The Effect of L-cystine on ACD and Blood Fluidity: A Replication Study

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Abstract

Blood fluidity can be impaired by a variety of factors, one of them being acetaldehyde, or ACD. ACD is one of the main metabolites of ethanol metabolization and is usually broken down by aldehyde dehydrogenase. However, the gene that encodes this enzyme, ALDH2, is often subject to defects, which results in the buildup of ACD. Notably, heavy drinkers with the defect are often at risk for digestive tract cancers. When in the body, ACD first increases the blood pressure and heart rate, but as the molecule affects smooth muscle in organs, both the blood pressure and heart rate decrease to dangerously low levels. In response to this effect of ACD, the amino acid L-cysteine was introduced into the present study by Otayama et al. to determine its effects on the concentrations of ACD. This study tested the effects of ACD on blood fluidity as well as the effects of L-cysteine on an ACD concentrated system. Overall, it was found that L-cysteine mitigates the effects of ACD on blood fluidity in a concentration dependent manner.

Introduction

Acetaldehyde, or ACD, is a highly toxic carcinogen and is produced through the process of the body metabolizing alcohol, or ethanol [2,3]. ACD has a variety of effects on the human body, including vasodilation, hypertension, increased skin temperature, flushing of the face and decreased blood pressure [1]. Consumption of alcohol increases blood viscosity which was a factor of interest in this study. Blood fluidity is an indicator of the effects of ACD and will be measured through a microchannel that simulates the flow of blood cells [4,6].

The observed effects are due to ACD stimulating the release of the hormone epinephrine and neurotransmitter norepinephrine from the sympathetic nerve cells and adrenal gland [1]. The

release of these compounds causes the cardiovascular effects seen with increasing ACD levels. Vasodilation and flushing of the face is caused by release of histamine and bradykinin [1]. The heart rate and blood pressure increases at first but as the ACD affects the smooth muscle in organs, the heart rate and blood pressure become abnormally low [1].

Typically, the body metabolizes ACD with aldehyde dehydrogenase 2 (ALDH2) which is extremely efficient to the point where ACD levels are undetectable [1,2,3]. However, a defect in the ALDH2 gene will result in ACD buildup, which poses a risk for digestive tract cancers [2,3]. This defect is common in Eastern Asians, affecting 30-50% of the population. Heavy drinkers who have this defect are at a higher risk for oral, pharyngeal, and esophageal cancers than those who do not have this defect [3]. In addition to being an extremely toxic metabolite, ACD is also difficult to study in humans because the liver immediately responds to significant levels of ACD in the body. The effects of ACD buildup in the body range from impairment of the central nervous system to many other parts of the body [1].

On the other hand, L-cysteine is a nonessential amino acid that is a building block of many proteins [2,3,5]. L-cysteine was likely part of early evolution because of the role it plays in the folding of proteins, pertaining to the secondary and tertiary structures, and that many living organisms have this amino acid [2]. The human genome has approximately 214,000 cys-coding sequences, showing its prevalence and versatility. L-cysteine has an -SH, or thiol, group which allows it to form disulfide bridges, making it relatively reactive [2,3,5].

There have been applications of L-cysteine where it is applied to oral cavities to reduce the levels of ACD, among many other applications [2]. However, the effects of the amino acid itself are controversial because there has not been enough clinical trials to identify them. While

research has shown that there are significant effects of L-cysteine, there is also a lot of research that shows that there are no significant effects of L-cysteine [2]. As pertaining to this study, cysteines in general were seen to have an antihypertensive effect on the body because they assist in controlling oxidative stress in the living system [5]. Even so, humans cannot intake cysteine directly because this has not been studied thoroughly enough [2].

Methods and Materials

Origin of blood samples:

Samples of blood were taken from 10 healthy, non-smoking male volunteers without any abnormal blood characteristics. The volunteers did not eat or drink for at least 2 hours before blood collection and did not consume alcohol since the night before. The blood was collected from the volunteers' antecubital vein via tubes containing a heparin solution (5%v/v 1000 IU/mL heparin).

Preparation of red blood cell suspension:

Red blood cell suspension (RBC suspension) is a solution that consists of saline, albumin, and red blood cells. In order to prepare this solution, the red blood cells are separated from the rest of the blood using a centrifuge. Blood from volunteers is added to a lymphocyte separation solution, then centrifuged at 400g for 30 minutes. The red blood cells are then separated from the plasma, buffy coat, and the lymphocyte separation solution. The RBC suspension is then prepared with 50% hematocrit level using phosphate buffered saline containing 0.1% albumin.

Preparation of blood samples:

The subjects' blood samples were not further modified.

Addition of ACD and L-cysteine:

A 10 microliter saline solution containing ACD and L-cysteine (when applicable) was added to 1000 microliters of either the blood sample or RBC suspension (10 microliters of saline was added in control experiments). The L-cysteine was added to the blood samples first when applicable, and mixed into the blood by inverting the tube for 10 seconds. ACD is then immediately added, and the tube is mixed again. Blood passage time is measured 5 minutes after adding ACD. The ACD concentrations tested were 1, 3, and 5 mM. The L-cysteine concentrations tested were 0.5, 1, and 2.5 mM. All experiments are performed at a temperature of 19°C because ACD has a boiling point of 20.2°C.

