Preclinical safety evidences of a novel therapeutic anti-HIV peptide

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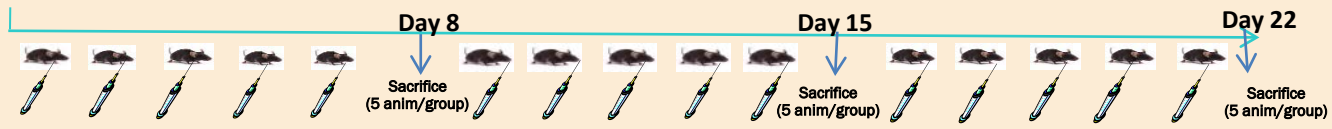
Abstracts

The main objectives for the development of drugs are efficiency and safety. Over the last years, the use of peptides as drugs has been increased. In terms of general safety, peptides have a comparatively small toxicological footprint due to, among others, their extremely high specificity for their target. Several anti-HIV peptides are currently in developing and a peptide has been approved and used since several years. The current HIV treatments require to be periodically administered; consequently, continuous administration regimens must be assayed during drug development in this field. The present work studies the effect of repeated doses of a new drug peptide candidate anti-HIV designated as CIGB-210, in mice C57BL6. A scheme of 15 repeated doses via subcutaneous was evaluated and three doses were assayed: 0.71 mg/Kg, 1.29 mg/Kg and 2.57 mg/Kg. Clinical evaluation related to appearance, behavior and others did not show differences in comparison with the control group. No alterations were observed in histopathology analysis, neither macroscopic nor microscopic. No damage in ex vivo splenocytes proliferation was detected by in vivo CIGB-210 treatment.

Materials and Methods

Line: C57BL6/ Sex: Female / Administration: Subcutaneous / Age: 8-10 weeks



Groups	Animals/group	Dosages (mg/kg)
I	15	Placebo
II	15	0,71
III	15	1,29
IV	15	2,57

Clinical evaluation during the assay

Clinical observation was performed from Monday to Friday during three weeks. Clinical signs related to behavior, appearance, functions and generals were evaluated.

Morfo-histopathologic analysis at macroscopic and microscopic level

The evaluation was performed at days 8, 15 and 22. Rodents were sacrificed by cervical dislocation and organs were kept in paraformaldehyde (4 %, v/v) until analysis. Features as size, color, consistency, weight and surface were examined. Incisions were also done to analyze internal appearance as solidity, content and brightness. Fragments of organs were included in paraffin, stained with hematoxilin-eosin and observed at optic microscope (Carl Zeiss, Germany; 10X and 40X).

Murine splenocytes isolation

Rodents were sacrificed by cervical dislocation and fresh spleens were perfused in RPMI 1640 medium (supplemented with 10% (v/v) fetal bovine serum, 50 µg/mL gentamicin). NH4Cl 0.83 % (v/v) was used to lyse erythrocytes. Viability and amount of spleen cells were determined by counting in Neubauer chamber through Trypan blue 0.4 % (m/v).

Proliferation assay

Proliferative response was meassured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) Cell Proliferation Assay. Spleen cells (2,5 x 105) were stimulated with Concanavalin A 1 µg/mL during 72 hours. MTT was added at 0.5 mg/mL and isopropanol was applied to dissolve formazan cristals.

Results

Clinical evaluation in mice treated with CIGB-210		
Behavior	Hyperactivity	N.O
	Lethargy	N.O
	Aggressive behavior	N.O
Appearance	Hair lost	N.O
	Medicine inflammation	N.O
	Induration	N.O
Functions	Excessive spittle	N.O
	Respiratory difficulties	N.O
Generals	Hurts	N.O
	Blood leakage	N.O
	Cyanosis	N.O
C57BL6 mice treated with CIGB-210 did not show differences in comparison with non treated group		

N.O: Not observed

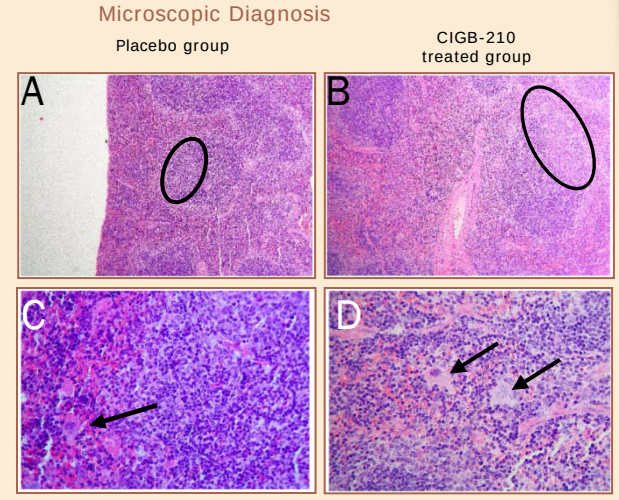
Hystopathology

Macroscopic Diagnosis

Organ	Placebo	0,71 mg/kg	1,29 mg/kg	2,57 mg/kg
Heart	5/5 N A	5/5 N A	5/5 N A	5/5 N A
M. L.. N.	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Thymus	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Spleen	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Stomach	5/5 N A	5/5 N A	5/5 N A	5/5 N A
S. intestine	5/5 N A	5/5 N A	5/5 N A	5/5 N A
L. intestine	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Liver	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Kidneys	5/5 N A	5/5 N A	5/5 N A	5/5 N A
Brain	5/5 N A	5/5 N A	5/5 N A	5/5 N A

M.L.N: Mesenteric Lympho Node; S. Intestine: Small intestine; L. Intestine: Large Intestine

Microscopic Diagnosis



Optical microscopy of spleen fragments of representative animals from placebo and CIGB-210 treated groups. Black elipsses enclose germinal centres (A and B); indicating specific antigenic stimulation of B lymphocytes; Hematoxilin-Eosin, 10X. Black arrows show megakaryocytes (C and D), indicating presence of extramedullary hematopoiesis; Hematoxilin-Eosin, 40X. Considering that both findings were present in placebo and CIGB-210 treated groups, it was determined that they were not a consequence of the administration of CIGB-210.

Not differences were detected among experimental groups.
•Normality Test (Kolmogorof-Smirnov); Variances homogenity test (Levene); One way ANOVA

Proliferative response ex vivo of spleen cells induced by Concanavalin A and evaluated by MTT method. Results are shown as proliferation index. Bars represent mean proliferation index of five animals per group. Error bars represent the standard error of the mean only in positive sense. CIGB-210 treatment did not affect the proliferative capacity of spleen cells.

Conclusions

- CIGB-210 treatment did not altered clinical parameters evaluated in this study.
- The examined organs were not impaired by the treatment with CIGB-210.
- CIGB-210 treatment in vivo did not affect the proliferation ex vivo of splenocytes from mice C57BL6.
- CIGB-210 treatment showed to be safe under conditions of the present study.

REFERENCES

• Uhlig T, Kyprianou T, Martinellia FG, Oppicia CA, Heiligersa C, Hills D, Calvo XR, Verhaert P. The emergence of peptides in the pharmaceutical business: From exploration to exploitation. EuPA Proteomic 2014; 4:58-69.