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#24

Passive Immunity in Parkinson's Disease

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INTRODUCTION AND OBJECTIVE

- Parkinson's Disease (PD) is the second most common neurodegenerative disorder
- Inspection of postmortem samples from patients reveal the presence of intracellular α synuclein (α-syn)-containing aggregates in neuronal cell bodies
- To check if clearance of these aggregates will help in disease treatment, the blood–brain barrier (BBB) needs to be interrupted to allow the passage of antibodies and drugs into the brain
- BBB prevents uptake of ~98% of small molecules and prevents the entry of most drugs from the blood into the brain – certain small molecule drugs may cross the BBB via lipid-mediated free diffusion
  - These drugs should have a molecular weight <400 Da and forms <8 hydrogen bonds to pass
- IgGs are known to poorly permeate the BBB (~0.1% of injected plasma immunoglobulins enter the brain)
- Recent research suggests that focused ultrasound briefly opens the BBB, thus allowing the passage of antibodies into the brain

- **<u>Objective</u>: To determine the kinetic distribution of intraperitoneal (IP)-injected antibodies into the blood as a prerequisite for using such injections in combination with ultrasound to optimize antibody entry into the brain to pave the way for future passive immunotherapy for PD.**

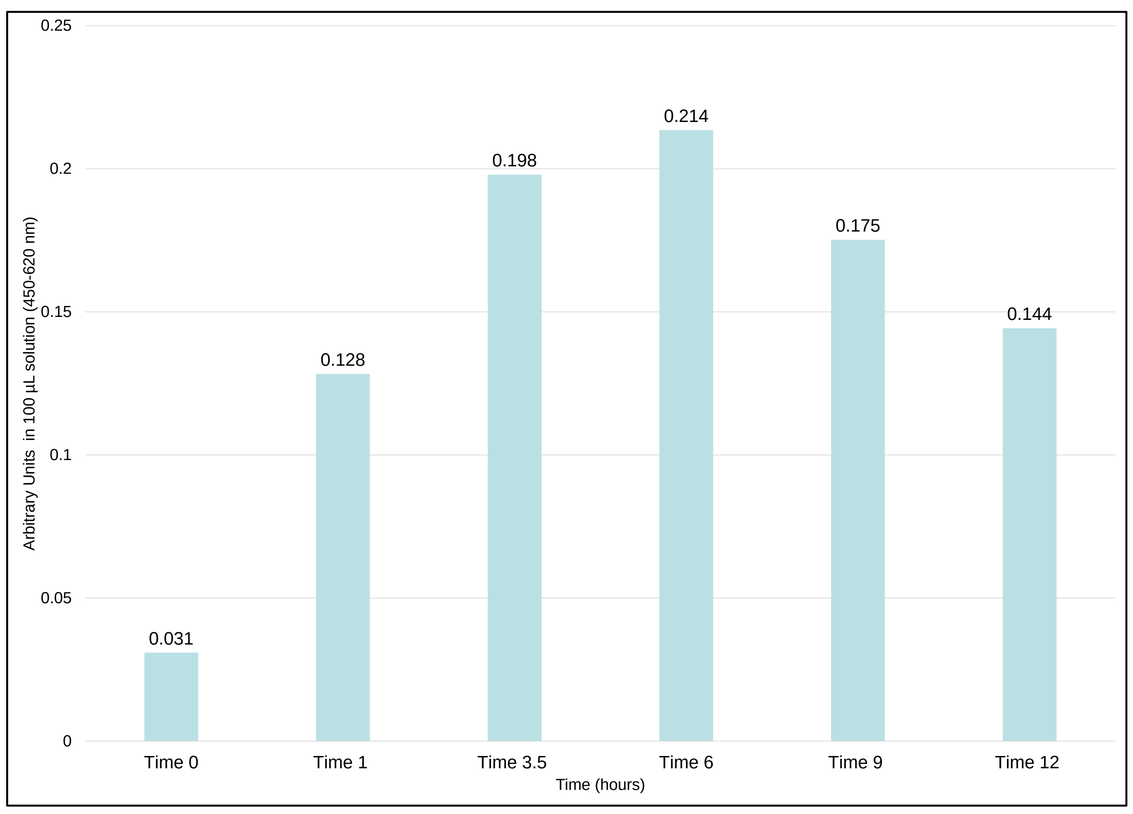
METHODS

- Two experiments:
  - Experiment one:
    1. Inject three mice IP with 1 mg mouse measles antibody for every kg of mouse
    2. Anesthetize mice 1-hour post-injection with ketamine / xylazine and draw blood
    3. Sacrifice mice 6 hours post-injection
    4. Separate plasma and measure mouse measles antibody with modified quantitative ELISA assay

