ARTICLE

As geneticists often point out, genetic manipulation is not new. Over the years, fruits and vegetables have been selected for sweetness, among other attributes. However, a lack of bitterness may have contributed to the current connection between diet and the increase in cancer rates, as Susan Pearson explains.

Bitter is better

The link between diet and cancer

According to Cancer Research UK, more than one in three of us will develop cancer at some time in our lifetime. This scary prediction reflects the rapid rise in cancer rates over the last few decades, as 270,000 people in the UK are being newly diagnosed with cancer each year. Although we still don't know the precise causes of most types of cancer, an unhealthy and unbalanced diet is considered the second biggest risk factor after smoking.

Cancer experts agree that around half of all cancers could be prevented simply through lifestyle changes; a message the government has been pushing with its healthy eating 'five-a-day' campaign. Launched in March 2003, the advice to eat at least five portions of fruit and vegetables daily to benefit our health is now very familiar. And it certainly seems that some of us are listening, as supermarkets are reporting increases in sales of fresh produce of 15–21% in the last year.

However, although the public has started to get the message about diet, cancer rates continue to rise, as our increased consumption of fruit and vegetables does not appear to be having much impact. There are certain isolated parts of the world where cancer is virtually unknown, so, if the link between diet and cancer is really so strong, it seems that our Western food is not offering us the cancer-protective nutrients we need. In Leicester, an independent research company has been working with scientists from De Montfort University to tackle just this question, and their results offer a significant and elegant explanation for what is happening in our food.

Cytochrome activation

The story begins in Aberdeen in the early 1990s where a small research team in the School of Medicine at Aberdeen University, headed by Professor Danny Burke, discovered what appeared to be a new CYP1 type of cytochrome P450 in human cancer cells. Cytochrome P450s are a big family of enzymes of major pharmaceutical significance because of their detoxification activity in the liver. They will metabolise almost any pharmaceutical compound, as well as many environmental toxins, transforming them into inactive substances. However, in some cases P450s respond by converting a pharmaceutically active drug into an equally pharmaceutically active metabolite. Some drugs, such as the chemotherapy agent cyclophosphamide, are entirely dependent on activation by these enzymes.

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In collaboration with Professor Bill Greenlee at the University of Massachusetts Medical School in the USA, the Aberdeen group was able to identify the cancer cell P450 as CYP1B1, a form recently discovered by Greenlee. What was significant about this finding was that immunohistochemical studies could locate CYP1B1 only in cancer cells – it was undetectable in the normal cells of the corresponding healthy tissue. This was definitely a different version from any P450 found in the liver or any other healthy cell.[1] Subsequent research showed that CYP1B1 protein is over-expressed in human tumours and is not specific to any particular type of cancer – it seems to be a feature of all cancer cells.[2]

Moving to Leicester to head the School of Pharmacy at De Montfort University, Professor Burke took the outline of this project with him. Here, the new Professor of Medicinal Chemistry, Gerry Potter, whose work is based on the development of anticancer drugs, began to look at how the CYP1B1 mechanism could be incorporated into a prodrug.

Rise of the prodrug

Anticancer prodrugs have no anticancer activity in themselves but they pass through the normal liver where one of the P450s (not CYP1B1) causes their conversion into active anticancer metabolites. While this conversion takes place in the healthy liver, metabolites move into the bloodstream from where they target cancer cells but also cause systemic side effects. What the Leicester team hoped to do was to design a prodrug that would not be activated by any of the P450s found in normal cells, but, if activated within cancer cells by the CYP1B1, might destroy or interfere with the growth of cancer cells without causing the side effects created by regular chemotherapy.

However, a piece of lateral thinking also took Potter's work in another direction. Cancer cells occur in our bodies every day and yet generally do not grow into malignant tumours; thus, something must be responsible for the death (apoptosis) of these cells. He postulated that the body might equip cancer cells with the seeds of their own destruction, a kind of Trojan horse that liberates a destructive force. What if CYP1B1 were that Trojan horse? And, if so, what could that destructive force be?

Any system that evolution might have produced as a primary system of control for cancer cells would have to be fuelled from somewhere. And since humans are fuelled by food, it seemed likely that ordinary nutritional components might provide the chemicals that create self-destructive properties in cancer cells, as there are cases where patterns of cancer incidence are clearly related to lifestyle (the classic example being the differences in diet and cancer incidence between California and Japan).

