IRON-FREE TRANSFERRIN **APOTRANSFERRIN** AS A NEW TREATMENT TO IMPROVE STROKE OUTCOME IN INTRACEREBRAL HEMORRHAGE

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Barcelona, October 8th 2024
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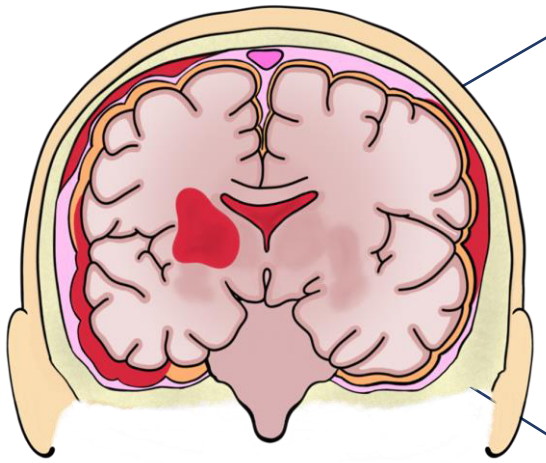
Background



Primary brain injury

- Mass effect
- Intracranial pressure
- Herniation
- Blood flow
- Tissue disruption
- …

BBB disruption

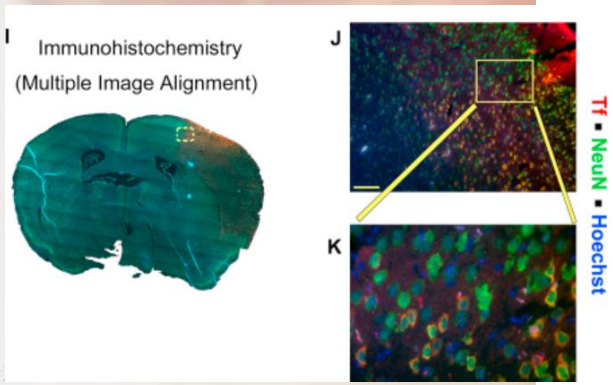
Neuronal damage

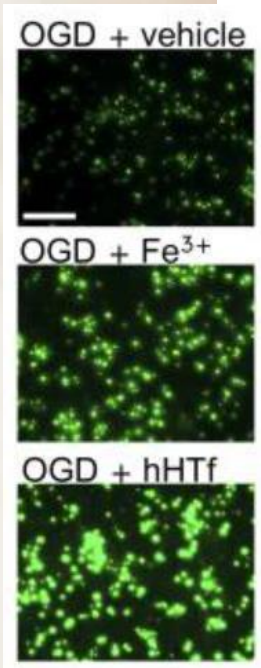
Cerebral edema

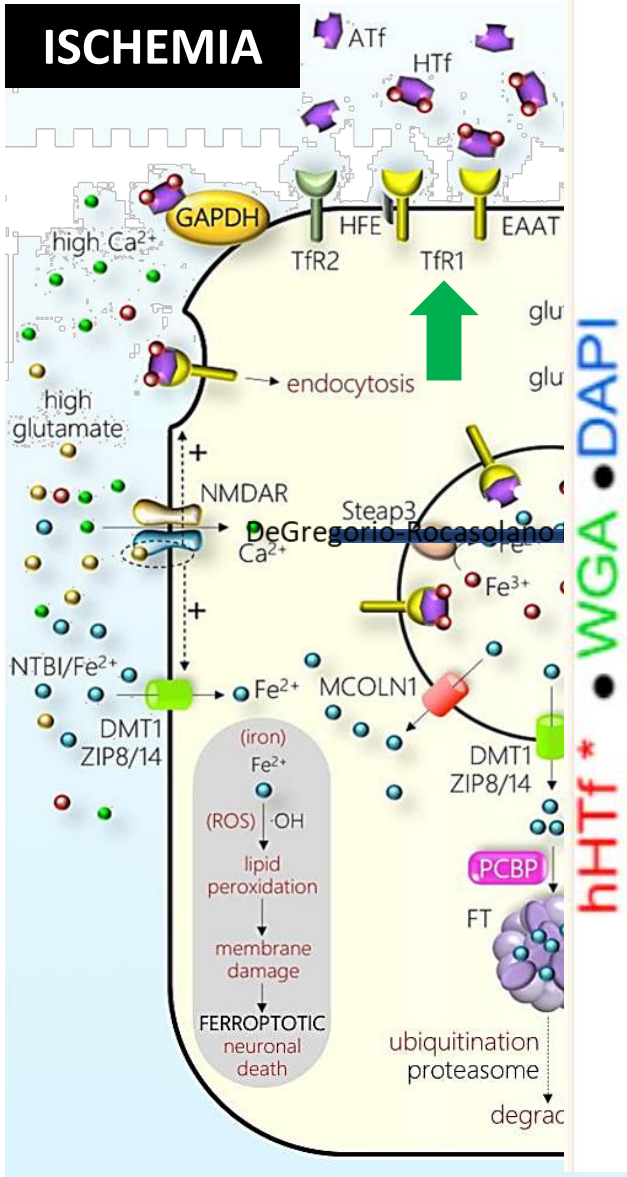
Secondary brain injury

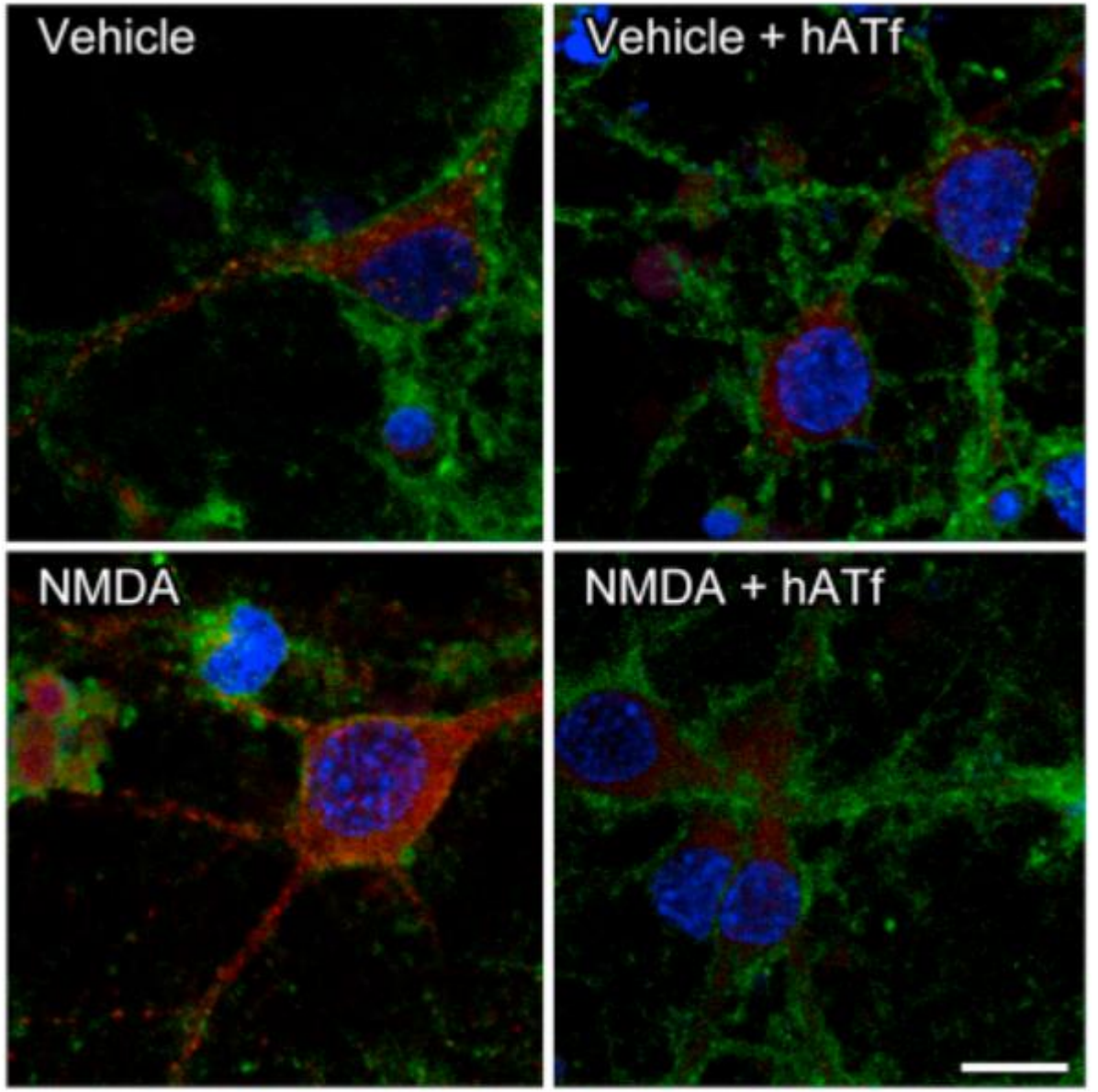
- Erythrocyte lysis
- Hemoglobin
- ROS
- Heme group & iron
- Inflammation
- …

Background









DeGregorio-Rocasolano N, et al Redox Biol. 2018
Millan M, et al. Antioxidants 2021

DeGregorio-Rocasolano N, et al Front Neurosci.. 2019

Background: ICH & IS share ferroptosis/iron-related mechanisms

Clinical Trial > Stroke. 2022 Apr;53(4):1149-1156. doi: 10.1161/STROKEAHA.121.035421. Epub 2021 Nov 18.

Effect of Deferoxamine on Outcome According to Baseline Hematoma Volume: A Post Hoc Analysis of the i-DEF Trial

Chenchen Wei [1 2 3], Jeffrey Wang [1], Lydia D Foster [4], Sharon D Yeatts [4], Claudia Moy [5], J Mocco [6], Magdy Selim [1]; i-DEF Investigators

Clinical Trial > Stroke. 2022 Jul;53(7):2204-2210. doi: 10.1161/STROKEAHA.121.037298. Epub 2022 Mar 21.

