

FRESH FRUITS FOR CARDIOVASCULAR DISEASE: EFFECTS OF FRUIT JUICES ON THE EXPRESSION OF CD36 AND LDL-R IN HUMAN MONOCYTIC CELLS, U937

Atherosclerosis is one of the leading causes of premature death in United States. Major risk factors for atherosclerosis include high cholesterol levels and oxidative stress. Epidemiological studies indicate that diet and exercise form the cornerstone in treating hypercholesterolemia and lower the risk of cardiovascular diseases. Among the various dietary components, fruits and vegetables have a high antioxidant capacity and can lower blood cholesterol. Our project tested the hypothesis that fresh fruit juices will lower oxidative stress and differentially regulate the CD36 and LDL-R receptors in human monocytic cells, U937. Cells were treated with fresh papaya juice for 24 hours and were analyzed for the expression of the two receptors by RT-PCR. Our results indicate that papaya juice was able to significantly inhibit CD36 mRNA expression with a concomitant increase in LDL-R mRNA expression. We are currently testing the antioxidant effects of papaya juice in U937 by measuring its capacity to inhibit the production of reactive oxygen species (ROS). Such studies are significant as they provide mechanistic insights as to why fruits and vegetables may offer an alternative strategy to prevent and/or lower cardiovascular disease risk.

INTRODUCTION

Atherosclerosis is the buildup of deposits of cholesterol and calcium on the inside of vessel walls. As the arteries harden and become more clogged, flow of blood is restricted. Depending on which artery becomes clogged, atherosclerosis can lead to different types of cardiovascular disease, including high blood pressure, stroke, and coronary heart disease, which includes myocardial infarction and angina pectoris. Atherosclerosis is a progressive, complex disease and is referred to as the "silent killer" because it can exist undetected for years.

There are several risk factors for atherosclerosis. They include high cholesterol levels, oxidative stress, high blood pressure, diabetes, physical inactivity, and obesity. Some possible methods of prevention would be to change lifestyles to avoid any of the risk factors. It has been shown that changes in diet, such as the reduction of intake of fatty foods, cholesterol, refined carbohydrates, and salt, may help eliminate some risk factors involved in heart disease. Exercise has also been shown to improve the cardiovascular system. If lifestyle changes fail to have an effect, other measures, including drug therapy, are usually recommended. Certain drugs can interfere with the rate-limiting enzyme of cholesterol biosynthesis, catalyzing the reduction of HMG-CoA, which can also cause decrease in superoxide formation in macrophages, leading to a decrease in oxidized LDL.

It is generally known that fruits and vegetables contain compounds known for their antioxidant properties, such as vitamin C and E, carotenoids, and flavonoids. These substances prevent the oxidation of biological molecules from

Student Researcher: Hezhi Gan
Mentor: Pratibha V. Nerurkar, PhD

free radicals. However, the mechanisms involved are unknown.

In Hawaii, *carica papaya*, or otherwise known as papaya, is one of the largest growing crops in our agricultural produce. In addition to this, because papaya has been known to contain many vitamins, enzymes, antioxidants, and consist of very little calories, we chose to focus our project on the investigation of the mechanism of papaya juice involved in preventing atherosclerosis. Therefore, our hypothesis is that papaya juice will reduce production of reactive oxygen species, using human monocytic cells, U937, and that papaya juice will also decrease the mRNA expression of CD36, while increasing the mRNA expression of LDL receptor. Hopefully, this will lead to the inhibition of foam cell formation.

MATERIALS AND METHODS

Preparation of Papaya Juice

Papaya was obtained from the local farmer's market, washed, skinned and deseeded. Papaya juice was prepared using a household juicer.

Cell Culture and Treatment

The human monocytic cell line, U937, obtained from the American Type Culture Collection (ATCC; Manassas, VA), was grown in RPMI media at 37° C and 5% CO₂ supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in suspension. Undifferentiated cells were treated with 0.5% to 20% of papaya juice for 24 hours. At the end of the experiments, media were harvested to measure cellular cytotoxicity.

From the University of Hawaii at Manoa; Honolulu, Hawaii.