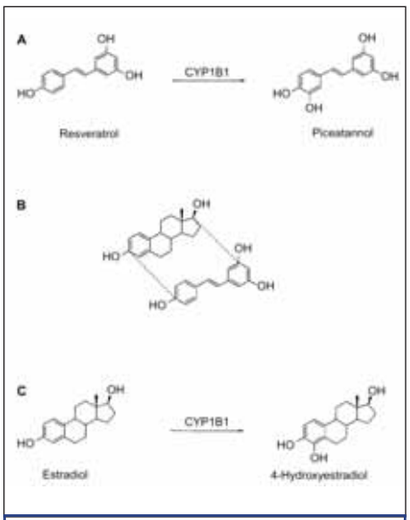


Fig 1. Molecular structures of resveratrol, piceatannol, oestradiol and 4-hydroxyoestradiol: a) the conversion of resveratrol to piceatannol catalysed by CYP1B1; b) mapping of the phytoestrogen on to the steroid framework of oestradiol; and c) the CYP1B1-catalysed aromatic hydroxylation of oestradiol to 4-hydroxyoestradiol (Reprinted by permission from Macmillan Publishers: *Br J Cancer* 2002; **86**; 774–8. © 2002).

As Danny Burke says: "This seemed a reasonable hypothesis, that it's our food that's been stopping us developing more cancers than we would otherwise get, provided you eat the correct food and metabolise it properly." Taking the research down this route, Potter and Burke's team began the work of identifying what the crucial chemicals in food might be.

Enter resveratrol

By 2002, Potter and his colleagues were able to publish a paper showing that resveratrol, a natural phytoestrogen found in grapes and red wine,[3–5] is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1.[6] Resveratrol has a stilbene core structure, as has piceatannol, which is structurally different from resveratrol only by the presence of an extra hydroxy group in one of its aromatic rings. Resveratrol is classified as a phytoestrogen because of its similar molecular structure to the endogenous oestrogen oestradiol. CYP1B1 was already known to have aromatic hydroxylation activity, and to catalyse the conversion of oestradiol to 4-hydroxyestradiol (Fig 1).

Owing to this relationship, the team reasoned that resveratrol might similarly undergo aromatic hydroxylation by CYP1B1, and that aromatic hydroxylation at the position corresponding to that of 4-hydroxyestradiol would generate the tyrosine kinase inhibitor piceatannol. This type of mapping had already been used by the team to design special CYP1B1-activated tyrosine kinase inhibitor prodrugs, based on the stilbene structure, for tumour selective cancer therapy.[7] It seemed that there was a similarity between the molecular structure of the anticancer prodrugs the group had designed in relation to CYP1B1 and the molecular structure of certain natural products that have cancer preventative properties.

The group hypothesised that CYP1B1 might have a functional role as a tumour suppressor enzyme, or 'rescue' enzyme. CYP1B1 would serve to activate certain non-toxic dietary components into a growth inhibitory substance specifically within tumour cells containing the CYP1B1 enzyme. High-performance liquid chromatography (HPLC) followed by GM-MS studies confirmed that resveratrol was metabolised by CYP1B1 to generate piceatannol.

Resveratrol was the first identified of a new group of over 20 natural dietary compounds, all shown to possess anticancer properties (unpublished observation). Discovered and named salvestrols by Potter, these are natural compounds produced by plants as a defence mechanism against pathogens such as pests and fungi. In pharmaceutical terminology, salvestrols conform to a pharmacophore, chemicals that have a pharmacologically active part of their structure in common, but which cannot actually be classified as the same chemical family of compounds, although the structure they do have in common will be biologically activated in the same way. The compounds in the salvestrol group are related by the way in which they are all metabolised by CYP1B1.

The confirmation of this mechanism left a big question mark. If CYP1B1 metabolism of salvestrols, which occurs naturally in our food, destroys cancer cells then why is cancer on the increase? Is something wrong with our food?

Nature's Defence

To look more closely at salvestrols in food and to create nutritional extracts containing active compounds that could be used therapeutically, the group at De Montfort linked up with independent natural products manufacturer Global Botanical Research (GBR), an expert in the design, formulation and licensing of plant-based extracts, and the types of chemistry the company had already developed fitted well with the work of Potter and his colleagues. This collaboration led to the foundation in early 2004 of a new self-financed research-based company, Nature's Defence, specialising in nutrition and plant chemistry.