Measurement of blood passage time:

Blood passage time in all experiments was measured using MC-FAN. MC-FAN is a device that simulates blood flow in the human body. Blood first enters an inlet, which then splits into many different smaller passages to imitate the small size of human blood vessels. The flow of blood through these flow paths can then be observed using a recording device that displays images of blood cells traveling through the passages as a microscope would. While the blood flow in each of the passages is relatively difficult to measure, the aggregate effect of many passages allows flow time to be quantitatively determined [7]. MC-FAN is chosen in this experiment for its reliability and reproducibility concerning the measurement of blood passage time [8]. The dimensions of the MC-FAN and microgrooves is 7 micrometers by 30 micrometers by 4.5

micometers, the passage time of the blood sample/RBC suspension is each measured twice, and the average is used for analysis.

Measurement of microchannel obstruction and WBC adhesion:

Images of the microchannel with the blood, ACD, and L-cysteine mixture flowing through were recorded and stored for later analysis. The percentage of microchannel obstruction and the number of adherent WBCs on microchannel terrace were then counted. An investigator who had no knowledge of the subjects then chose 5 images of 20 microliters of blood and analyzed them.

Statistics:

The data was analyzed using Microsoft Excel for Windows and macOS. An average and standard deviation was taken for each combination of blood, ACD, and L-cysteine. The ANOVA and the Dunnett methods were used to detect points of significant difference, using $p<0.05$ as statistically significant.

Results

Statistical analysis indicated that the presence of ACD in blood has a significant effect on blood flow. The Dunnett method shows that there is a significant difference in blood fluidity between 3mM ACD and 0mM ACD, and a significant difference between 5mM and 0mM ACD, but no significant difference in blood fluidity between 1mM ACD and 0mM ACD. Overall, this shows that ACD decreases blood fluidity.

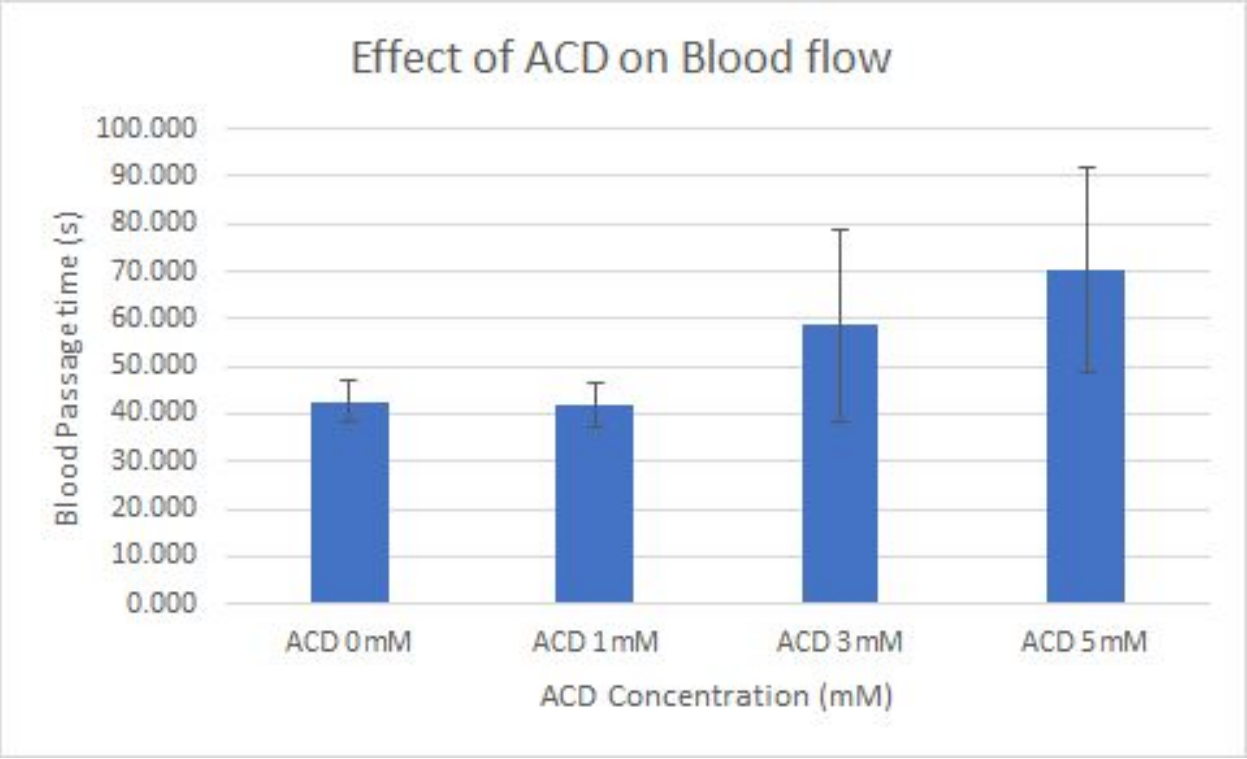

Fig. 1A

Passage time for 100 µL blood after addition of ACD.

