

Biofuel Enzyme Reactions Kit

A ThINQ!™ Investigation

Science Case Study

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Science Case Study

“Could Washing Down Your Pills with Grapefruit Juice Be Bad Medicine?”

Part 1 – Setting the scene.

Dr. Marilyn Castor was excited to begin clinical studies examining the effectiveness of grapefruit juice for increasing the potency of certain drugs in humans. For decades, scientists and medical professionals have been aware that special molecules — called **furanocoumarins** — within grapefruits, limes, Seville oranges, and pomelos can significantly change the way drugs are broken down within the small intestine and used by the body. For example, in patients who drink grapefruit juice when taking certain cholesterol-lowering medications, much higher levels of the drug accumulate in their bloodstreams. As patients take their medication each day, the amount of drug in their blood rises. For many patients, this results in toxic side effects such as muscle breakdown and liver damage. When patients stop consuming grapefruit juice with their medication, the medication works as expected and returns to normal levels in the blood.



Researchers determined that furanocoumarins from the grapefruit changed the activity of an enzyme called **CYP3A4** found in the intestine. The role of CYP3A4 is to break down various chemicals in the intestine, including medications, so the body can use or eliminate them. The furanocoumarins in grapefruit juice can have negative or positive effects on a drug regimen. On the one hand, the CYP3A4 and other enzymes in the gut can break down drugs before they enter the bloodstream, requiring higher doses to achieve a therapeutic benefit. On the other hand, inhibition of this process can lead to increased levels of a drug in the bloodstream, which may allow a drug that otherwise is metabolized quickly to become effective. But it may also lead to toxic levels of the drug accumulating in the bloodstream. When patients take certain medications with grapefruit juice or while eating grapefruit, their CYP3A4 enzymes are not able to break down the medication as usual and more medication is absorbed into the patient's bloodstream.

1. Describe how you think furanocoumarins from grapefruit might be interacting with the CYP3A4 enzyme in the gut. Draw a diagram to support your explanation.



Scientific investigations have found that many medications interact with grapefruit in a similar way, but results vary from one person to the next. Now pharmacists clearly mark all prescription medication bottles with a warning label if the medication interacts with grapefruit.

Dr. Castor believed that the interaction between grapefruit and CYP3A4 could actually be useful in some cases. Her work examined how enzymes like CYP3A4 break down drugs that suppress the immune system of patients who have recently received an organ transplant. Immunosuppressant drugs are useful in these cases because they prevent the body's immune system from attacking the newly transplanted organ. However, Dr. Castor found that the drugs have limited activity in the body, so patients are required to take higher doses more often. She theorized that if the medication were paired with grapefruit juice, the effect of the medication might be amplified so that patients could take lower dosages less often. She needed to determine the **therapeutic dosage** (the amount of drug required to alleviate symptoms) and avoid using a **toxic dosage** (the amount of drug that causes negative side effects). From previous studies she knew that the most effective dose of the medication was 2 milligrams (mg), three times per day. This would maintain an ideal therapeutic dosage at 5 micrograms per milliliter ($\mu\text{g}/\text{ml}$) circulating in the bloodstream over 24 hours.



Dr. Castor's research question is:

How does grapefruit juice affect the amount of medication circulating in patients' blood over 24 hours?

2. What data would Dr. Castor need to gather in order to answer her research question?

Part 2 – In vivo experimental results.

Among 40 patients, Dr. Castor gathered the data shown in Table 1:

Table 1. Amount of drug found in the bloodstream of each patient after 24 hours, $\mu\text{g}/\text{ml}$

	Group 1	Group 2	Group 3	Group 4
	10	13	1	1
	11	10	4	2
	12	1	1	1
	10	8	3	1
	7	2	3	3
	5	2	1	4
	8	9	2	2
	8	4	4	5
	11	3	1	1
	11	11	2	1
Average	9.3	6.3	2.2	2.1

Dr. Castor's initial blood sample data from patients are shown in Table 1. Each group comprised ten patients. All patients were prescribed 2 mg of medication once a day. What liquid and how much of it was taken with the medicine determined the experimental condition.

Group 1: With 200 ml grapefruit juice.

Group 2: With 100 ml grapefruit juice and 100 ml water.

Group 3: With 200 ml navel orange juice.

Group 4: With 200 ml water.

3. What do Dr. Castor's data suggest about the effect of grapefruit juice on drug concentrations in the bloodstream of patients and how might that change the recommended dosage of a drug?