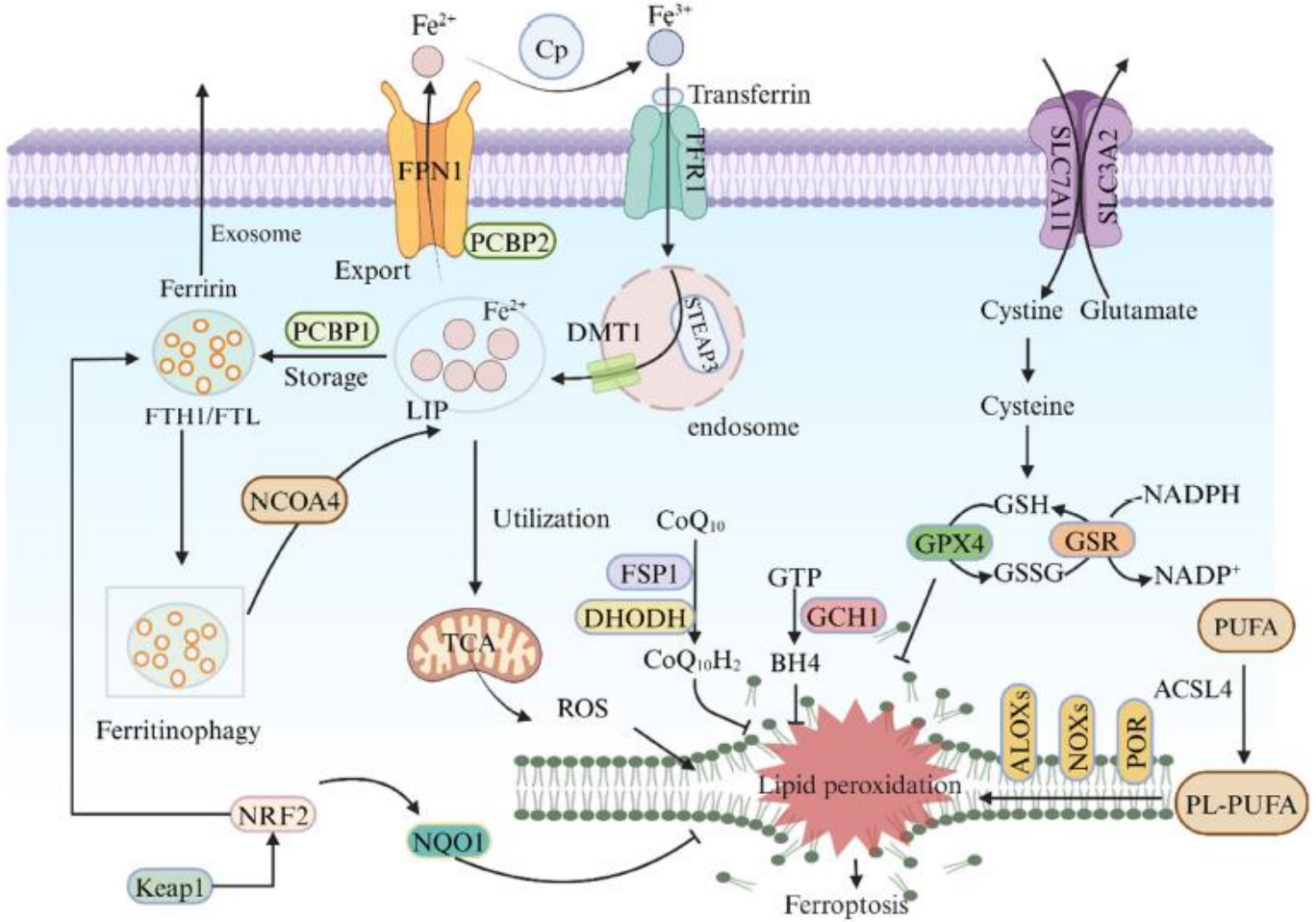
Effect of Deferoxamine on Trajectory of Recovery After Intracerebral Hemorrhage: A Post Hoc Analysis of the i-DEF Trial

Lydia Foster [1], Laura Robinson [2], Sharon D Yeatts [1], Robin A Conwit [3], Amjad Shehadah [2], Vasileios Lioutas [2], Magdy Selim [2]; i-DEF Investigators

> Antioxidants (Basel). 2021 Aug 10;10(8):1270. doi: 10.3390/antiox10081270.

