What is the Effect of Omega-3 Fatty Acids on the Vascular Permeability of the Cellular Junctions in Bovine Blood Cells as a Model for Cerebral Cavernous Malformations?

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Abstract

Cerebral Cavernous Malformations occur when a deletion mutation in the CCM1/2/3 genes cause the loss of the KRIT1 protein. The protein maintains the structural integrity of the endothelial cell junctions, and the lack thereof leads to the structure being compromised, causing the bursting of the blood vessels in the brain. The compromised cell junctions lead to increased vascular permeability, which this study aims to increase. To model these conditions, two types of bovine blood cells were used, one extracted from a cow supplemented with increased omega-3 fatty acids in the diet, and one fed a regular meal. Both types of blood were placed in different concentrations of hypertonic solutions that model the mutated cells. The corn gluten meal that has high fat concentrations is hypothesized to affect the cell membrane of the cell as it is made up of lipids. This should increase the vascular permeability between the cell junctions of the blood cells, as there is an increased thickness of the cell membrane, making up for the compromised structure (increased distance between cells) of the cell junction due to the mutation. The results were analyzed through both quantitative (statistical tests) and qualitative tests (comparison of cell structure and size) The hypothesis was proven correct, making the use of omega-3 fatty acids a promising frontier for combating the effects of the deletion mutation in the CCM1/2/3 genes.

Question

What is the effect of Omega-3 Fatty Acids on the Vascular Permeability of Cellular Junctions of Bovine Blood Cells as a Model for Cerebral Cavernous Malformations?

Hypothesis

The addition of the increased Omega-3 fatty acid in the diet will affect the membrane dynamics, and decrease the distance between bovine blood cells and their average size in a hypertonic solution compared to a cow fed a regular meal.

Literature Review

The increase of Omega-3 fatty acids present in the diet of the cows should have an effect on the membrane dynamics of the plasma membrane. This is due to the fact that The plasma membrane is composed of primarily lipids, and a change in the diet plays a direct role in the composition of the membrane. (Plewes et. al 2018)

The fish meal is high in Omega 3 fatty acids compared to the regular corn-meal diet, which acts as the control (White et. al 2012)

Cerebral Cavernous Malformations occur due to the high vascular permeability between the cell junctions. If the structure of the cellular membrane that makes up the junctions is strengthened, this will in turn also strengthen the cellular junctions. This is true for both ways; the first through improving the structural integrity, and due to the decreasing distance between two cells due to the increased thickness of the cell membrane. (Dejanna, Lasserve, Weinstein 2009)

Variables

Independent Variable: Fish Meal Omega-3 Fatty Acid Diet with 5% DMI
Dependent Variable: Bovine Blood in different solutions

- 0.9g/100mg (Isotonic)
- 1g/100mg (Hypertonic Sol 1)
- 1.5g/100mg (Hypertonic Sol 2)
- 2g/100mg (Hypertonic Sol 3)

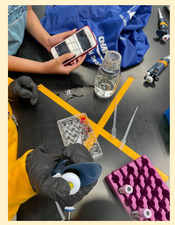
Control Group: Regular Corn Meal Diet with 6%DMI

Methods

Sample Preparation

1.) 2 Non-lactating Angus Cows (Bos Taurus) between 2 and 8 years of age were fed two different diets. One of them was supplemented with fish meal at 5% DMI (Dry Matter Intake). The fish meal is a rich source of eicosapentaenoic acid and docosahexaenoic acid, both forms of Omega-3 fatty acids. The other was fed a regular corn-gluten meal at 6%DMI.

2.) Samples were collected 20 days after supplementation, and stored in potassium salt vacutainers after collection to prevent clotting.



Solution Preparation

There are four solutions prepared in total, one isotonic, and three different concentrations of sodium in a hypertonic solutions.

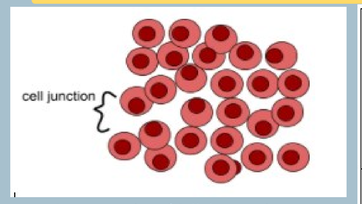
1) Measure out 4 different beakers of 100g of water.
2) Use a scientific scale to measure 0.9g, 1g, 1.5g, and 2g of salt respectively.
3) Pour each measurement in its designated beaker, and mix each solution with pipette with caution to prevent cross-contamination.

Bloodwork

1) Use a micropipette with a new tip to measure 1000µL of the isotonic solution (first solution that contains 0.9g of salt)
2) Dispense into a well in a 24 well plate, and dispose of the tip
3) Refill tips, and measure 20µL of the control blood, and start a stopwatch immediately after release.
4) Swirl the well plate, and move the well-plate under a light microscope, and calibrate until desired focus is achieved. Take pictures at 1:30.
5) Repeat steps 1-4, and substitute control corn-gluten blood for fish meal blood, and dispense in the well vertically below the previous for convenience.
1) Repeat steps 1-5 with each different solution; (1g, 1.5g, and 2g) Place controls Horizontally side by side, and trials vertically opposite.

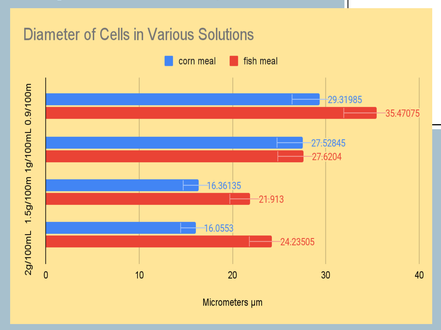
Organization of Results

1) Take caution to label all image files correctly with correct solution, concentration, and sample.
2) Use software to e the data. (size, concentration, shape, distance, etc.) The specific software that was used in this study was a free version of the ImageJ software. Other applications such as MIPAR may also prove to be suitable.