ACD concentration compared to 0mM ACD	p value
1mM	0.682
3mM	0.026
5mM	0.000898

p values for Fig. 1A

Statistical analysis indicated that the combined presence of ACD and L-cysteine in blood has a significant effect on blood flow. The Dunnett method shows that there is a significant difference in blood fluidity between 0 mM of both ACD and L-cysteine and all other concentrations except 5 mM ACD with 2.5 mM L-cysteine.

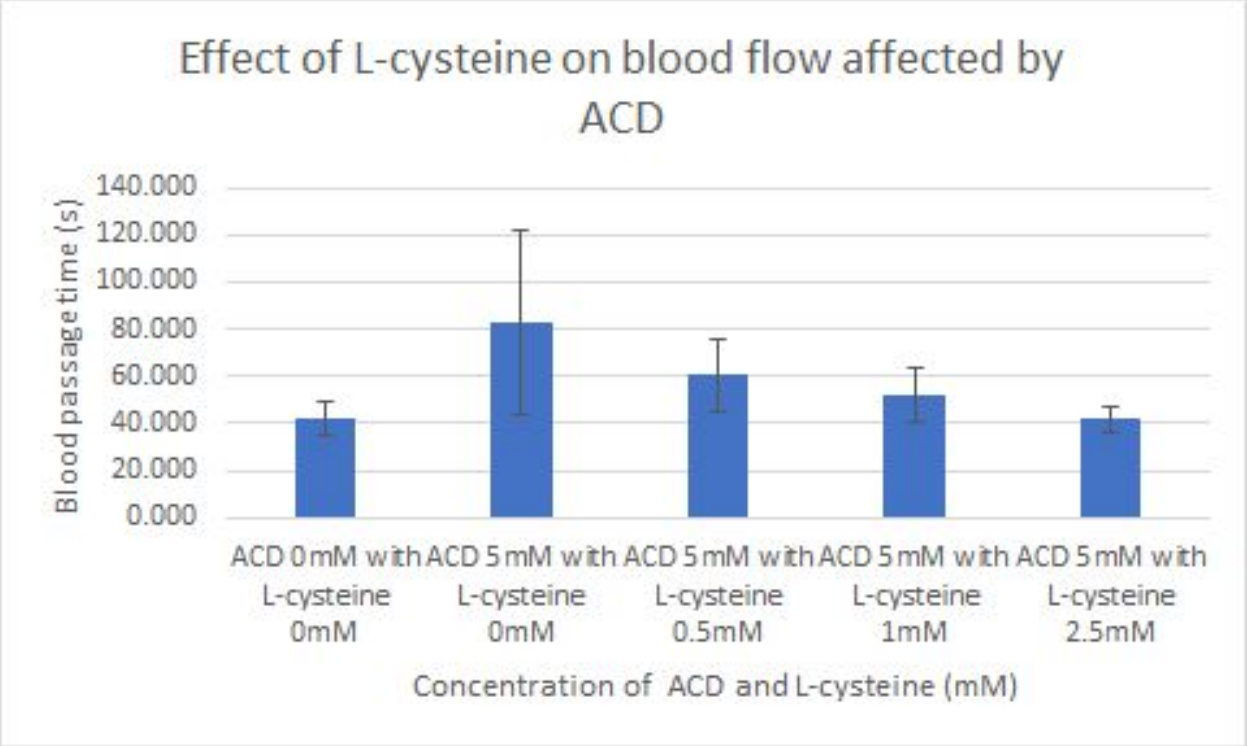

Fig. 1B

Passage time for 100 μL blood after addition of ACD and/or L-cysteine.

ACD and L-cysteine concentration (compared to 0mM ACD and L-cysteine)	p value
5mM ACD with 0mM L-cysteine	0.00403
5mM ACD with 0.5mM L-cysteine	0.00304
5mM ACD with 1mM L-cysteine	0.0302

5mM ACD with 2.5mM L-cysteine	0.999

p values for Fig. 1B

Statistical analysis indicated that the presence of ACD in blood had a statistically significant effect on the percentage of microchannels obstructed. The Dunnett method showed that there was a significant difference in the obstruction of microchannels between 3mM ACD and 0mM ACD, and a significant difference between 5mM and 0mM ACD, but no significant difference in the obstruction of microchannels between 1mM ACD and 0mM ACD. Overall, this showed that ACD increases the percentage of microchannels obstructed