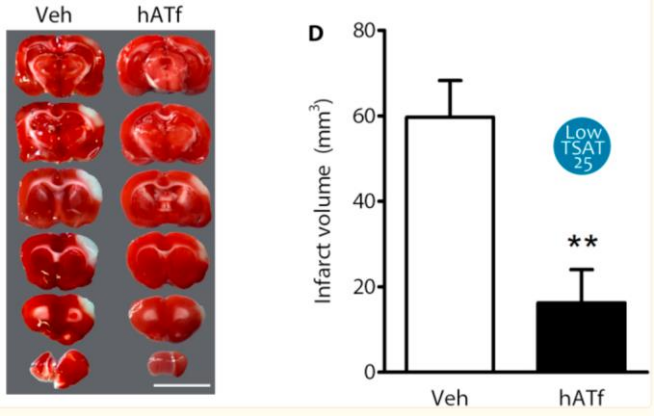
Targeting Pro-Oxidant Iron with Deferoxamine as a Treatment for Ischemic Stroke: Safety and Optimal Dose Selection in a Randomized Clinical Trial

Mònica Millán [1], Núria DeGregorio-Rocasolano [1 2], Natàlia Pérez de la Ossa [1], Sílvia Reverté [1], Joan Costa [3], Pilar Giner [4], Yolanda Silva [5], Tomás Sobrino [6], Manuel Rodríguez-Yáñez [7], Florentino Nombela [8], Francisco Campos [6], Joaquín Serena [5], José Vivancos [8], Octavi Martí-Sistac [2 9], Jordi Cortés [10], Antoni Dávalos [1], Teresa Gasull [1 2]



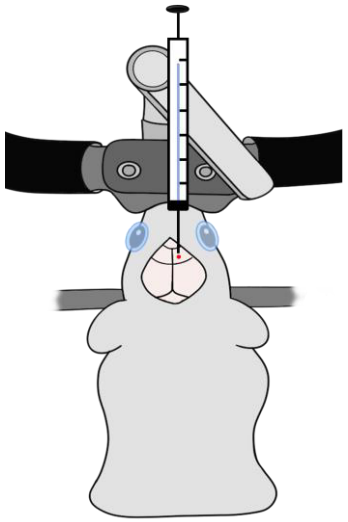
Zhaohui Chai, Jiesheng Zheng& Jian Shen. CNS Neurosci Ther. 2024

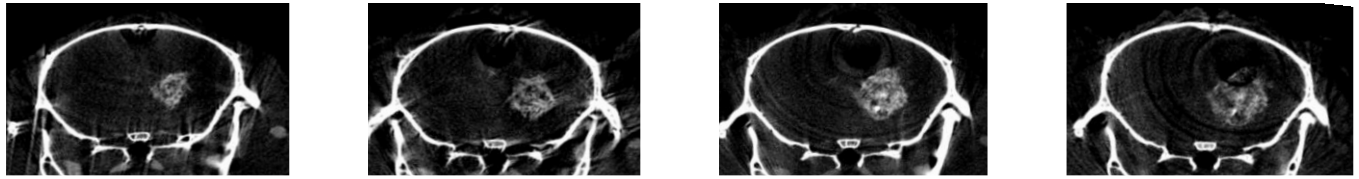
Hypothesis

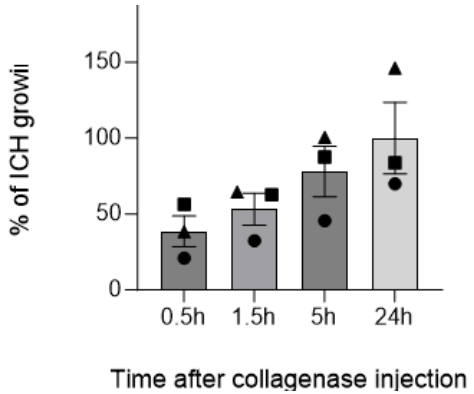


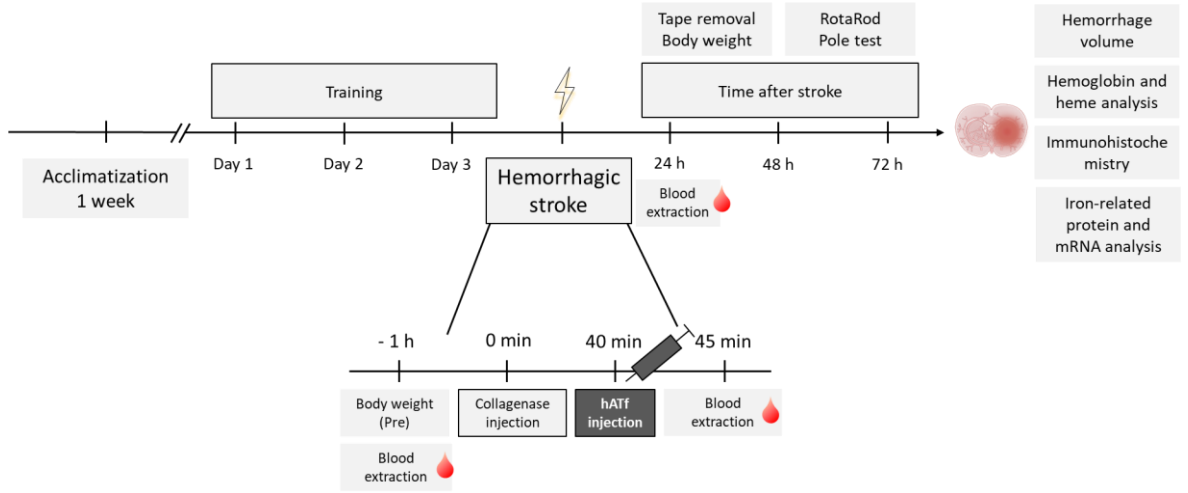
Apotransferrin is able to improve stroke outcome in intracerebral hemorrhage

