Inter-alpha inhibitors: the bacon of blood proteins

An investigation into the in-vitro interactions between Inter-alpha inhibitors and erythrocyte hemodynamics

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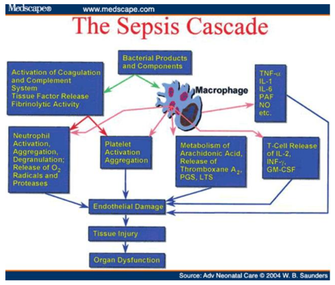
Purpose

The purpose of this research is to investigate the effects of IAIP on different parts of the inflammatory cascade, more specifically red blood cell aggregation, sedimentation and morphology. Research in animal subjects show that IAIP is effective in treating a vast range of inflammatory diseases, but little is known on how or why. The purpose of my research is to examine the direct effect IAIP has on the conditions of blood introduced by inflammation.

Background

Inflammation:

- Inflammation is a response from body tissues to danger signals such as pathogens, damaged cells, or irritants used to protect itself from further harm.
- Inflammation is necessary for survival as it facilitates tissue repair.
- While minor inflammatory responses are crucial for all instances of healing, severe inflammatory responses could fatally damage the body.
- Sepsis is an example.
- Sepsis is common and often deadly. It still remains the primary cause of death from infection, despite advances in modern medicine.



- When an inflammatory response has been triggered, IAIP levels have been found to drop.
- When protease levels do not equal protease inhibitor levels, sepsis related tissue damage occurs and it is known to be fatal.

IAIP:

- Inter-alpha inhibitor protein (IAIP) is a naturally occurring blood protein found in high concentrations in all mammals.
- Current research suggests that IAIP plays a large role as an anti-inflammatory in the body.
- IAIP increases the survival rates of mice with sepsis by around 90% by suppressing the proinflammatory cytokine activity.
- IAIP reduces the mortality rate of mice with severe sepsis even with delayed administration.
- IAIP increases the survival rate of mice from 0% to 71% in anthrax.
- IAIP also reduces the effects of histone induced injuries.
- It is currently known that IAIP has therapeutic values in treating inflammation, however it is not know what part of the inflammation cascade it targets.

Materials

- Nikon diaphot microscope
- Dextran 580 kDa purchased from Sigma Aldrich
- Inter-alpha-Trypsin inhibitors purified by Prometic
- Phosphate buffered saline
- Hydrogen peroxide
- Hemocytometer
- Erythrocytes produced by finger prick
- Depleted plasma by centrifuge from blood bank
- Fibrinogen by centrifuge from blood bank
- Eppendorf pipettes, sizes 20µl, 100µl, 200µl, and 1ml
- Jitterbug microplate incubator
- Torvex 3000 lab mixer
- ThermoFisher Scientific microplate
- iPhone for photography

Hypothesis

Due to its prior success with neutrophils, I suspect that IAIP will prevent aggregation, reduce sedimentation rate and reduce form factor in affected erythrocytes.

Methodology

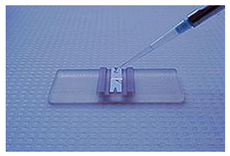
Preparation of materials:

- A dextran solution of 10mg/ml was created for aggregating cells.
- This amount of dextran was found through testing different amounts.
- Blood was taken via finger prick.
- A drop of blood was then put in a 1 ml tube with PBS and centrifuged at ~1800 rpm for about a minute.
- The tube was then taken out and the PBS was removed, without disturbing the blood pellet and PBS was refilled to 1 ml.
- The process was repeated 3 times with the last time refilling the blood solution up to 250 microliters with PBS.
- This process was used to remove all IAIP from the system to prevent inaccurate results.

Testing for the effect of IAIP on RBC aggregation:

- A microscope was used to take photos to inspect both aggregation and the morphology of cells.
- A hemocytometer was used with the microscope to count the number of cell aggregates per defined area.
- RBCs were put into a microplate and dextran and plasma were added to aggregate the cells. Then, IAIP was added in differing amounts to test for effectiveness.
- To use the hemocytometer, 10 microliters of solution were pipetted into the slide, which disperses through capillary action.
- The hemocytometer was then put under the microscope for inspection and data was recorded.

Hemocytometer shown here

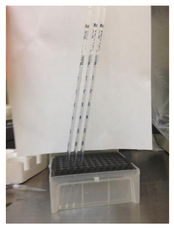


Testing for the effect of IAIP on RBC morphology:

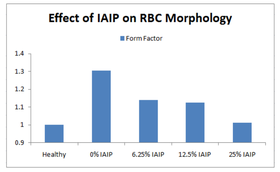
- Hydroperoxides were used to distort RBCs.
- RBCs were put into a microplate with hydroperoxides which distorted RBCs to be oval instead of perfect circles
- IAIP was then introduced in differing amounts to test for its ability to reverse the damage done by oxidation
- The microplate was then put under the microscope and photos were taken
- The photos were later analyzed for form factor

Testing for the effect of IAIP on RBC sedimentation:

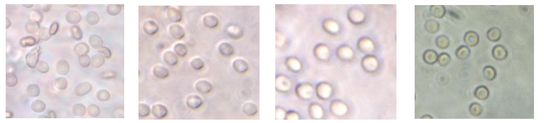
- Traditional methods are very expensive and time consuming.
- I created a new ESR device that could measure sedimentation rate quicker and cheaper.
- The device consisted of a glass tube with markings on the side filled with a 2.5% glycerol solution and was sealed at the bottom with clay.
- Differing amounts of dextran were used to test the ESR device for accuracy.
- The blood solution then dropped in through the top and the cells made their way downwards with speeds depending on the degree of aggregation.
- Data was then recorded on the sedimentation rates of various solutions with differing amounts of IAIP in it.



