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Nutrition and the Prevention and Treatment of Cancer: Association of Cytochrome P450 CYP1B1 With the Role of Fruit and Fruit Extracts

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Recommendations for a healthy or prudent diet include a large number of daily servings of fruits and vegetables, and these 2 classes of food are widely believed to assist in cancer prevention. One potential mechanism that is rarely mentioned in nutritional studies involves the cytochrome P450 enzyme CYP1B1, which appears to have the unique properties of being a universal cancer marker overexpressed in cancer cells and having the capability of converting various phytochemicals and synthetic chemicals into substances cytotoxic to these cells. Although these particular features of CYP1B1 have not gone unnoticed, there has been relatively little research aimed at exploiting them. Furthermore, therapeutic and preventive strategies currently being considered based on vaccines against the enzyme or inhibition without the generation of cytotoxins can be questioned because they do not take advantage of the unique properties of this enzyme. In addition, a few relevant case histories have been published that use specially designed fruit extracts containing substrates with demonstrated cytotoxic metabolic products, and these reports provide an initial confirmation of the potential of exploiting the unusual properties of this enzyme for cancer therapy.

Keywords: CYP1B1, fruit extracts, cytotoxins, phytochemicals, natural cancer therapy

It is generally believed that fruits and vegetables confer some protection against numerous of types of cancer. The challenge has been to surmise which constituents are important and then search for evidence of effectiveness, mechanisms, and biologic plausibility. Antioxidants have been major candidates, but intervention studies have proved inconclusive. Classes of constituents thought to be candidates as protective agents have many individual chemical members. Furthermore, the action may be both multifactorial and synergistic, vastly increasing the difficulty of eventually achieving significant mechanistic understanding. Thus, it is challenging to provide evidence-based justification for preventive or therapeutic protocols that emphasize certain types of fruit or vegetable intake or use single supplemental compounds or fruit and/or vegetable extracts. Diets include complex mixtures of micronutrients and manmade chemicals that are thought to potentially include both carcinogens and anticarcinogens as well substances that may inhibit or enhance innate protective mechanisms. It seems implicit in our very existence that we have evolved a system, presumably complex, that surveys the presence of adventitious cancer cells and destroys them. The role of dietary micronutrients in this process is clearly of great interest.

The cytochrome P450 enzyme CYP1B1 is rarely considered in discussions of the bioactivity of plant-based agents that affect cancer, but it is a potentially important if not key factor. The P450 CYP proteins form a large ubiquitous family of enzymes that catalyze a multitude of reactions and are involved in the oxidative metabolic activation and detoxification of many endogenous and exogenous compounds. The cytochrome protein P450 1B1 (CYP1B1) is involved in NADPH-dependent monooxygenation of a variety of substrates. Included are steroids, fatty acids, and zenobiotics. One mechanism for the transcriptional activation of this particular cytochrome P450 involves polycyclic aromatic hydrocarbons acting via the Ah receptor complex.[1] The enzyme CYP1B1 is implicated in metabolic processes that involve some compounds in fruits and produce substances that are suspected of anticancer activity.[2] Also there is evidence that this antitumor activity can be targeted because the enzyme is overexpressed in tumors and appears absent or present at low levels in normal tissue. Although overexpression of several other P450 enzymes at the protein or

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messenger RNA (mRNA) level has been observed in tumor tisues,[3] CYP1B1 appears to be the most extensively studied and characterized and the most interesting in the context of this review.

The action of CYP1B1 is frequently regarded as procarcinogenic, and the list of carcinogens known to be activated by this enzyme is long.[4] However, the substrates CYP1B1 activates to carcinogens are mainly manmade chemicals. This is a complex problem because once cells have been transformed and overexpress CYP1B1, the ability of this enzyme to generate substances capable of inflicting genetic damage in already cancerous cells through the metabolism of exogenous manmade chemicals can be viewed as either irrelevant or potentially beneficial. To quote Potter et al, "it does not matter if carcinogens are activated in cancer cells since they are already cancerous."[5]

The estrogen 17β-estradiol (E2) is an endogenous substrate for CYP1B1 and other P 450 enzymes and a ligand for the estrogen receptor. This dual role as substrate and ligand has implicated E2 in the development of breast cancer by being simultaneously involved in causing DNA damage and stimulating cell proliferation and gene expression. The 2-OH catechol estrogen (catalyzed by P450 1A1, 1A2, and 3A) and the 4-OH catechol estrogen (catalyzed by P450 1B1) are thought to be important.[6,7] In particular, the CYP1B1 catalyzed formation of 4-OH catechol estrogen is generally viewed as potentially significant in breast cancer carcinogenesis, but mechanistic details are complex and there are many unanswered questions.[6]