Results

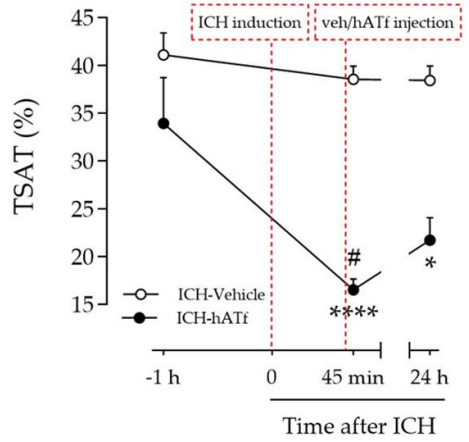


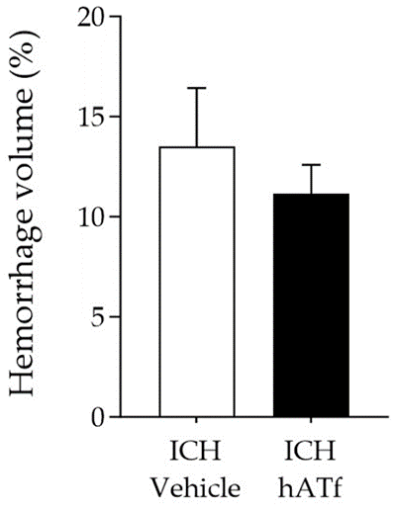

0.5h
1.5h
5h
24h

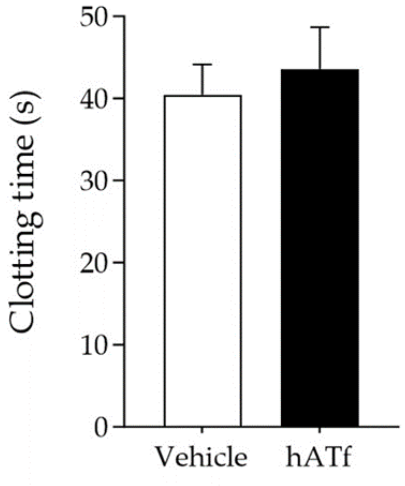

% of ICH growth
150
100
50
0
0.5h
1.5h
5h
24h
Time after collagenase injection

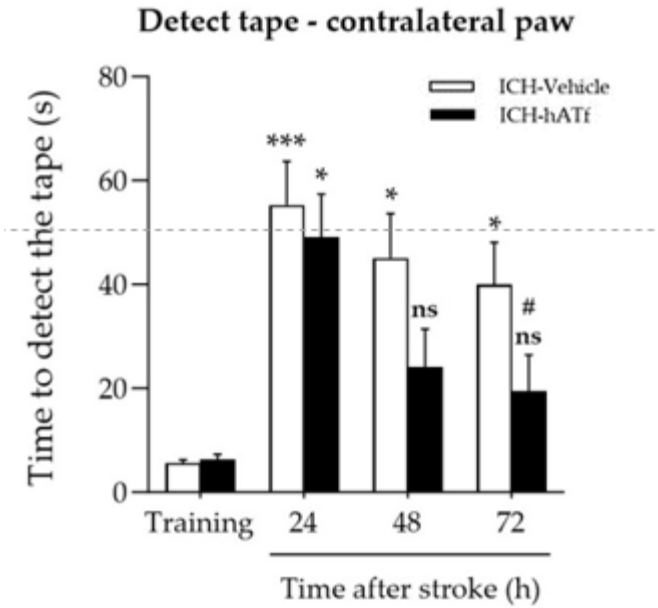

Tape removal
Body weight
RotaRod
Pole test
Hemorrhage volume
Hemoglobin and heme analysis
Immunohistochemistry
Iron-related protein and mRNA analysis
Training
Time after stroke
Acclimatization 1 week
Day 1
Day 2
Day 3
Hemorrhagic stroke
24 h
48 h
72 h
Blood extraction
- 1 h
0 min
40 min
45 min
Body weight (Pre)
Collagenase injection
hATf injection
Blood extraction
Blood extraction

Results

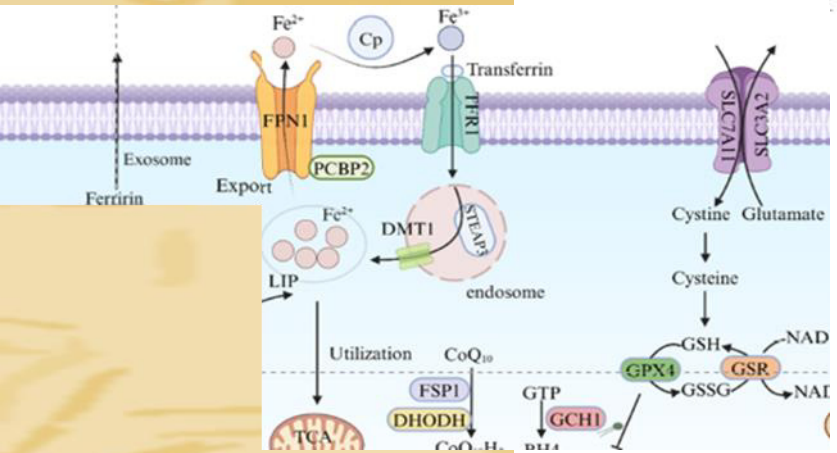

ICH induction
veh/hATf injection
TSAT (%)
45
40
35
30
25
20
15
ICH-Vehicle
ICH-hATf
#
****
*
-1 h
0
45 min
24 h
Time after ICH