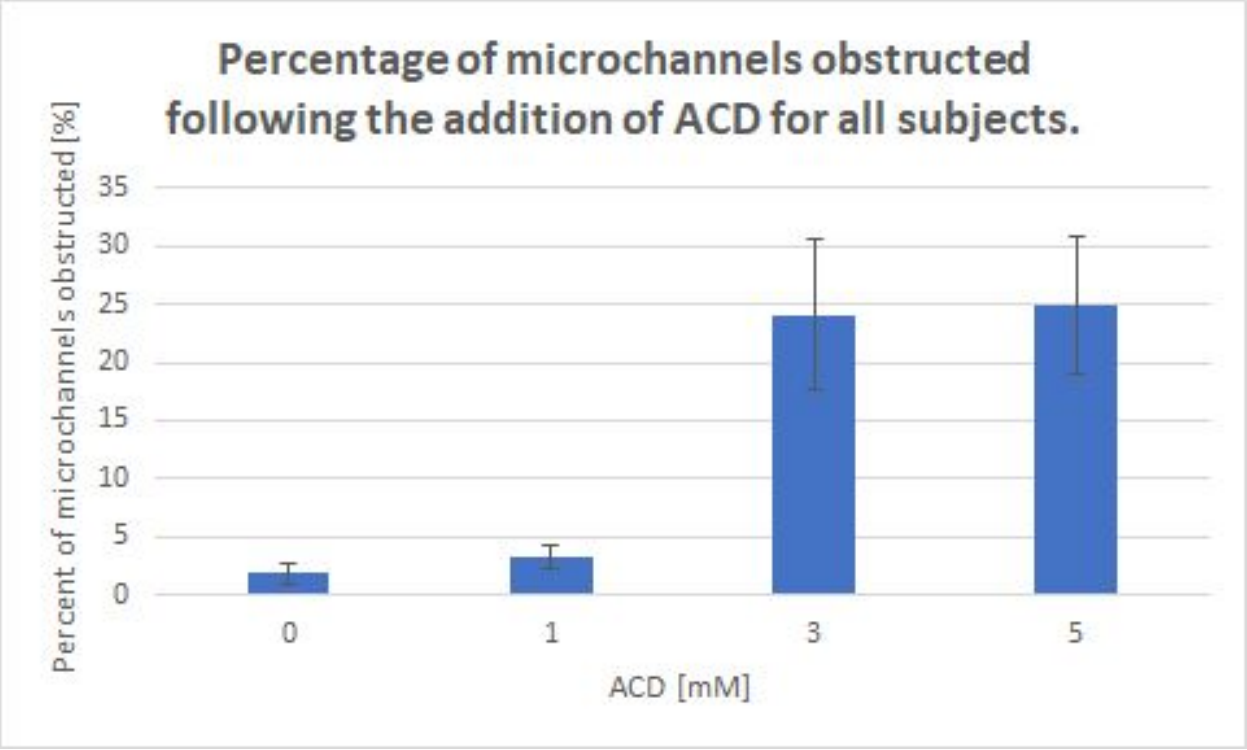

Fig. 2A

Percentage of microchannels obstructed after ACD addition.

ACD concentration compared to 0mM ACD	P value
1mM	0.302
3mM	0.00333
5mM	0.00103

p values for Fig. 2A

Statistical analysis indicated that the combined presence of ACD and L-cysteine in blood had a statistically significant impact on the percentage of obstructed microchannels. The Dunnett method showed that there was a significant difference in the obstruction of microchannels between 0 mM of both ACD and L-cysteine and all other concentrations except 5 mM ACD and 2.5 mM L-cysteine. This showed that the presence of L-cysteine decreased the obstruction of microchannels.

