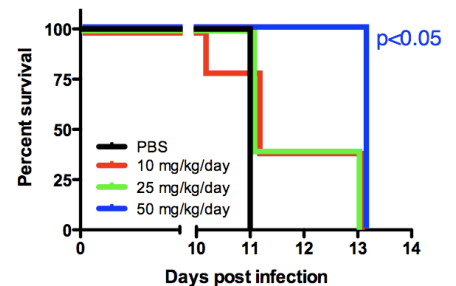
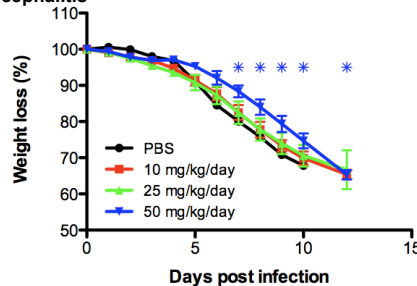
- The glutathione transporter has a preferential expression in CNS and BBB and is present from mice to man.
- Glutathione (GSH) is a safe targeting ligand:
  - Endogenous tripeptide
  - Functional food ingredient and antioxidant
  - Supportive therapy in cancer and HIV treatments
  - Excipient in parenteral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

## RESULTS

### Efficacy in hamsters with viral encephalitis

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 ( $P < 0.05$ ).



### 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.

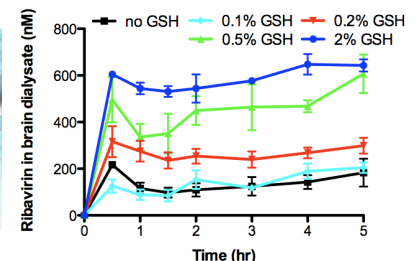
### Pharmacokinetics in rats with microdialysis

Figure 3: **Left:** Brain uptake is increasing with higher amounts of GSH, even though the plasma  $AUC_{0-5}$  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.

	Ratio AUCs GSH vs. no GSH	
	Plasma	Brain
No GSH	1,0	1,0
0,1% GSH	1,0	1,0
0,2% GSH	1,2	2,0
0,5% GSH	1,0	3,1
2% GSH	1,0	4,4



### 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.

## METHODS

### Experimental design

- Inoculation of immune competent hamsters with MODOC virus (MODV) 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).
- Dosing schedule:
  - Day 0: immune competent hamsters received an i.p. injection of MODV (LD90) or PBS (control and safety study).
  - Days 1-12: daily intra-cardiac 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).
  - Daily: evaluation of mortality and disease severity (weight and clinical symptoms)

### Liposomes

Liposomes of DPPC (55%), cholesterol (41%), rhodamine-PE (0,04%) mPEG-DSPE (4,4%) and GSH-PEG-DSPE (0 - 2%) were prepared by a post-insertion of GSH-PEG-DSPE into preformed liposomes containing ribavirin. Ribavirin GSH-PEG liposomes were 90 nm and contained 10 mg/ml ribavirin (encapsulation efficiency of 8-12%).

### Pharmacokinetic analysis in rats

Brains On-line (Groningen, the Netherlands) performed a brain microdialysis study in the medial prefrontal cortex of freely moving healthy male rats. The animals received i.v. injections of ribavirin GSH-PEG liposomes at 50 mg/kg ribavirin (n=3-4). On the outside, liposomes contained different percentages of GSH ranging from 0 to 2%.

<sup>1</sup>to-BBB technologies BV, Leiden, the Netherlands (gaillard@tobb.com),

<sup>2</sup>REGA Institute for Medical Research, KU Leuven, Belgium