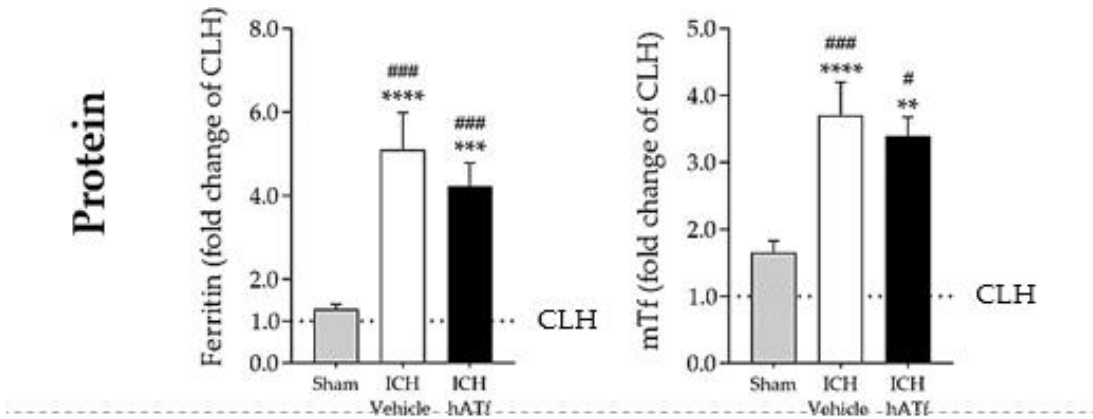

Hemorrhage volume (%)
20
15
10
5
0
ICH
Vehicle
ICH
hATf

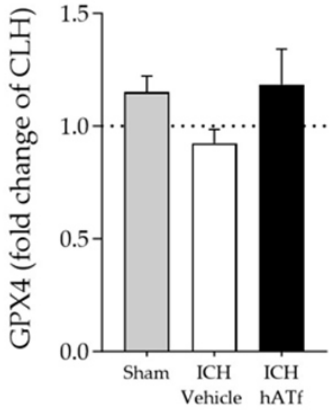

Clotting time (s)
50
40
30
20
10
0
Vehicle
hATf

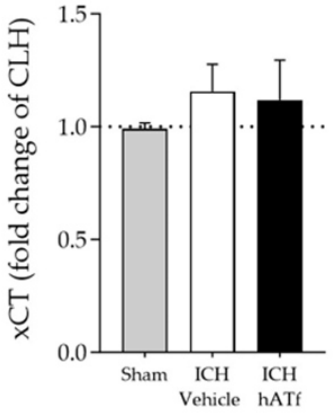

Detect tape - contralateral paw
Time to detect the tape (s)
80
60
40
20
0
ICH-Vehicle
ICH-hATf
***
*
*
*
ns
#
ns
Training
24
48
72
Time after stroke (h)

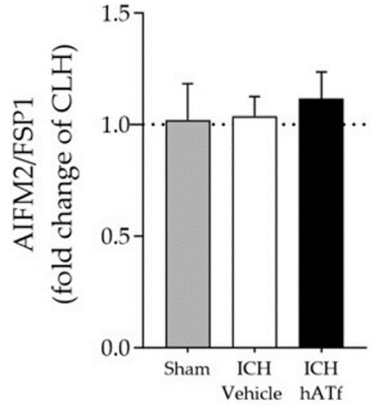
Results


Fe2+
Cp
Fe3+
Transferrin
FPN1
TFR1
SLC7A11
SLC3A2
Exosome
Ferritin
Export
PCBP2
Fe2+
DMT1
STEAP3
endosome
Cystine
Glutamate
LIP
Cysteine
Utilization
CoQ10
GSH
NAD
GPX4
GSR
GSSG
FSP1
GTP
DHODH
GCH1
TCA