If it turns out to be true that the procarcinogenic aspects of this enzyme are not as significant as the anticancer activity, except perhaps in special cases such as smokers, then the current view that appears to downplay or ignore the beneficial role of CYP1B1 discourages the pursuit of a potentially fruitful line of investigation related to the unusual properties of this enzyme. An enzyme overexpressed in tumors that can metabolize phytochemicals from food and as well certain synthetic chemicals to produce localized cytotoxicity perhaps deserves a much higher level of interest than it currently appears to receive.[2] Recognition of the potential importance of this beneficial aspect requires careful assessment of proposals to inhibit or inactivate the CYP1B1 enzyme for purposes of either primary or secondary prevention. Nevertheless, the development of aromatase inhibitors for the treatment of breast cancer has been described as representing the paradigm of success for P450 CYP inhibition in cancer therapy.[8]

Overexpression of the CYP1B1 Enzyme in Tumor Cells and Tumor Tissue

Murray and coworkers appear to be the first to demonstrate that the CYP1B1 protein is present and overexpressed in human tumor tissue.[9,10] Included were connective tissue and breast, bladder, brain, colon, esophagus, kidney, liver, lung, lymph node, ovary, skin, small intestine, stomach, testis, and uterus tissues. No protein was detected in normal tissue. As Murray et al[1] pointed out, ideally the presence of CYP1B1 protein should be confirmed by detecting the functionally active enzyme, and finding CYP1B1 mRNA should not be confused with the presence of the enzyme. Most subsequent studies from other laboratories have confirmed the observation that the CYP1B1 protein is overexpressed in tumor tissue.[11-17] However, it now appears that the enzyme is also present, in most cases at low or very low levels, in some tissues presumed normal,[15,18-22] and it has been suggested that the assays of Murray et al[10] and McFadyen et al, [23] which failed to detect the enzyme in normal tissue, lacked the required sensitivity.[21] However, in some cases, only nuclear staining has been observed in normal tissues, which raises the issue of requiring cytoplasmic presence to describe tissue as containing the active enzyme.[19] Overexpression of this enzyme has also been found in tumor tissue that resulted from metastasis from a primary tumor, in this case from ovarian cancer.[17] But the important point is that this enzyme is highly overexpressed in tumor tissue and tumor cells, and this provides a target for tumor-selective therapy and prevention, an observation that has not gone unnoticed.[2,4,5,8,24,25] In fact, this offers the potential for using a so-called magic bullet with very low risk of systemic toxicity, a still unrealized goal of most forms of chemotherapy.

The overexpression of the CYP1B1 enzyme presumably involves 2 regulation steps, one transcriptional and the other posttranscriptional (ie, translation to ultimately yield the protein). The important observation requiring explanation is that there are no consistent differences in CYP1B1 mRNA levels between tumor and normal tissue, suggesting posttranscriptional regulation.[26] There appears to have been very little research thus far concerning the regulation processes in this particular member of the P450 family. It has been suggested that the overexpression in tumor tissue could be explained by a high level of expression of one or more micro-RNAs in normal tissue that silence translation and protein synthesis.[26]

Fruits, Vegetables, and CYP1B1 in Cancer Prevention and Treatment

The evidence that fruit and vegetable consumption reduces the risk of developing cancer has received considerable attention. A large set of meta-analyses of cohort and case-control studies published in 2006 indicated limited but significant evidence for a cancer-preventive effect associated with the consumption of fruits and vegetables for sites that included the mouth and pharynx, esophagus, stomach, colon–rectum, larynx, lung, ovary, bladder,

and kidney but with inadequate evidence for other sites. Statistically significant odds ratios as low as 0.5 were found.[27] Also, in the past few years there has been considerable research in this context concerning various types of berries, with significant risk reduction benefits observed.[28,29] One reviewer cites evidence considered overwhelming that indicates that edible small and soft-fleshed berry fruits have beneficial effects against several types of human cancer.[29]