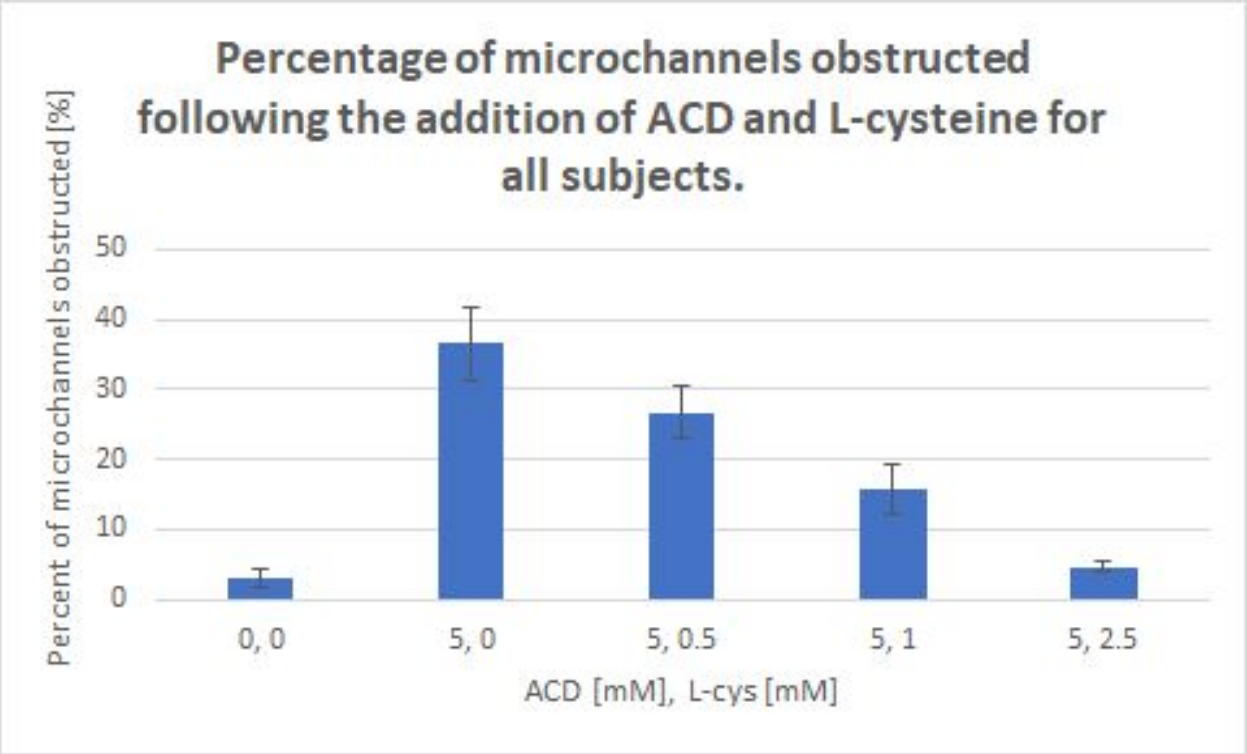

Fig. 2B

Percentage of microchannels obstructed after ACD and/or L-cysteine addition.

ACD and L-cysteine concentration (compared to 0mM ACD and L-cysteine)	P value
5mM ACD with 0mM L-cysteine	0.00000791
5mM ACD with 0.5mM L-cysteine	0.00000938
5mM ACD with 1mM L-cysteine	0.00319
5mM ACD with 2.5mM L-cysteine	0.252

p values for Fig. 2B

Statistical analysis indicated that the presence of ACD in blood had a statistically significant effect on the number of adherent WBCs. The Dunnett method showed that there was a significant difference in the number of adherent WBCs between 3mM ACD and 0mM ACD and all other concentrations of ACD. Overall, this showed that ACD increases the percentage of microchannels obstructed.

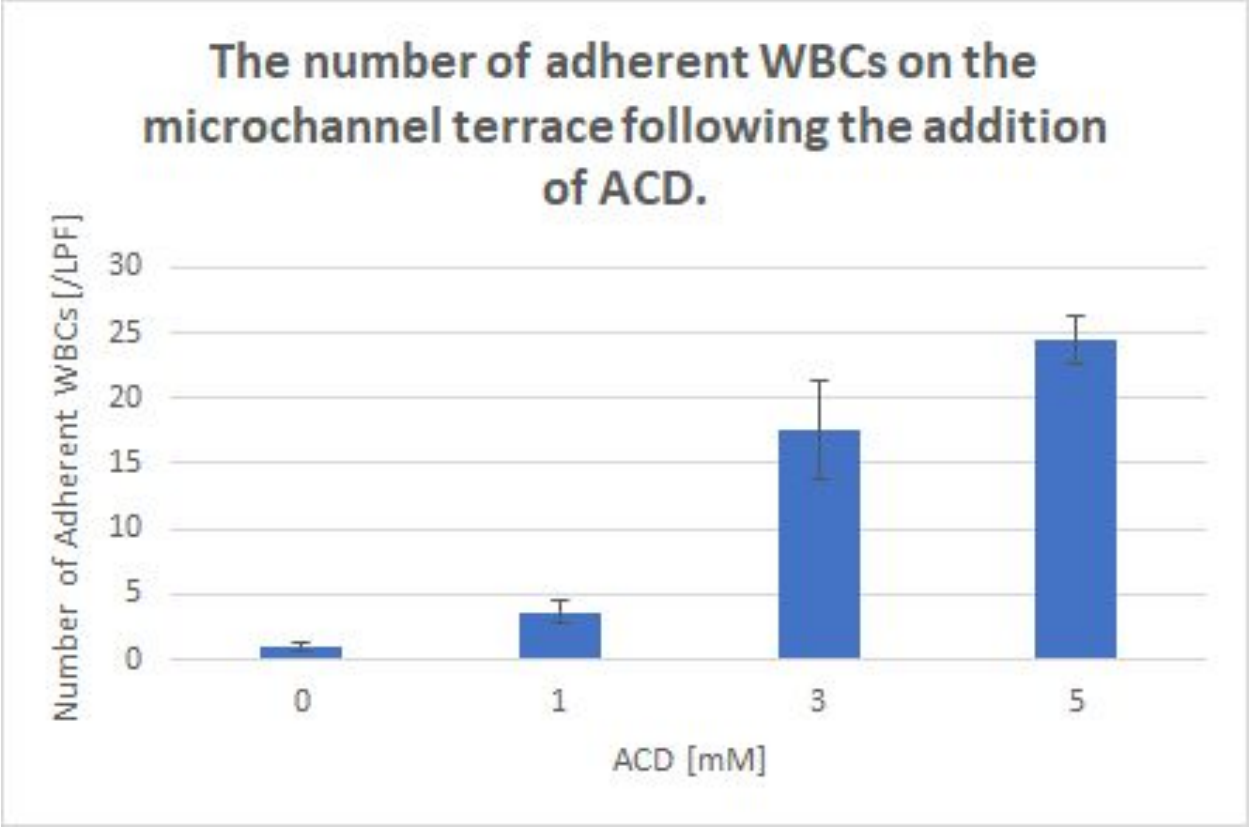

Fig. 2C

Number of adherent WBCs on microchannel terrace after addition of ACD.