Cytotoxicity Assay

The release of LDH into the culture media was used as a measure of cell death due to damaged membrane and was measured fluorimetrically using the commercial CytoTox-ONE[®] Assay kit (Promega) according to manufacturer's direction. LDH released into the culture medium is measured with a 10-minute coupled enzymatic reaction that results in the conversion of resazurin into resorufin. The reagents are not toxic to healthy cells.

Reactive Oxygen Species Assay (ROS)

Cells were treated with papaya juice for 4 hours. The media were discarded and replaced with new media containing 20 μ M of a fluorescent dye (dichlorodihydrofluorescein diacetate—H₂DCFDA). Cells were incubated for 30 minutes at 37°C. H₂DCFDA was discarded, and cells washed with 200 μ L of cold PBS. 200 μ L of cold PBS was added into each well before being read.

Extraction of RNA

Cells were treated with papaya juice for 24 hours. After treatment, cells were collected by centrifugation and 1 mL of Tri Reagent was added to the cells, which lyses (breaks open) the cells. RNA was extracted with standard phenol-chloroform procedure. Nucleic acids were precipitated with an equal volume of iso-propanol, washed with 75% ethanol and dissolved in DEPC-treated water. RNA was stored at -20°C freezer until further analysis.

Semi-quantitation of LDL-R and CD36 Gene Expression

mRNA expressions of both LDL-R and CD36 were determined by RT-

PCR. Total RNA was extracted using Tri Reagent as mentioned above. Two μ g of RNA was reverse transcribed into complementary DNA (cDNA). PCR was performed according to published protocol.

RESULTS AND DISCUSSION

In our first experiment, we measured cytotoxicity and cell death in different concentrations of papaya juice using a cytotoxicity assay kit. Toxicity significantly decreases at 1% and 2% of papaya juice, while at higher concentrations, toxicity significantly increases. Based on this data, for future studies, we used non-toxic doses of papaya juice.

In our second experiment, we measured production of reactive oxygen species in different concentrations of papaya juice. There was a non-significant decrease of ROS with papaya juice. A decrease in reactive oxygen species may result in less oxidation of LDL to oxidized LDL.

In our third experiment, mRNA expression of CD36 was measured using reverse-transcription polymerase chain reaction (RT-PCR). There was a significant decrease in CD36 expression at 2% papaya juice. This decrease in CD36 expression may result in a decrease in uptake of oxidized LDL in the cells.

We also measured mRNA expression of LDL-R using reverse-transcription polymerase chain reaction (RT-PCR). Papaya juice significantly increased mRNA expression of LDL-R, with 1% showing the highest increase. This increase in LDL receptor expression may result in greater lowering of plasma LDL.

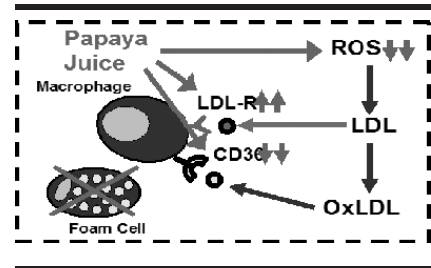


Fig 1. Illustration of hypothesis that fresh fruit juices will lower oxidative stress

CONCLUSION

As this data demonstrates, it is known that papaya juice decreases the production of reactive oxygen species, decreases expression of CD36, while increasing expression of LDL-R. Henceforth, because all three factors play a large role in foam cell formation, an increase in LDL-R would allow greater reduction of plasma LDL, a decrease in CD36 would limit the upregulation of oxidized LDL, and a decrease in production of ROS would inhibit the formation of oxidized LDL. (Figure 1) This would help in eliminating the amount of LDL available for oxidation and decreasing the chances of foam cell formation.

Even though our data supports our hypothesis, the ROS data are preliminary and further research is needed to confirm our findings. In addition, because these data correspond to *in vitro* studies, we would need to study the effects of papaya juice *in vivo* in animals and humans.

ACKNOWLEDGMENTS

The author would like to thank: Laurel Pearson, Steven Lim, Ellen Linden, Leah Midles, and Yun Kyung Lee; the NIH/NIDDK/Charles Drew University and Dr. George Hui; and Dr. Pratibha Nerurkar.