In connection with the possible role of CYP1B1 in fruit-mediated cancer prevention and treatment, a breakthrough occurred a few years ago that involved the phytochemical resveratrol, found in grapes, cranberries, and peanuts, which is well known to have cancer chemopreventive and therapeutic potential operating by a number of suspected mechanisms.[30] It was observed that resveratrol was converted by this enzyme into piceatannol, a cytotoxic anticancer agent.[5] CYP1B1 was obtained from 2 different sources: human lymphoblast-expressed CYP1B1 microsomes and a CYP1B1 transfected *Escherichia coli* enzyme preparation. Both gave the same results. Piceatannol is a potent tyrosine kinase inhibitor that acts on a variety of kinases involved in cell proliferation. It is also interesting in this context that resveratrol exhibits a direct inhibitory effect on CYP1B1 but does not inactivate it.[31] This research provided a new mechanistic vista for targeted cancer therapy. The observation also provided a proof of principle for the exploitation of what had been described as the most exciting development in cancer research in 25 years, that is, the recognition of the overexpression of this enzyme in tumor cells.[10] It also provided a plausible mechanism for at least some of the protective power of fruits because they contain compounds, especially polyphenols, some of which could undergo metabolic reactions catalyzed by CYP1B1 to yield cytotoxins.

Potter and coworkers[5,32] have hypothesized that CYP1B1 may function as what they term a *rescue enzyme*, which uses nontoxic dietary micronutrients as prodrugs, chemicals that are metabolized to active drugs, in this case targeted to destroy cancer cells. Potter and coworkers suggest that these prodrugs had their origin in plant–animal warfare where plants developed agents to combat both animal and microbiological threats, and animals and eventually humans evolved to use some of these chemicals as part of their natural defense system, including preventing cancer.[33] These investigators have identified a number of such compounds that act as prodrugs for this tumor-specific enzyme. Consistent with the hypothesis, the investigators also found that these prodrugs occur at low levels in modern agriculture where pesticides and fungicides eliminated the incentive for plants to synthesize such compounds. However, these prodrugs occur in high concentrations in fruits and vegetables that are organically grown.[2] This observation is consistent with other studies that have examined the impact of pesticides and disease control spray programs on the levels in plants of phenolic compounds, including resveratrol.[34,35] Unfortunately, with one exception,[36] Potter and coworkers do not appear to have published the identity of the compounds they have found to be active or the details of their methodology.

Phytochemicals that function as prodrugs for CYP1B1 frequently have a sharp or bitter taste, a problem that has been the target of both plant breeders and the food processing industry. This has provided an additional explanation for the decline in the levels of these phytochemicals in modern agricultural products and processed foods. Potter and coworkers point out that this decline is coincident with the increase in the incidence of cancer.[2]

The discovery of CYP1B1 followed by the elucidation of its unusual tumor specificity and potential to produce cytotoxins, which was pioneered by Graeme Murray, Gerald Potter, M. Danny Burke, and their coworkers, may significantly aid in identifying, among hundreds if not thousands of dietary phytochemicals, those that are the most active in either preventing or treating cancer. But only a very small fraction of the menagerie of potentially beneficial candidates will in fact be metabolized by CYP1B1 or other tumor-specific enzymes to generate substances that halt cell proliferation, induce apoptosis, or in some other way deal with the presence of tumor cells. It would appear that these particular prodrugs, either natural or synthetic, can and are being identified by screening, using established procedures and cancer cell lines.[2,33] However, confirmation with animal studies may be important.[37]

CYP1B1 Activity in Premalignant and Normal Tissue

One very important question in the context of this review concerns the presence of CYP1B1 in normal and premalignant tissue.[1,33] Studies on tissue from surgically removed prostates have provided some interesting insights. Carnell et al[11] examined 33 prostatectomy specimens using a specific monoclonal antibody for the enzyme. No CYP1B1 was found in normal prostate tissue, but CYP1B1 was present in the cytoplasm of tumor cells but not in the surrounding stromal tissue. The enzyme was also detected in premalignant prostatic intraepithelial neoplasia and in noncancerous tissue associated with benign hyperplasia (BPH), metaplastic prostatic urothelium, and hyperplastic prostatic urothelium. The observation that similar levels of the enzyme were present in BPH and tumor tissue is consistent with the findings of Tokizane et al,[38] who found levels in the former that were about half that found in tumor tissue. It was concluded that these observations imply a possible link between CYP1B1 and malignant progression. This observation also raises interesting questions regarding the relationship between BPH and prostate cancer because pathologically BPH is not considered a precursor for

prostate carcinoma.[39] Polymorphisms in CYP1B1 have also been associated with increased risk of prostate cancer.[40]