ACD concentration compared to 0mM ACD	P value
1mM	0.0115

3mM	0.000287
5mM	0.000000000189

p values for Fig. 2C

Statistical analysis indicated that the combined presence of ACD and L-cysteine in blood had a statistically significant impact on the number of adherent WBCs. The Dunnett method showed that there was a significant difference in the obstruction of microchannels between 0 mM of both ACD and L-cysteine and all other concentrations except 5 mM ACD and 2.5 mM L-cysteine. Overall this showed that the presence of L-cysteine decreased the number of adherent WBCs.

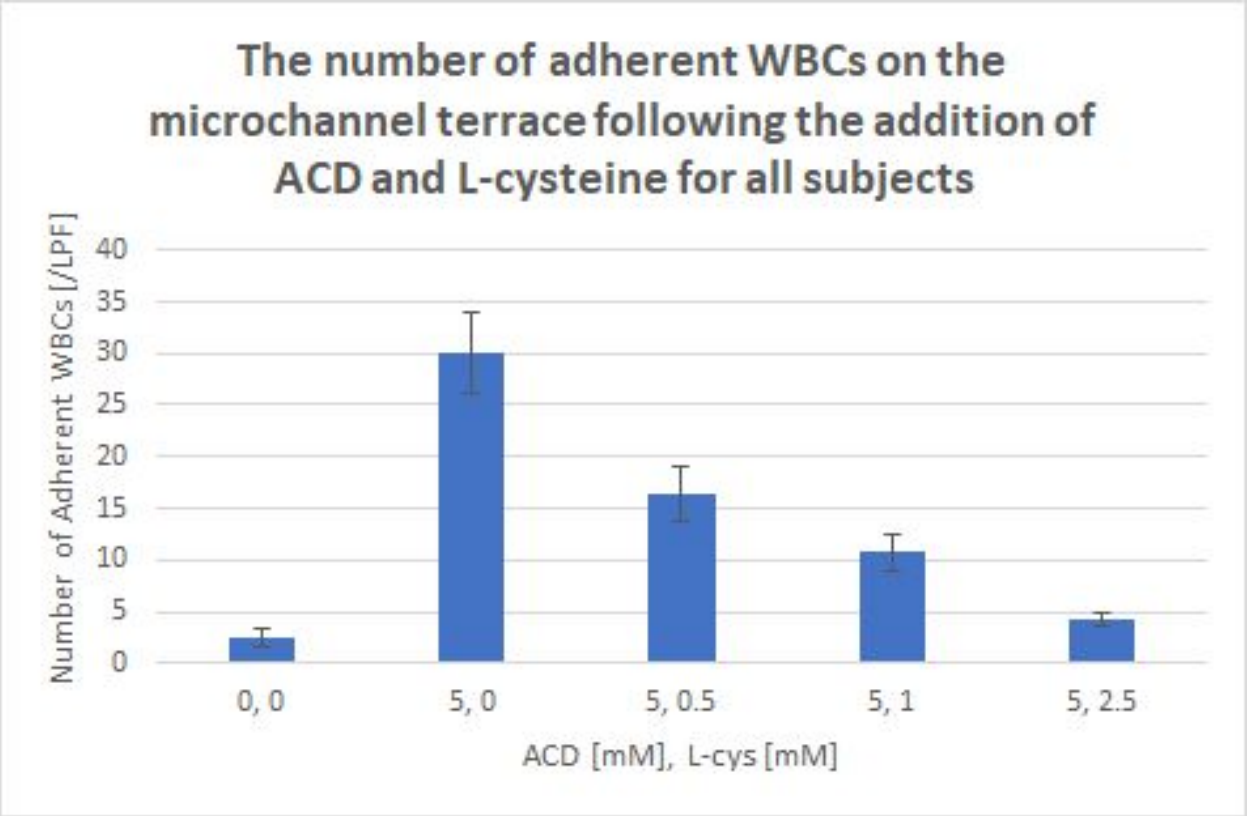

Fig. 2D

Number of adherent WBCs on microchannel terrace after addition of ACD and/or L-cysteine.

ACD and L-cysteine concentration (compared to 0mM ACD and L-cysteine)	P value
5mM ACD with 0mM L-cysteine	0.00000223
5mM ACD with 0.5mM L-cysteine	0.000109
5mM ACD with 1mM L-cysteine	0.000621
5mM ACD with 2.5mM L-cysteine	0.111

p values for Fig. 2D

Fig. 3

Measurement of flowpath with MC-FAN, demonstrating a decrease in concentration of ACD with the addition of L-cysteine. (A) This is the microchannel image with 0mM ACD AND 0mM L-cysteine, (B) this is the microchannel image with 5mM ACD AND 0mM L-cysteine, (C) this is the microchannel image with 5mM ACD AND 2.5mM L-cysteine.

The addition of ACD to the RBC suspension showed no significant effect on the fluidity of RBC suspension (p value 0.824).