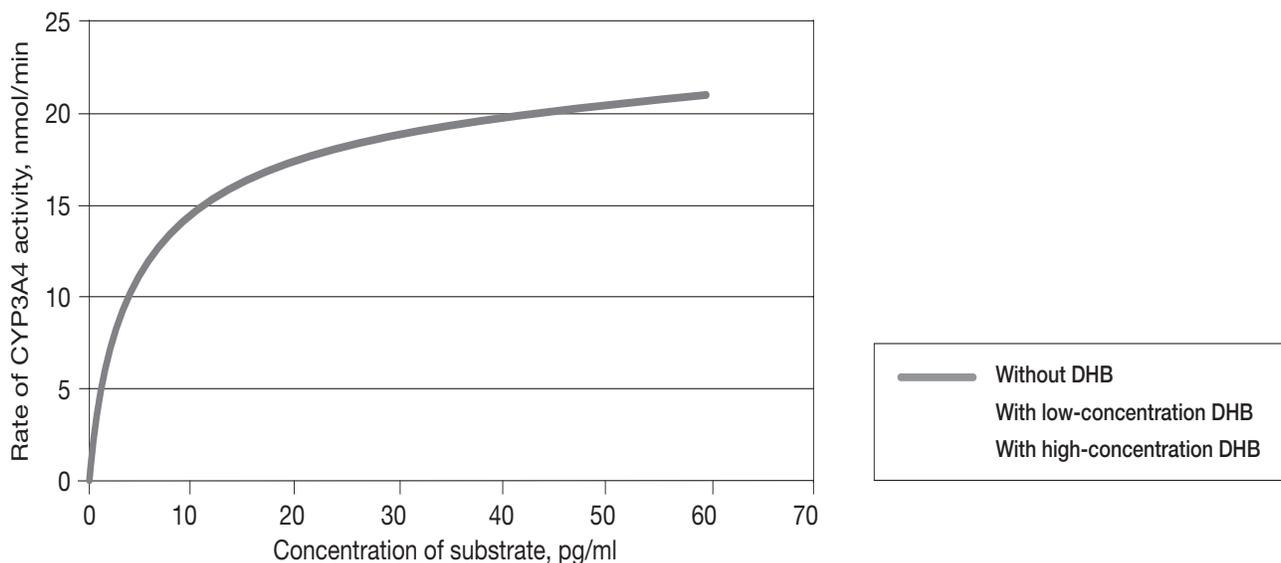
4. For patients in Group 2, what might explain the difference in results from one patient to the next?

5. What might explain differences in enzyme concentration in the body? (Hint: could external factors, such as grapefruit juice, explain such changes?)

Dr. Castor was intrigued by her findings and wanted to further understand how grapefruit juice contributes to greater amounts of medication in the bloodstream. Enzyme rate reaction was a perfect choice to determine how furanocoumarins interact with the CYP3A4 enzyme. She decided to conduct her experiment in vitro so she could better control the variables of the reaction.

According to Dr. Castor's literature search, a furanocoumarin found in grapefruit juice called dihydroxybergamottin (DHB) is most commonly used for in vitro analyses. Dr. Castor ordered enough DHB from a chemical company to conduct three experiments that tested CYP3A4 activity with a substrate in the presence of no DHB, high-concentration DHB, and low-concentration DHB. She measured the rate at which CYP3A4 enzyme metabolized the substrate in all three conditions.

6. In the graph below, the control experiment is depicted for you (no DHB). Draw what you expect the in vitro data to demonstrate in terms of rate of enzyme reaction in the presence of low- and high-concentration DHB.



Part 3 – In vitro experimental results.

Figure 1 illustrates Dr. Castor's data:

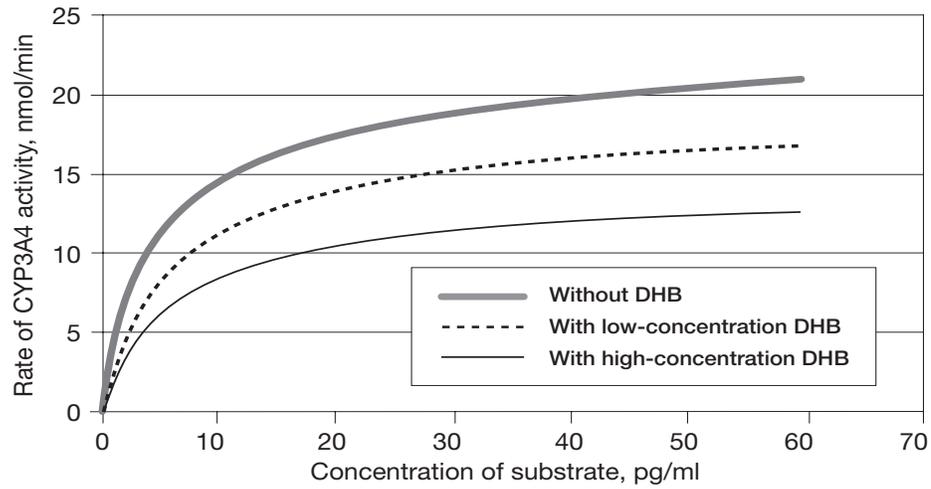


Fig 1. Effect of DHB on CYP3A4 activity. Rate of reaction of CYP3A4 in the absence of DHB and in the presence of high- and low-concentration DHB.

7. What do the data in Figure 1 suggest about CYP3A4 enzyme function in the presence and absence of DHB? Provide your reasoning.

8. Draw a diagram that explains the mechanism by which furanocoumarins could be affecting CYP3A4 activity in the body such that more medication stays in patients' bloodstreams.

9. Given the data Dr. Castor generated in her studies (Table 1 and Figure 1), how might she answer her research question? Be sure to use data to support your thinking.

Dr. Castor's research question is:

How does grapefruit juice affect the amount of medication in patients' blood over 24 hours?

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