One important issue concerns fact that normal tissue levels of CYP1B1 mRNA or protein may be a significant factor in the initiation of carcinogenesis triggered by exogenous procarcinogens. This is one of the reasons for proposed preventive measures involving the inhibition of this enzyme.[8,41] CYP1B1 and a number of other P450 enzymes play a role in deactivating or reducing the effectiveness of chemotherapeutic agents, and this has also stimulated research into inhibition.[25,41] Most of the enzymes suggested for targeting have multiple functions, they are not tumor specific, and their inhibition could well produce undesirable side effects. In the case of CYP1B1, there also appears to be a conflict in strategy between inhibition without producing cytotoxic metabolites and exploiting the ability of this enzyme to catalyze the production of targeted cytotoxins. There also appears to be a strategic conflict between this type of inhibition and the postulated role of this enzyme that may have evolved over a million or so years to provide natural surveillance for and destruction of adventitious cancer cells. However, substances that are metabolized by the enzyme to produce cytotoxins may also act as inhibitors, as appears to be the case with resveratrol.[31] The same strategic concerns can be raised for using vaccines against CYP1B1.[42]

Enzyme action becomes increasingly complex in the presence of several competing substrates, and the optimum exploitation of CYP1B1 will require consideration of a number of factors. In addition, dietary substrates no doubt in some cases act as inhibitors without producing cytotoxic metabolites or inactivating the enzyme. However, given that CYP1B1 is a cancer marker, there should be merit in any approach that eliminates as many cells as possible that carry this marker, especially because those that are not associated with established tumors appear to have low prevalence and their elimination may be accompanied by few side effects. This is clearly an area that deserves attention.

Human Studies

The nature of CPY1B1 as a catalyst for hydroxylation that enabled this enzyme to produce from resveratrol a known and well-characterized cytotoxin prompted the investigation of other chemicals, including polyphenols of similar chemical structure, as candidates for tumor-specific induced cytotoxicity. Potter's group has been active in identifying a number of candidates among both naturally occurring substances and synthetic chemicals.[2,36] In collaboration with an organization that is part of Nature's Defence, a British holding company, these investigators have been involved in developing fruit extracts containing high concentrations of CYP1B1 active compounds, and a number of hydrophilic and lipophilic compounds yielding cytotoxic metabolites have been identified and made available over the counter. There do not appear to have been clinical trials reported, either randomized or otherwise, and the identity of the compounds appears proprietary.

However, these extracts have been used by individuals in the belief that the extracts are a significant approach to therapy for existing cancer; some interesting anecdotal results have been collected, and 5 case reports have been published.[33] All involved advanced and/or terminal cancer cases that included melanoma, lung, prostate, bladder, and breast. In all cases the positive response was rapid and dramatic and, for some, apparently curative. Unfortunately, these results, although published in a peer-reviewed publication, have surely gone almost entirely unnoticed because the journal is not monitored by Medline (PubMed) and probably is absent from many library subscription lists. For those who accept that case histories are important and can be informative, these results suggest that the therapeutic strategy implied by the unusual properties of CYP1B1 may indeed be significant, especially because the agents used were selected to maximize the cytotoxic power of the metabolites in question. The preparations used in these case studies were formulated from extracts of such common fruits as blackcurrant, blueberry, strawberry, and tangerine (peel) and were both hydrophilic and lipophilic. No adverse reactions were reported, but this was merely a collection of anecdotal results. These preparations are marketed under the name *Salvestrols*, a term coined by Potter to reflect the view that they represent substrates for a rescue enzyme.

These case study results can viewed as an initial indication that the therapeutic implications of the test tube and cell culture research, which involve screening for substrates that can be transformed into effective cytotoxins, are indeed correct. The case studies of course bypass the conventional steps between experimental studies and human evidence-based therapeutic use, but it can be argued that this is justified given the problems of human studies using phytochemicals and the fact that the therapeutic agents were simply extracts from fruits that are part of the normal human diet. However, intakes potentially optimum for therapeutic purposes appear to be unknown.

Although there do not appear to be any human clinical trials that address exploitation of the tumor specificity of CYP1B1, two phase I trials of prodrugs targeted mainly on the P450 CYP1A1 are underway, one involving a compound called *phortress* and the other an aminoflavone. But CYP1A1 is not tumor specific, and drugs targeting specifically CYP1B1 would presumably offer greater safety. One such drug, DMU-135, is currently under development. It is a prodrug metabolized by CYP1B1 into a potent tyrosine kinase inhibitor. It has been shown to prevent gastrointestinal tumor formation in a mouse model without any sign of toxicity.[8,43]

Implications for Fruit Extracts and Concentrates

These results point to the potential benefits of the fruit concentrates widely available today as supplements. These include blueberry, bilberry, cranberry, grape seed, pomegranate, green tea, and whole grape, as well as cocoa and other polyphenol concentrates, cruciferous vegetable extracts, and concentrated resveratrol. It is reasonable to assume that some or all of these extracts and concentrates have chemical components that are metabolized by CYP1B1, with some yielding cytotoxins. Furthermore, the problem attributable to modern agriculture of low concentrations of compounds that might be effective in the context of this review is to some extent overcome by the high levels of phytochemicals achieved by extraction and concentration. Presumably, these extracts carry little or no risk, although this is of course unknown and it is unlikely that toxicity from high doses will ever be studied because these are natural products. The issue appears to be simply a question of ingesting increased if not abnormally large amounts of certain phytochemicals that are already consumed in human diets.