- Experiment two: set up four-time groups (N=3), time = 0, 3.5, 9, and 12-hours post-injection
  1. Inject three mice IP with 1 mg mouse measles antibody ÷ kg mouse, except for time 0-hour cohort
  2. Sacrifice mice at respective post-injection times
  3. Separate plasma and measure mouse measles antibody with modified quantitative ELISA assay

RESULTS

Figure 1: 1 mg / kg injection of mouse measles antibody (Ab; N=3)



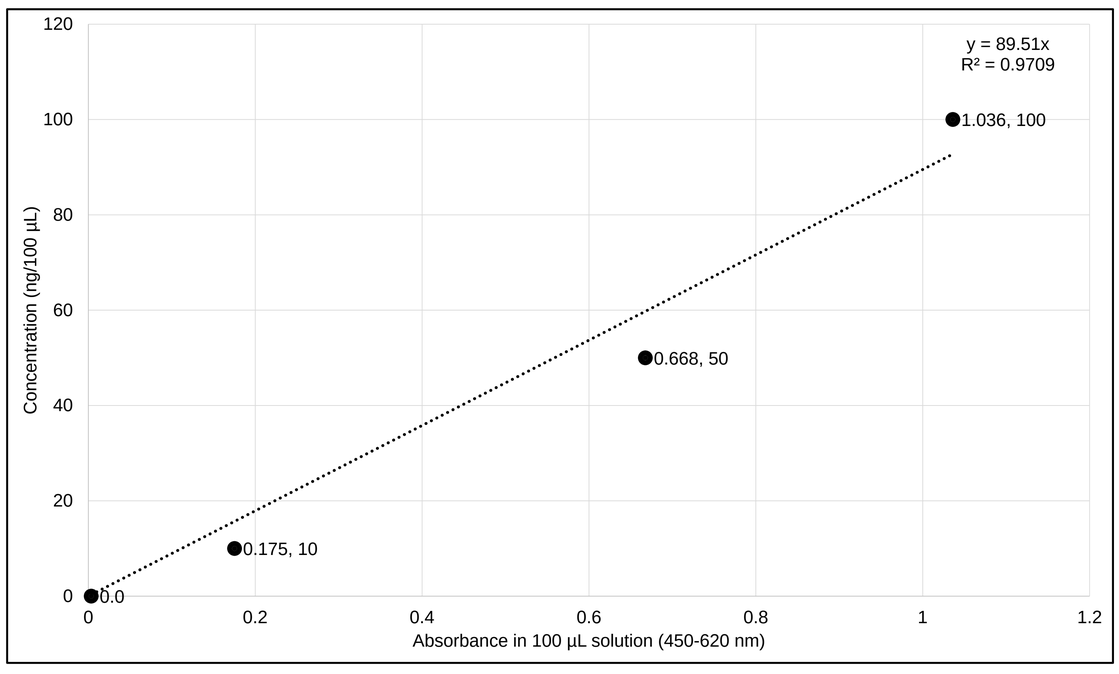
Results are means of arbitrary absorbance units in blood (1:10 dilution)

Table 1: Time-course of blood IgG concentrations

	0-hours post-injection	1-hour post-injection	3.5-hours post-injection	6-hours post-injection	9-hours post-injection	12-hours post-injection
Absorbance (450 – 620 nm) of 1:10 dilution of plasma (100 µL solution)	0.031	0.128	0.198	0.214	0.175	0.144
Concentration (ng in 100 µL solution) (adjusted for background)	0.000	8.718	14.951	16.345	12.913	10.146

Arbitrary absorbance units were converted into IgG concentrations using a standard curve (Fig. 2)

Figure 2: Standard curve



Linear relationship between arbitrary absorbance units (x-axis) vs. IgG concentrations (Y-axis)

CONCLUSIONS

- Currently, tests that determine accurate pharmacokinetics of IP-injected antibodies against non-mouse antigens are currently unavailable in the literature
- This knowledge proves important because passive immunotherapy is exploding as a therapeutic means for neurodegenerative disorder
- Because the mouse measles antibody and the α synuclein antibody are both IgG1 antibodies, they share the same pharmacokinetic factors
- To measure the levels of the antibody with a standard market ELISA assay deemed difficult because most are qualitative assays
- We modified and validated a quantitative human measles ELISA assay
- Highest level of IgG1 antibodies was measured at 6 hours post-IP injection with a concentration of approximately 163 ng in 10 µL of plasma
- Since approximately 30,000 ng of measles antibody was injected-IP and approximately 163 ng of antibody was recovered in blood, then we know that to change antibody concentration in blood by 1 ng requires approximately 185 ng of injected antibody
- Future studies will investigate the improvement in penetrance of antibodies into the brain with focused ultrasound, as well as investigate the location of these antibodies by attaching them to gadolinium and scanning with MRI

ACKNOWLEDGEMENTS

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