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GRAPEFRUIT JUICE FURANOCOUMARINS AND P450 CYTOCHROME CYP3A4

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Abstract. **It has been reported in several studies that the blood levels of some drugs can be significantly higher in patients who have consumed grapefruits or grapefruit juice. These drugs have in common the fact that they are metabolized in the intestine by an enzyme of the P450 cytochrome family, CYP3A4. This enzyme is located in the liver and in the nutrient absorbing cells lining the intestine (enterocytes). The increase in drug bioavailability is due to the removal from the intestine of the CYP3A4 enzyme by grapefruit compounds called furanocoumarins (FC) and some of their homo and hetero dimers. The mechanism responsible for these increases in drug blood levels by grapefruit juice has been largely elucidated. Under normal conditions, intestine CYP3A4 catabolizes many xenobiotics including some drugs. In extreme cases, most of an ingested drug is destroyed and only a very small amount may reach its target. Following grapefruit absorption, the FCs are modified by CYP3A4 and the resulting FC metabolites bind the enzyme, tagging it for destruction. New CYP3A4 enzymes will reappear about three days later, in new enterocytes, when the old cells are replaced. When CYP3A4 is absent from the intestine, the transfer of some drugs into the blood stream is no longer inhibited and has been shown to rise significantly. Even among those drugs, the effect of grapefruit on blood level increases has been shown to range from zero to up to ten folds in a few cases. To clarify this problem a web site has been created, which classifies drugs according to their level of interaction with grapefruit (http://www.druginteractioncenter.org). It is therefore important for a patient to consult a physician and also such a site to determine if the drugs taken are affected or not by grapefruit. In most cases, a specific condition can be treated with a drug not affected by grapefruit.**

In 1991, Bailey et al. (1991) discovered that the blood level of the drug felodipine was significantly higher in patients who had consumed grapefruit juice. The same phenomenon was later found to occur with various drugs and particularly some of the calcium channel blockers and some of the cholesterol lowering drugs (statins). Further studies showed that the increase in drug bioavailability was due the elimination from the intestine of an enzyme, of the P450 cytochrome family, CYP3A4. Cytochrome P450 enzymes are monoxygenases that play a major role in the metabolism of a wide array of xenobiotics including drugs, chemical carcinogens, insecticides, petroleum products, and other environmental pollutants (Lewis, 2003). Cytochrome P450s catalyze an oxidation of the xenobiotics and result in mostly beneficial detoxification. In some instances, a cytochrome P450 may produce cytotoxic or even carcinogenic metabolites of an absorbed chemical. For example, the liver CYP1A2 transforms some tobacco compounds into carcinogens (Le Marchand et al., 2004).

Furanocoumarins (FC) are a broad class of compounds synthesized in various plants, mainly the Apiaceae and Rutacea, and found in fruits and vegetables ranging from grapefruit to parsnip. They have been shown to have adverse effects on a wide variety of organisms, ranging from bacteria to mammals, but particularly insects (Li et al., 2003, 2004). In animals, FCs are metabolized by many different cytochromes P450s. The interaction between plant FCs and insects has been extensively examined, particularly the co-evolution of FC and P450s in wild parsnip (Li et al., 2004). The FCs found in wild parsnip (*Pastinaca sativa*) play a dominant role in the resistance of this plant to its principal enemy the parsnip webworm (*Depressaria pastinacella*) (Zangerl and Berenbaum, 2004). Diversification of cytochrome P450 monooxygenases (P450s) is thought to result from antagonistic interactions between plants and their herbivorous enemies. As mentioned earlier, in humans several cytochromes P450s have been shown to metabolize a wide array of chemicals from pollutants to medications (Chang and Kam, 1999).