It is also well known that many individuals fail to consume adequate amounts of fruits and vegetables, not only because of dietary preferences but also because of seasonal availability and cost. Organically grown heritage varieties of fruits are obviously available to only a very limited number of individuals. Fruit extracts obviously offer a potential solution to this problem and provide the opportunity for exploiting what has been suggested as an essential natural cancer defense system that has evolved over eons. Extracts with demonstrated cytotoxic metabolites generated by CYP1B1 would appear to be very interesting in this connection, and the case studies provide some guidance regarding the issue of dose. Thus there appears to be urgent need for expanded research regarding fruit extract efficacy, dose dependence, and safety for both prevention and targeted therapy with special attention to those extracts containing high levels of phytochemicals that are demonstrated to be specific and theoretically beneficial substrates of CYP1B1.

An impediment to progress in this field is simply that randomized clinical trials will probably only be carried out for patentable compounds, and in the context of primary prevention, such studies of necessity involve very large numbers of participants, long follow-up, and great expense. It seems likely that such trials are far from imminent. On the other hand, clinical trials involving therapy can be much smaller and provide results over a much shorter period. However, for ethical reasons, such trials probably would at least initially involve only cases deemed untreatable by conventional medicine, especially if natural phytochemicals are involved, but there will always be some who reject treatment, especially chemotherapy, and would become candidates for such trails. It is possible that therapy targeting CYP1B1 would not interfere with conventional therapy and that the combination would not raise serious ethical issues.

Conclusions

Schaefer et al[33] summed up the attributes of the natural approach to prevention and therapy using plant-based phytochemicals that are transformed by CYP1B1: (a) Toxicity appears negligible because the toxins produced are confined to tumor cells and exhausted during apoptosis; (b) dietary substances are involved; (c) the mechanism of action provides a clear link between diet and cancer and should inspire dietary change; and (d) CYP1B1 appears to be a universal cancer marker, and the associated defense mechanisms can be used regardless of the oncogenic origin of the cancer.

The above discussion appears to provide compelling evidence for the need to accelerate research regarding this remarkable enzyme. Many fundamental issues apparent in this review clearly merit investigation. These include the role of CYP1B1 in the early development of cancer, the significance of its presence in premalignant tissue and apparently normal tissue, and the mechanisms and timing associated with the regulation and development of overexpression in cancer cells. Applications to prevention and therapy are clearly in the beginning stages, but the anecdotal evidence of the efficacy of targeted therapy in advanced cancer, although obviously very limited, should stimulate a strong interest in more extensive human trials, especially because the case studies tend to confirm that the efficacy is independent of the cancer type being treated. Individuals who reject conventional cancer therapy potentially could serve as subjects in studies that could yield rapid answers to many of the issues raised in this review.

Much research is needed to clarify the appropriate approach to exploiting CYB1B1 for both primary prevention, targeted therapy, and secondary prevention. Inhibition by compounds that do not necessarily go on to produce cytotoxicity and, as well, the development of vaccines against CYP1B1 appear to be the main focus at present. Implementation of both of these approaches, although perhaps justified in some situations, would interfere with what appears to be an important natural action of this enzyme, which presumably evolved prior to the introduction of manmade carcinogens into the environment or the inhalation of carcinogens via smoking. It can be argued that an approach focused on prevention consistent with the present state of knowledge is to simply provide this enzyme with substrates derived from a diet high in organically grown fruits or, in the absence of such produce, a diet rich in fruits that is augmented with fruit extracts and concentrates. Individuals who have failed to

respond to standard treatments or those who refuse such therapy may be candidates for treatment with commercial preparations already screened as potent cytotoxic yielding substrates of CYP1B1, and these preparations may also be interesting candidates for preventative strategies when used at low doses.

In view of the research cited in this review, human trials of selected natural and synthetic substrates for CPY1B1 should be awaited with considerable anticipation and impatience.

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