Representation of Cellular Junctions

Visual differences can be observed from the images recorded form the microscope from different solutions in the control and treated blood.



Bar graph comparing the diameter size between the cells in different solutions, isotonic, and hypertonic. The one isotonic solution has 0.9 grams of salt per 100 mL of water, and there is 1g, 1.5g, and 2g or salt per 100mL of water for the hypertonic solutions.

0.008963461318	0.008963461318	0.000269877781	0.00005416077547
isotonic 0.9g/100mL	hypertonic 1g/100mL	hypertonic 1.5g/100mL	hypertonic 2g/100mL

A t test was conducted to determine statistical significance. Four different t tests were conducted between the 4 different isotonic and hypertonic solutions. The t test was conducted between the treatment of the fish meal supplement with Omega-3 Fatty Acids, and the treatment of the corn gluten meal that is fed normally, and was used as a control in our experiment. All the t tests proved to be statistically significant due to all results being less than 0.05.

1		34.928	34.409	18.248	34.059	13.892	19.799	18	21.095
2		30.594	22.627	20	28.2	14.318	21.095	21.095	25.71
3		21.541	27.857	18.248	27.295	15.232	28.601	15.556	31.765
4		37.577	34.928	20.396	36.139	17.205	28.844	20	23.345
5		24.166	39.294	27.893	24.698	12.207	34.828	8.944	24.352
6		25.06	45.343	20.396	25.632	13.454	19.105	10.817	20.881
7		25.298	36.056	36.056	31.321	18.439	14.213	14.318	26.926
8		14.56	34.928	29.155	28.792	16.643	28.46	17.088	30.364
9		25.612	42.048	32.016	27.459	16.763	21.541	12.166	25.612
10		33.287	31.623	38.013	26.401	12.369	25.456	20.616	26.571
11		29.732	32.802	34.059	27.295	18.358	19.416	18.248	9.899
12		41.037	36.77	25.942	20	19.698	21.633	9.22	18.974
13		50.933	32.558	28.46	25	20.518	20.125	13.038	27.459
14		23.324	45.343	25.298	25.179	16.763	19.849	18.028	17.029
15		22.361	36.878	31.89	27.459	21.954	13.038	31.78	15.133
16		31.113	50	32.016	36.878	17.205	14.213	24.698	30.414
17		25.612	37.202	23.087	28.46	12.369	28.46	11.045	20.518
18		24.166	27.857	32.062	22.091	17.029	15.652	10.296	33.601
19		21.633	34.059	31.257	27.203	17	22	13	21.932
20		43.863	26.833	26.077	22.847	15.811	21.932	13.153	33.121
21									
22									
	avg	29.31985	35.47075	27.52845	27.6204	16.36135	21.913	16.0553	24.23505
		iso c	iso t	hyp 1 c	hyp 1 t	hyp 1.5 c	hyp 1.5 t	hyp 2 c	hyp 2 t

Representation of all the data collected through a software called ImageJ. ImageJ was used to convert the images to binary, and then have each cell automatically measured by the software. The two shortest points from the cells circumference were measured for each cell. After a change in the scale from pixels to micrometers were made, the first 20 cells for each sample were transferred to a spreadsheet, and averages were calculated. The Bar Graph on the left is also derived from this data.

Results

The results are as followed based on analysis:

We took 20 measurements of cells per picture, leading in 20 cells per 8 columns. The first two columns showed both the treated blood and control blood in a isotonic solution, which is why the measurements of the diameters is relatively larger than the rest of the measurements. The lower amounts of saline in the solution led to less shrinkage due to the fact that there was not that much water travelling out of the cell. You can see that even in the isotonic solution, the treated sample had a significantly larger diameter size. This was true for all solutions. The hypertonic solution with 1g/100mL had a pretty close average. Through more trials in the future we would hope to see a larger statistical difference. With the increased concentrations of salt in the last solutions, you can see a very large difference in the averages of the diameter proving our hypothesis true.
You can also see this qualitatively in the images taken through the microscope, there is fewer cells completely shrunken in the greater concentrations in the treated solution compared to the untreated.
Lastly, we performed 4 t tests between both the isotonic and the hypertonic solutions. The tests proved to be statistically significant as they showed a visible difference between the control and treated blood effect on the size and the structure of the cells as each t test produced
a numbered t value less than 0.05.

Conclusion

The hypothesis was supported, and this was seen in multiple ways. Through the visible difference in the images taken through the light microscope you can see that the density of the cells is significantly larger in the treated cell sample, and you can see that the overall size of the cell is less shrunken and wrinkled in the treated sample. This can especially be seen in the higher concentrations of the hypertonic solution. Through our quantitative measurements taken by ImageJ, you can see that the overall diameters of the cell, taken from the two closest points of the cells circumference, have lower values for the control group than the treated version. This is also more clearly seen through the averages taken from each data set. In every concentration of sodium to water, the control group mixed inside was always lower than the treated version. Finally, this can also be seen through the t test that was conducted. There were 4 different t tests conducted, one for every concentration of sodium to water. The t tests compared the diameters of the cells between the control group and the Omega -3 treated groups in the different solutions. Every t test proved to have a statistical relevance due to the fact that they all had p values of less than 5%. This shows that there was a significant change between the Omega -3 treated blood, and the regular blood. Our hypothesis was proven because vascular permeability was shown to have increased with the treatment in all of our results. This shows that the use of Omega - 3 fatty acids, especially through the form of eicosapentaenoic acid and docosahexaenoic acid are a promising frontier for future exploration in managing the symptoms of Cerebral Cavernous Malformations by increasing the surface area of the cell membrane, thereby decreasing vascular permeability of cellular junctions, and of the cell itself.

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that couldn't be compiled into this poster;
the QR code displayed to the right will lead you to our full list.