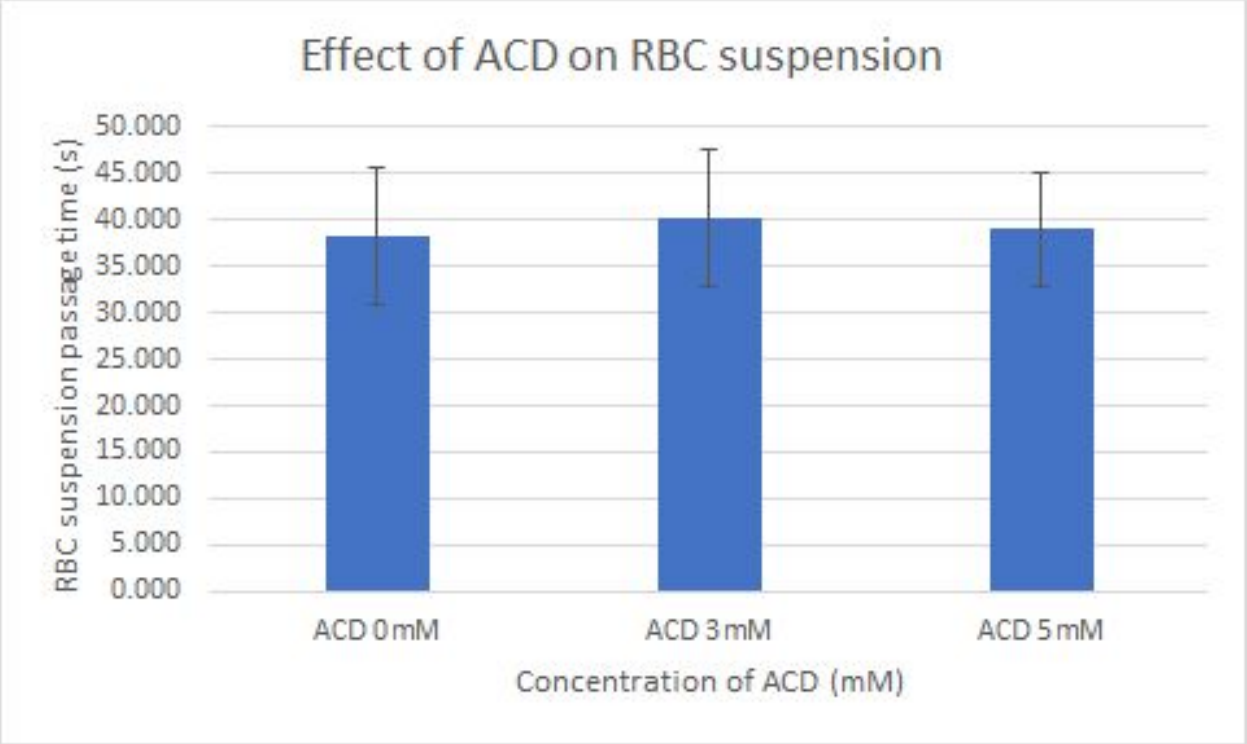

Fig. 4A

Passage time of RBC suspension after addition of ACD.

The addition of L-cysteine to the RBC suspension showed no significant effect on the fluidity of RBC suspension (p value 0.835).

