Drug Delivery to the Blood Brain Barrier

Promising Strategy Based on Herpes Simplex Peptide

Peptide gH625, derived from glycoprotein H of *Herpes Simplex virus* 1, is able to traverse the membrane bilayer and to link a cargo. We evaluated the capacity of gH625 to enter and accumulate in neuron and astrocyte cell lines, and his ability to cross the **blood brain barrier** (BBB) in rats. Our results show that gH625 pass through the BBB and taken by neuronal cells. *This can be considered useful to develop gH625-based nanocarriers as an efficient drug delivery system through the blood brain barrier.*

**Introduction**

The high impermeability and selectivity of the **blood brain barrier** (BBB) prevent the transport of many therapeutic molecules into the brain and thus make ineffective the drugs administration for the treatment of neurological disorders. The development of new strategies for the delivery of therapeutic agents across the BBB is fundamental for the treatment of many brain diseases [1].

**Cell-penetrating peptides** (CPPs) are short and usually basic amino acid rich peptides derived from proteins that are able to cross biological barriers, and they represent a new tool to functionalize carriers in order to deliver therapeutic molecules to the brain [2]. Although the mechanism of CPPs cellular uptake is still unclear, it’s known that their endosomal entrapment may limit their utility. Recently, great attention has been devoted to the hydrophobic peptides that efficiently traverse biological membranes, promoting lipid-membrane reorganizing processes [3].
gH625, a peptide of nineteen amino acids derived from the glycoprotein H of *Herpes Simplex* virus 1, is able to traverse the membrane bilayer and its translocation does not seem to involve classical endocytic pathways [4]. Although several studies have demonstrated that gH625 is an efficient carrier for bioactive cargoes *in vitro* [5, 6], these data do not guarantee that gH625 can be used into a pharmaceutical delivery platform. The aim of the current study was to evaluate whether gH625 can enter into brain cells *in vitro* and whether it crosses the BBB when administered to rats *in vivo*.

**Materials and Methods In Vitro Analysis**

The human neuroblastoma (SH-SY5Y) and glioblastoma-astrocytoma cell lines (U-87 MG) were used to assess the uptake of gH625 by fluorescence and spectrofluorimetric analysis as described elsewhere [7]. The gH625 was labeled with 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (gH625-NBD) and the cells were treated with 1 or 5µM gH625-NBD for 2 hours. Images were acquired using an AxioCam MR c5 (Zeiss) with differential interference contrast, a FITC channel (λ<sub>ex</sub> 488 nm; λ<sub>em</sub> 515 nm) and a DAPI channel (λ<sub>ex</sub> 350 nm; λ<sub>em</sub> 461 nm). The plates for spectrofluorimetric assay were analyzed with an Infinite 200 M spectrofluorimeter (Tecan, Switzerland) equipped with a fluorescence filter (λ<sub>ex</sub> 485 nm; λ<sub>em</sub> 560 nm).

**Ex Vivo Analysis**

Adult male Wistar rats were treated with a single intravenous administration of vehicle or a single administration of gH625-NBD (40 µg/100 g b.w.). Brains were then used for immunofluorescence analysis as described elsewhere [7]. Colocalization studies were carried out with primary antibodies anti-SMI-71, anti-GFAP, or anti-tubulin III to determine if the gH625-NBD signal colocalized with main component of the BBB. The secondary antibodies were Dylight 594 for anti-
SMI-71 and AlexaFluor 594 for anti-GFAP and anti-tubulin III. Images were acquired using the AxioCam MR c5 with $\lambda_{\text{ex}}$ 488 nm/$\lambda_{\text{em}}$ 515 nm and $\lambda_{\text{ex}}$ 594 nm/$\lambda_{\text{em}}$ 618 nm filters, and then analyzed with ImageJ 1.48 software or Zen 2012 (Zeiss). The Deconvolution-lab plugin was used to deconvolve image channels using the Tikhonov-Miller algorithm [8] and the Colocalization Colormap plugin was used to evaluate the degree of correlation between pairs of pixels in the red and green channels, resulting in distribution of the values for the normalized mean deviation product (nMDP) and the index of correlation as the fraction of positively correlated pixels in the image [9]. To evaluate the green fluorescence inside and outside the BBB vessels, the mean gray values of ten sections of controls (vehicle or gH625 without NBD; n=3) and treated animals (n=3) were measured within and outside the region of interest (ROI) of the BBB vessels. These values were then divided for the correspondent ROI area ($\mu m^2$) and the ratio between the outside and inside values was indicated. The two-tailed unpaired t-test was used, and the values were considered statistically significant at $P<0.01$.

Results

In Vitro
The fluorescence and spectrofluorimetric analysis show that gH625 was efficiently internalized in both cell lines. NBD alone was almost unable to penetrate into the cells, as previously reported [10]. Indeed, SH-SY5Y and U-87 MG cells efficiently accumulated gH625 (fig. 1 A and B). At low concentration (1 μM), approximately 30% of gH625 administered was internalized, while uptake was nearly complete (80%-90%) at a concentration of 5 μM (fig. 1 C and D).

Ex Vivo
The evaluation of fluorescence colocalization demonstrated that gH625 accumulates in the blood vessels of the brain in treated rats and that the mean green fluorescence outside the blood vessels is 3-fold change compared to control (fig. 2 C). Colocalization was found mainly between gH625 and the endothelial cells of BBB (fig. 2 A). Although there was low or null colocalization with astrocytes surrounding the BBB (fig. 2 B), it was of interest that some neurons in the cerebral cortex were labeled for gH625, indicating the passage of gH625 through the BBB (fig. 2 D). The nMDP distribution values and the index of correlation (fig. 3) suggest that gH625 labeling is correlated mainly with BBB than to astrocytes.

Conclusion
The treatment of many neurological disorders requires the development of systems able to efficiently deliver drugs through the BBB. Peptide-based carriers have attracted considerable attention in the field of targeted drug delivery due to their
high binding affinity and specificity for targets.

We investigated the capacity of a CPP, gH625, to penetrate brain cells and to cross the BBB in rats. Our in vitro studies show that both SH-SY5Y and U-87 MG cells take up more than 90% of administered gH625, indicating that internalization of gH625 into these cell lines is an efficient process.

The results in vivo show that gH625 substantially reaches the brain BBB vessels and it is efficiently accumulated in endothelial cells of the BBB. We also evaluated whether gH625 had actually crossed the BBB or was simply trapped within endothelial cells of the brain. In particular, although we did not detect any association with astrocytes, which are located between the inner endothelial BBB and outer neuron parenchyma, we were able to find the presence of gH625 in neurons, thus suggesting that gH625 can reach neurons from systemic blood.

These data show for the first time that a memranotropic peptide can be incorporated in vitro by neuron or astrocyte cells and has the ability in vivo to accumulate in the brain.

Therefore, new gH625-based nanocarriers could be regarded as a promising strategy to design delivery systems for administration of therapeutic molecules to the brain.

Acknowledgments
This work was supported by Progetto FARO (2012/0043756) and by the Ministero dell’Istruzione, dell’Università e della Ricerca (BAP114AMK).

References

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