Discussion

Cytochrome P450. The nomenclature of cytochrome P450s, for example CYP3A4 is a code assigned to each P450 amino acid sequence reflecting the principle of sequence similarity. It does not have any involvement with such criteria as enzymatic properties, phylogenetic assignment or chronology (Fig. 1) (Nebert and Gonzales, 1987; Nelson et al., 1993). Group II P450s are distributed widely in life, in eubacteria (family CYP102), cyanobacteria (CYP110), fungi (CYP52, CYP53 and CYP56), insects (CYP4 and CYP6) and mammals (CYP3, CYP4 and CYP5). Many group II P450s catalyze the hydroxylation of linear chains such as alkanes (CYP52), alcohols and fatty acids (CYP4, CYP5, CYP102); *Aspergillus niger* CYP53 carries out para-hydroxylation of benzoate; yeast CYP56 is possibly involved in oxidation of tyrosine residues; insect CYP6 metabolizes a wide range of toxic compounds.

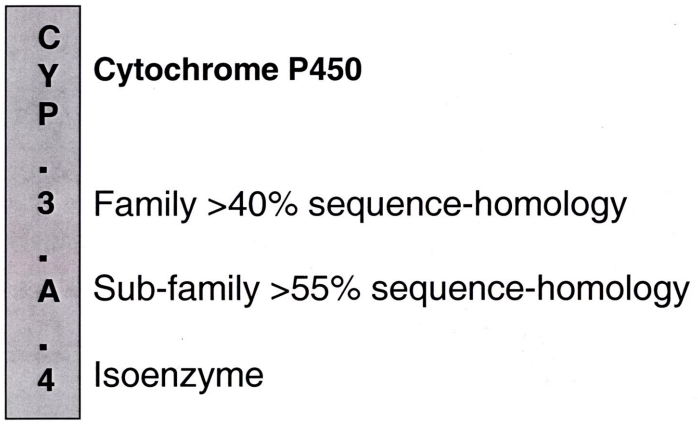

Fig. 1. Nomenclature of cytochrome P450.

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It should be emphasized that the enterocyte level of CYP3A4 in human can vary up to 60 folds. It is influenced by race, age, sex and particularly hormonal levels (Lin and Lu, 2001). Therefore, the bioavailability of a given drug is highly variable from individual to individual. This explains in part why different patients may have to take very different amounts of the same drug to obtain the similar physiological action.

Furanocoumarins. Furanocoumarins (FCs) often display a certain level of toxicity (Beier, 1990) and have sometimes been called plant's natural insecticides. The immediate precursors for FC synthesis are umbelliferone and isoprene. Two categories of FCs are produced; the linear FCs have the furan ring in line with the benz-2-pyrone nucleus, while the angular FCs have the furan ring oriented at an angle to the nucleus (Fig. 2). Angular FCs are found in vegetables such as wild celery and parsnip. Citrus contain linear FCs. FCs have been found in grapefruit, sour orange and lime, but they are not present in sweet oranges, and tangerines. The main citrus FCs are seen in Fig. 3a. Besides the monomers, various homo and hetero dimers have been characterized in grapefruit. Dimers are formed by a link either tail to tail or tail to head (Fig. 3b).

Study of the effects of processing on grapefruit juice FCs showed that the concentration of several FC decreases during storage (Cancalon, 2004). The mechanism responsible for FC degradation was investigated by HPLC and LC/MS. Paridisin C, 6',7' dihydroxybergamottin and to a lesser extent bergamottin have been shown to be affected by storage. The reaction is associated with an increase in the amount of bergaptol present in the juice. This transformation has been shown to be a pH, temperature dependent, nonenzymatic hydrolysis involving the removal of the FC's geranyl side chain. Because of this process, significant differences in FC composition can be found in shelf stable and refrigerated grapefruit juices.

Furanocoumarin drug interaction. Bioavailability is a major problem with drugs taken orally and reaching the blood stream through the intestine. In many cases, P450 enzymes destroy a very large fraction of the drug. For example up to 90% of the ingested drug Saquinavir is destroyed before reaching its target (Kupferschmidt et al., 1998). If the FCs destroy CYP3A4, a much larger amount of drug may reach the blood stream, and with some drugs this increase may lead to an overdose with negative consequences. Reversely, the GJ effect could be beneficial with some expensive, poorly bioavailable drugs since a small amount ingested could produce a blood level similar to that due to a much higher intake.