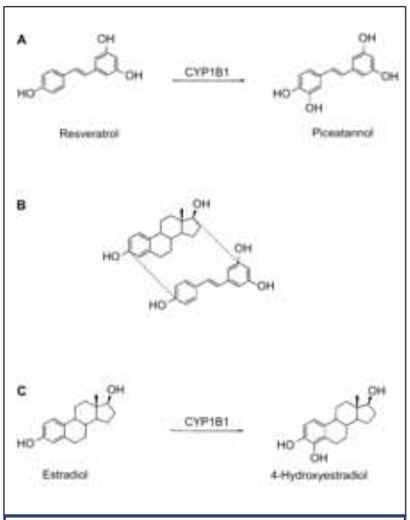
> 'Analysis of fresh fruit and vegetables from wide-ranging sources has revealed huge discrepancies in levels of salvestrols'

Nature's Defence has now analysed around 2000 plant food sources, with disturbing results. Analysis of fresh fruit and vegetables from wide-ranging sources (supermarkets, farmers' markets, organic, non-organic and many processed foods) has revealed huge discrepancies in levels of salvestrols. Apparently identical crops might contain high levels of salvestrols, or none at all. Most notably, methods used to grow fresh produce and differences between varieties appear to have a big impact. Organic fruit and vegetables are often, but not always, found to contain much higher levels of salvestrols than food produced by conventional modern intensive farming, which tends to contain low levels or none at all. The majority of plant-based processed food also contains few if any salvestrols.

Anthony Daniels, managing director of Nature's Defence, explains why this might be. "Firstly, salvestrols are produced by plants as a natural defence against disease. When outside pathogens are removed from the environment by the pesticides used in modern farming methods, the plants simply stop making them. Secondly, the way salvestrols manifest in flavour is as bitterness. Over the last 50–60 years agriculture has focused on breeding sweeter, less bitter-tasting varieties, so has effectively been breeding most of the salvestrols out of our food. What's more, consumers have also now come to expect fruit and vegetables that are consistent in size, colour and shape, and thus many of the old varieties are disappearing. It seems that it is not just the use or non-use of pesticides that will determine salvestrol levels in fresh produce."

The last two decades have also seen many of us become heavily reliant on highly processed food, but this processing also has an impact, often removing salvestrols or losing the ability to keep them intact. A seemingly innocent example is olive oil. Testing a snapshot of 10 different brands revealed only one with any significant levels of salvestrols. This was a cloudy organic oil that had been produced using traditional stoneground methods. The other oils, organic or otherwise, were all cold-pressed virgin oils, and it appears that cold pressing is not able to extract the salvestrols into the oil. In other cases, salvestrols are removed deliberately. For example, 'natural' fruit juices often have any 'bitterness' (in effect, the salvestrols) extracted to make them taste sweeter. Food labelling regulations require that what has been added to our food be stated, but not what has been removed.

Based on these findings, Nature's Defence has been producing therapeutic dietary



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supplements in the form of capsules containing a blend of bio-available salvestrols from fruit, using varieties that contain high levels of the compounds. Working with cancer groups around the country and with local oncologists, feedback has been encouragingly positive, and has even included testimonials from patients experiencing the disappearance of or reduction in their tumours.

Nature's Defence did not set out originally to produce publishable results; rather, the key aim was always to seek out compounds in food that are involved in the CYP1B1 reaction. But with increasing interest in its supplements from patients, and a huge bank of data on salvestrols, the company is now aiming for peer-reviewed publication to complete the scientific credibility of its work, although this is some time away.

Salvestrol-sure food

In the meantime, as a result of its conclusions on the low salvestrol content of today's fruit and vegetables, the company has been directing its research towards the salvestrol content of older varieties of produce. Collaborative projects are now being set up with Kew Gardens and the Eden Project to help identify varieties that may be fast disappearing. Another collaboration has been set up with a local Leicestershire farmer to grow what the group term 'salvestrol-sure food', which include varieties that are known to have a high salvestrol content and have been grown without the use of pesticides (Fig 2).

The group emphasises that although conventionally grown food is full of useful nutritional properties, eating 'salvestrol-sure food' is the crucial factor. Anthony Daniels believes: "If Potter and Burke's discoveries are correct then we are seeing a very clear link between our research and the problems in the food chain that are causing cancer rates to increase. Scientists generally look for reasons for the induction of cancer from environmental carcinogens, but, while they are undoubtedly part of the problem, we don't think they're the main issue." ■

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Susan Pearson (not to be confused with Sue Pearson) is a freelance writer specialising in medical and environmental issues. For further information on Nature's Defence research and supplements log on to www.naturesdefence.com or www.fruitforce.co.uk.