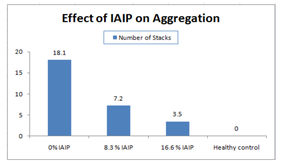
Results



	0% IAIP	25% IAIP
Mean	1.305	1.01125
Variance	0.011546667	0.000678333
Observations	16	16
Hypothesized Mean Difference	0	
df	17	
t Stat	10.62706734	
P(T<=t) one-tail	3.15154E-09	
t Critical one-tail	1.739606716	
P(T<=t) two-tail	6.30312E-09	
t Critical two-tail	2.109815559	

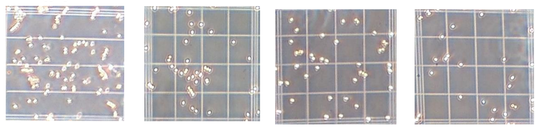


0% IAIP 6.25% IAIP 12.5% IAIP 25% IAIP

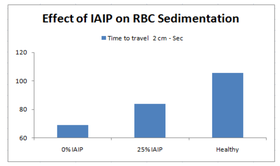


# of aggregates per 100nl of solution

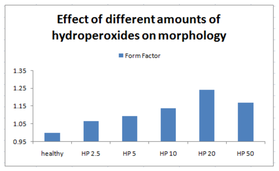
	0% IAIP	16.6% IAIP
Mean	18.1	3.5
Variance	49.88421053	6.368421053
Observations	20	20
Hypothesized Mean Difference	0	
df	24	
t Stat	8.705554356	
P(T<=t) one-tail	3.42402E-09	
t Critical one-tail	1.710882067	
P(T<=t) two-tail	6.84804E-09	
t Critical two-tail	2.063898547	

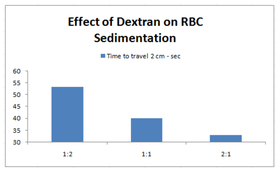


0% IAIP 8.3% IAIP 16.6 % IAIP Healthy



	0% IAIP	25% IAIP
Mean	69.2	83.8
Variance	13.2	55.7
Observations	5	5
Hypothesized Mean Difference	0	
df	6	
t Stat	-3.933038947	
P(T<=t) one-tail	0.003842924	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	0.007685848	
t Critical two-tail	2.446911846	





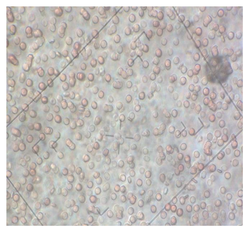


Figure 1 after treatment

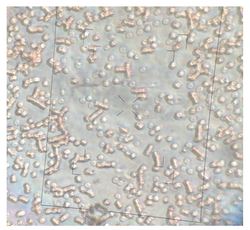


Figure 2 before treatment

After 20 minutes of incubation, the effect of IAIP is clear. Before treatment, cells are aggregated as shown in **Figure 2**. After treatment (20 microliters of IAIP), they are aggregated as shown in **Figure 1**. Most signs of aggregation are no longer visible, and the erythrocytes now resemble healthy erythrocytes.

Analysis

The use of IAIP significantly reduces chemically induced erythrocyte aggregation, sedimentation, and morphology.

- For aggregation, IAIP was able to almost entirely reverse the effects of dextran, a chemical. This is very promising and shows that IAIP will have an even greater effect as an anticoagulant in inflammation.
- IAIP was also able to almost entirely reverse the damage caused by hydrogen peroxide, a ROS (Reactive Oxygen Species), which is commonly released during inflammation.
- Further evidence in sedimentation rate shows that IAIP was able to significantly reduce aggregation.
- Connecting the dots, IAIP shows that it can promote smoother blood flow and higher tissue perfusion in inflammatory diseases.
- Also, IAIP is a serine protease inhibitor, which means that it protects against organ injury from proteases, showing significant therapeutic potential.

Discussion

- The results combined with previous information known about IAIP suggest that IAIP would be successful in treating a vast number of acute inflammation cases with no side effects.
- From this study and other research, it can be understood that IAIP targets many parts of the inflammatory cascade.
- This makes it a viable treatment even if administered late into the cascade.
- For example, death in sepsis occurs when there is insufficient tissue perfusion to the brain, but IAIP serves both as an anticoagulant and a protease inhibitor, which allows it to reduce organ damage and to facilitate healing through smoother blood flow.
- Traditional methods of thinning blood in sepsis include NSAIDs, or if severe enough, using the sepsis six.
- Methods in the sepsis six include introducing new RBCs and oxygen into the system.
- If IAIP was introduced, it would increase the tissue perfusion ability of the residing RBCs and aid the new RBCs in even higher tissue perfusion
- IAIP does not affect platelet activity, making it even more suitable as an inflammatory disease treatment

Future Research

- Potentially, a micro-capillary machine could be used to test for the efficiency before and after treatment for passing through micro-capillaries.
- In the future, research could be done in vivo to further confirm the in vitro evidence provided here.
- Investigation of the effect of IAIP on RBC adhesion to the endothelium will also be done.