Once ingested the FCs enter the enterocytes lining the intestinal walls. They react with CYP3A4 in normal enzymatic reactions affecting the furan ring. They generate various metabolites particularly epoxyfurans and ketoenals (Fig. 4). This entire reaction takes about one hour. If the process was to stop here, the drug interaction problem would not exist.

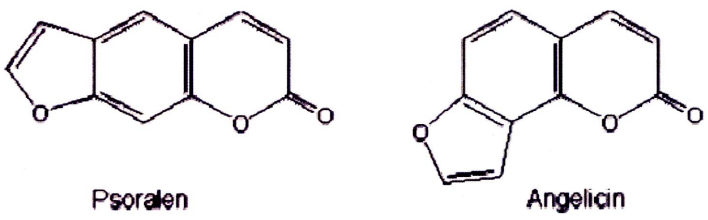
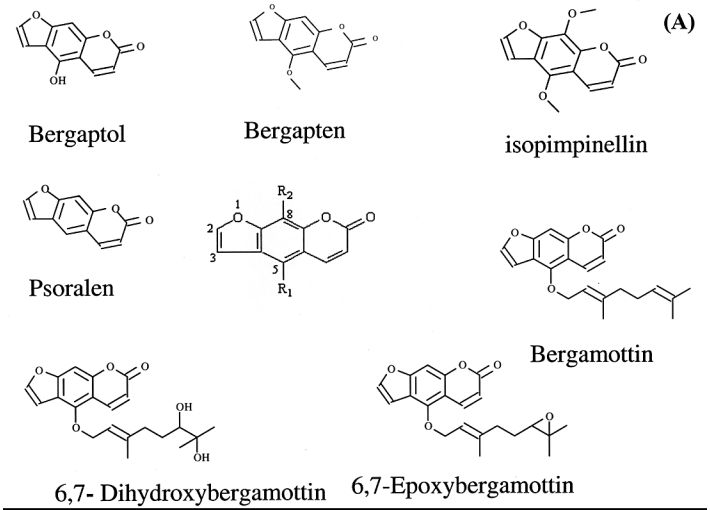


Fig. 2. Basic structure of linear and angular furanocoumarins.



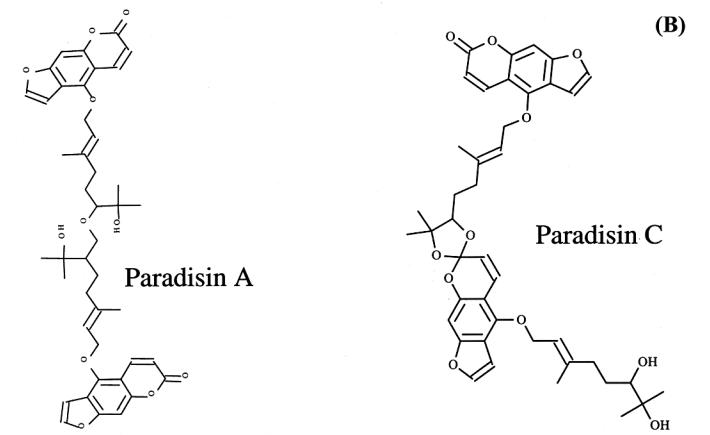


Fig. 3. (A) Major furanocoumarins found in citrus. (B) Examples of tail-tail and tail-head dimmers.