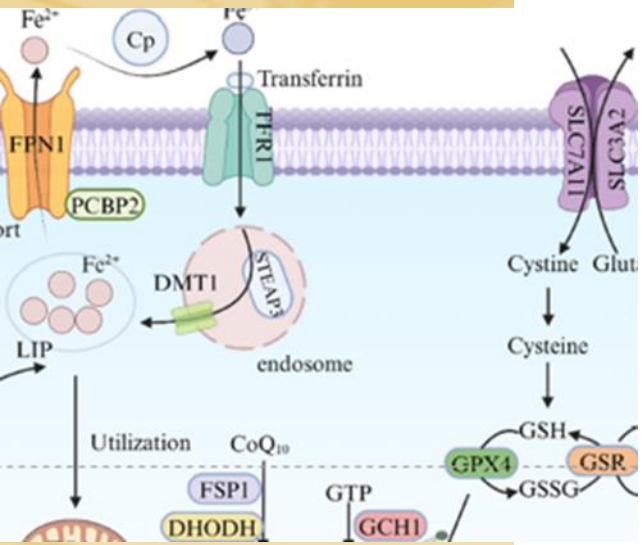

Protein
Ferritin (fold change of CLH)
8.0
6.0
4.0
2.0
1.0
0.0
###
****
###
***
Sham
ICH Vehicle
ICH hATf
CLH
mTf (fold change of CLH)
5.0
4.0
3.0
2.0
1.0
0.0
###
****
#
**
Sham
ICH Vehicle
ICH hATf
CLH