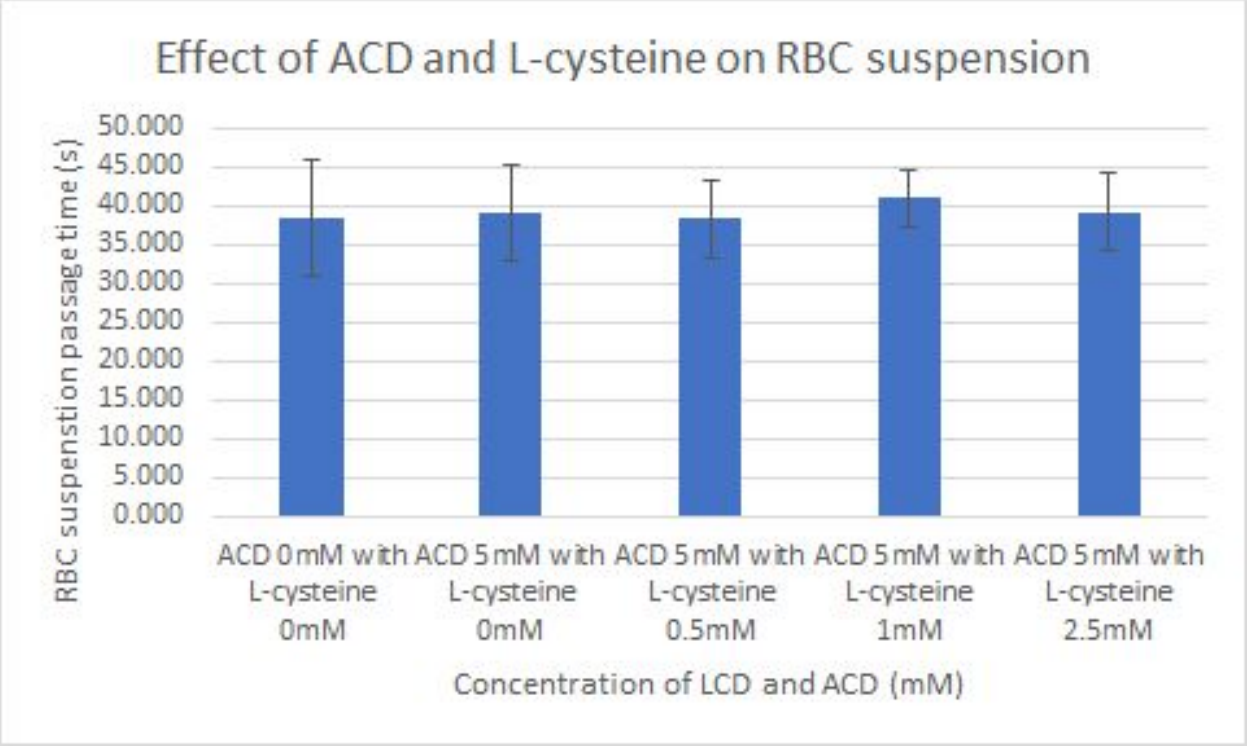

Fig. 4B

Passage time of RBC suspension after addition of ACD and/or L-cysteine.

Discussion

Previous research has found a correlation between alcohol and blood flow, as well as suggesting L-cysteine's role of treating alcohol poisoning. Therefore, this paper hypothesizes that the presence of acetaldehyde (ACD) in blood decreases blood fluidity and therefore increases blood passage time, while adding L-cysteine to blood already affected by ACD will increase blood fluidity, therefore decreasing blood passage time. The paper by Otoyama et al. further hypothesizes that the reason why ACD decreases blood fluidity is because ACD induces the adhesion of certain particles found in blood, or because ACD affects the deformability of red

blood cells. The data found in this study shows that ACD does decrease blood fluidity, primarily caused by the adhesion of white blood cells. In addition, L-cysteine increases blood fluidity that was previously impaired by ACD back up to normal levels.

Two primary conjectures explaining why ACD would decrease blood fluidity were that ACD either inhibited the red blood cells' ability to deform, or that ACD causes the increased adhesiveness of other particles in the bloodstream [9]. Decreased deformability would cause RBCs to become rigid, making blood passage more difficult. WBCs, monocytes, or other particles found in blood are also made more adhesive by ACD [10], which could make blood passage more difficult. Based on the results of this study, it is concluded that monocyte adhesion was the main factor in decreasing blood fluidity, and that reduced red blood cell deformability either did not occur, or did not have a significant impact on blood fluidity. Figure 4 showed that neither ACD nor L-cysteine affected the fluidity of red blood cell suspension, a solution composed of red blood cells and saline, which was separated from any other particles found in blood. These results show that ACD does not affect the red blood cell directly. However, as seen with all other figures, ACD does change the fluidity of human blood, and is shown to cause particles in the blood to stick (Figures 2 and 3).

The results also show that L-cysteine is effective in returning blood flow to normal levels. A possible reason for why this happens is that L-cysteine directly eliminates ACD from the blood [3]. A way for this to happen is for L-cysteine, through a yet unknown mechanism, to directly and covalently bond with ACD, creating 2-methylthiazolidine-4-carboxylic acid [3]. A study reviewing the effects of L-cysteine from multiple databases have also shown a pattern of L-cysteine being capable of eliminating ACD from other areas of the human body [2]. Additionally, the results from Figure 4 imply that L-cysteine has no direct effect on red blood

cells themselves, as neither the addition of ACD nor L-cysteine affected the fluidity of the suspension. However, this does not exclude the possibility that L-cysteine affects other particles in the blood, i.e. how ACD affects the adhesion of white blood cells.

Given that acetaldehyde is a substance that is both dangerous, being a carcinogen [3], and being relatively common, as it is a byproduct of the metabolism of alcohol [1], it is then important to know how acetaldehyde affects the human body. Further study is needed to determine through what mechanism ACD changes the adhesive properties of white blood cells and other particles. Knowing how this happens may offer insight into other ways that mitigate ACD's effect of decreasing blood fluidity. Additionally, this study was conducted in an *in vitro* environment with little diversity between the study's volunteers. Conducting *in vivo* analogues of this study with a more diverse group of volunteers may uncover factors that affect ACD's initial presence in blood, and the human body's reaction to ACD's presence in blood. Such factors could not be completely replicated outside of the human body. Additionally, the possibility of L-cysteine directly eliminating ACD should be explored further, such as the quantity of L-cysteine required to completely negate the effects of ACD, or the mechanism of which L-cysteine eliminates ACD. If L-cysteine proves to be successful in eliminating ACD in the human body, it could offer a way of treating alcohol poisoning by introducing L-cysteine into a patient's bloodstream, as ACD is responsible for many of the negative effects of alcohol [1].

In summary, this *in vitro* study suggests that ACD decreases blood fluidity, and L-cysteine reverses the effects ACD has on blood flow.

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