However, the released FC metabolites, in turn, bind irreversibly CYP3A4. The enzyme is then tagged for destruction and is rapidly catabolized (Fig. 4) (Lin et al., 2005). The intestinal enterocytes have a very short life span and are replaced in

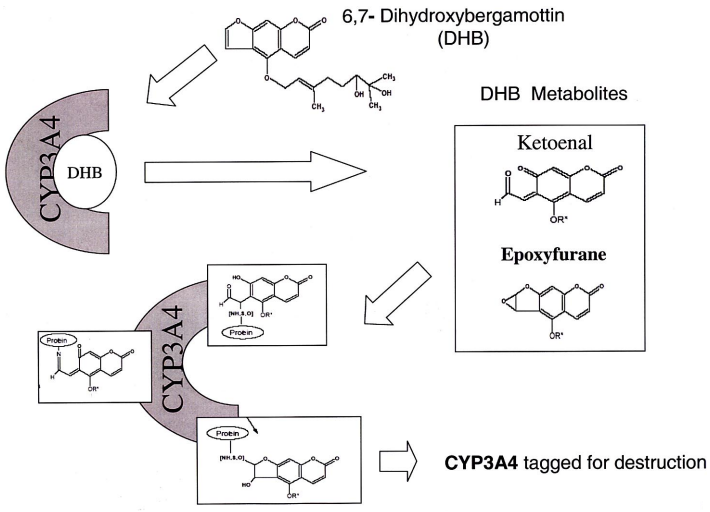


Fig. 4. Mechanism of furanocoumarin inhibition of CYP3A4. From Lin et al., 2005.

three to seven days. Following GJ ingestion, CYP3A4 is not replaced in the affected enterocytes, and it is necessary to wait about three days for new enterocytes containing CYP3A4 to be formed.

Not all FCs are equally effective. They have been ranked by order of inhibitory potency: paradisin C > 6',7'-dihydroxybergamottin > bergamottin > bergapten > bergaptol with comparative strength of 260, 44, 20, 20, 1 (Ohnishi et al., 2000). More recently Row et al. (2006 a, b) examined the physiological activity of various FCs and concluded that a hydroxylated geranyl side chain in the 5 position was essential for good activity. The intact furan ring is also needed to generate the active metabolites. The lactone ring however was shown to have little activity (Fig. 5).

Grapefruit and drug interaction. Among all the medications taken by mouth only a limited number are metabolized by CYP3A4 and could potentially be affected by grapefruit. All other drugs follow different pathways and are unaffected. Even among the drugs metabolized by CYP3A4, the effect of grapefruit can be highly variable and ranges from zero to a ten-fold rise in blood level. To clarify this problem a web site was established by Tufts University and the University of Florida (http://www.druginteractioncenter.org). The site reviews the drugs affected by grapefruit and classifies them as having a low or no interaction, a medium and a high interaction. Besides consulting with a physician, it may be useful for a patient to examine such a site to assess the influence of a particular medication with grapefruit.

Conclusion

The problem of grapefruit-drug interaction cannot be ignored, but it is important to put it in the proper perspective. Its potential negative effect is limited to a small number of drugs and in most cases, such as statins, there are similarly acting drugs unaffected by grapefruit. Therefore, patients consuming grapefruit and their physicians should in most cases be able to find a drug that can treat their specific disease. The possibility of using grapefruit and furanocoumarins to boost the bioavailability of costly medications should also be kept in mind.

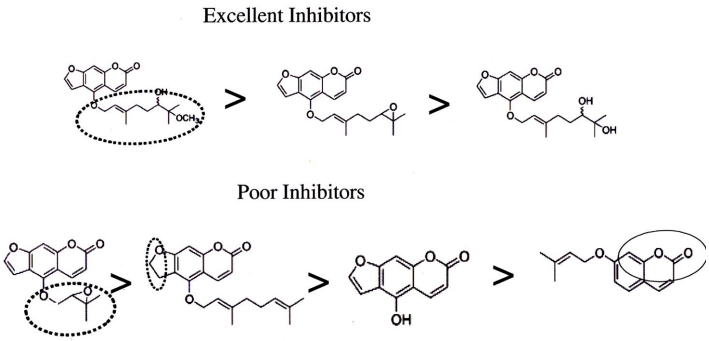


Fig. 5. Areas of the furanocoumarin molecule essential for promoting drug interaction. From Row et al., 2006a.

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