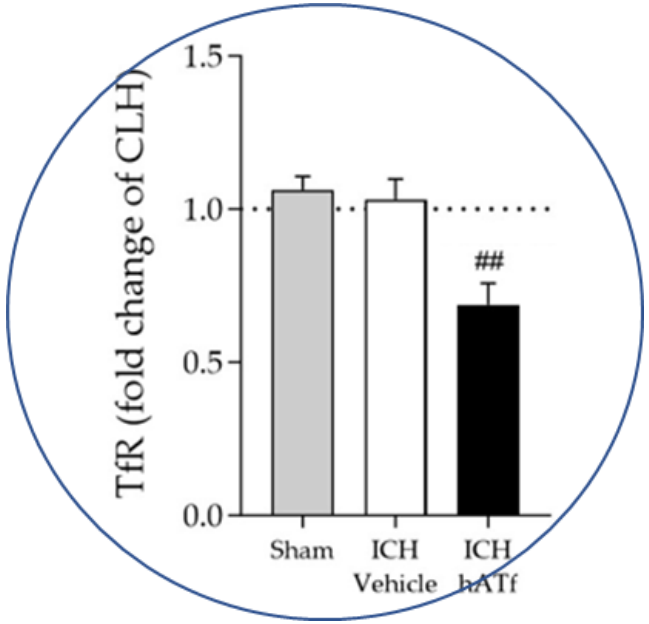

GPX4 (fold change of CLH)
1.5
1.0
0.5
0.0
Sham
ICH Vehicle
ICH hATf

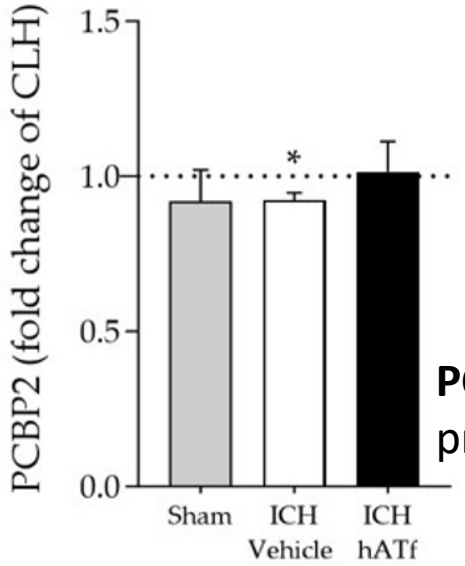

xCT (fold change of CLH)
1.5
1.0
0.5
0.0
Sham
ICH Vehicle
ICH hATf


AIFM2/FSP1
(fold change of CLH)
1.5
1.0
0.5
0.0
Sham
ICH Vehicle
ICH hATf

Results

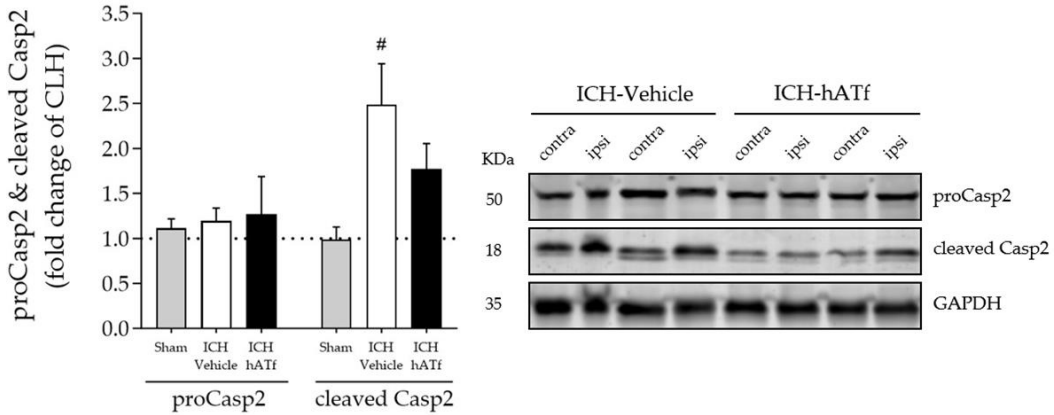




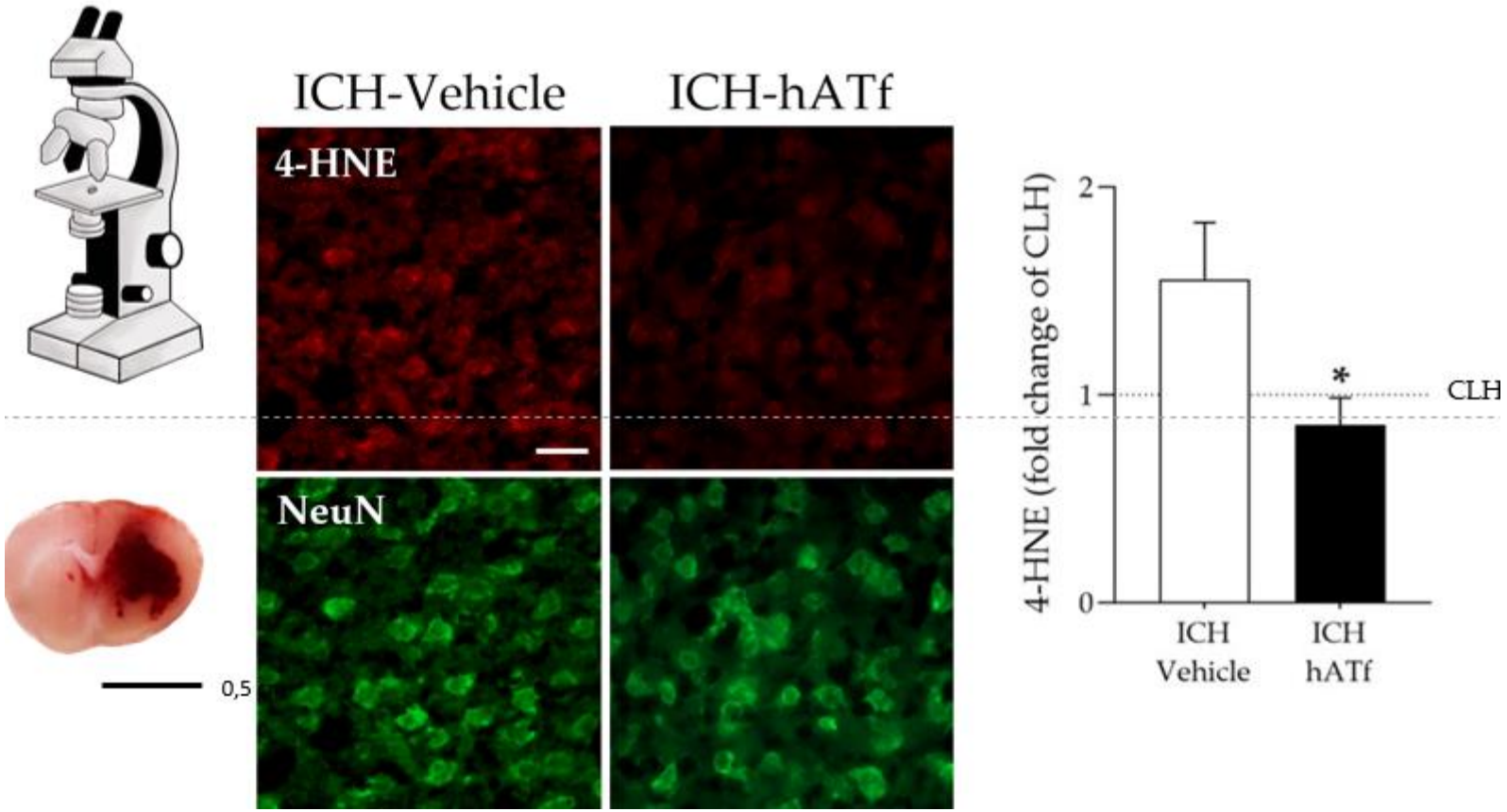


PCBP2 = Antiferroptotic and proapoptotic effect

APOPTOSIS RELATED-PROTEIN



Caspase 2 = upstream caspase effector


ICH-Vehicle
ICH-hATf
4-HNE
NeuN
0,5
4-HNE (fold change of CLH)
2
1
0
CLH
*
ICH
Vehicle
ICH
hATf

Conclusions

1) Apotransferrin administered intravenously during the ICH bleeding period does not reduce hematoma size or have any effect on clotting.
2) Apotransferrin immediatelly reduces TSAT in blood.
3) It reduces the ferroptotic marker TfR in brain tissue, with TfR1 being the main gate of iron entry in the neurons.
4) It normalizes the activity of PCBP2 and the activation of caspase 2.
5) Apotransferrin improves the sensorimotor performance of the ATf-treated mice, which is associated with a reduction of the free radicals in the areas near the hematoma.

Thank you

RICORS-ICTUS

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CMCiB Comparative Medicine & Bioimage Centre of Catalonia

Germans Trias i Pujol Hospital
Institut Català de la Salut

INc Institut de Neurociències

UAB Universitat Autònoma de Barcelona

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