Identification and Cancer Therapeutic Properties of Microfloral Anthocyanin Metabolites

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ABSTRACT Colon cancer is very common, but those who consume anthocyanins, common red pigments in wine, grapes and other fruits, appear to have lower rates of colon cancer. We investigated the fate of grape anthocyanins in the colon by using a pig model of the lower intestine and observed the rapid conversion to three simple phenols. These were studied, along with metabolites reported by others, for their effect on cell proliferation and apoptosis, and we observed promising results; cell proliferation was suppressed and apoptosis enhanced. Thus, these metabolites are promising anti-cancer agents and the effect of anthocyanins on colon cancer appears to be mediated by these bacterial metabolites.

Introduction

Colon cancer is the third most common form of cancer in the US, with its highest occurrence in North America and Europe, and lowest in India. It often begins with benign adenomatous polyps, with symptoms of pain, diarrhoea, weight loss, and bleeding. The mechanisms that lead to colon cancer are multi-faceted, and include behaviours such as smoking and diet, but at a molecular level, many inflammatory factors are important, such as reactive oxygen species, TNF-α, the COX enzymes and NF-κB (Singh et al., 2008).

Current prevention and treatment includes the use of colonoscopy to detect the presence of emerging polyps and cancers, surgery to remove any detected tumours, as well as chemotherapy and/or radiation for follow-up treatment. There is some use of NSAIDS as anti-inflammatory COX-2 inhibitors, such as celecoxib.

Of the plant phenolics, the anthocyanins are the most interesting. Consumption of foods high in anthocyanins have been shown to be protective, and some effects in in vitro and in vivo experiments have been demonstrated (Kang et al., 2003; Yi et al., 2005; Cooke et al., 2006). There are also reports of decreased risk for moderate wine drinkers in epidemiological studies (Anderson et al., 2005). However, low levels are absorbed into the bloodstream, and it is well established that polyphenols are metabolized by bacteria/enzymes in the large intestine (He et al., 2005). So, our goal was to
determine grape anthocyanin gut metabolites and to see if these substances had any promising anti-cancer activity.

**Procedures**

The experiment started with an ethanolic extraction of phenolic compounds from cabernet sauvignon grapes and the removal of organic acids and sugars with an Amberlite XAD-7 column. The purified material was fractionated with 60 ml solid phase extraction (SPE) C-18 cartridges: eluting anthocyanins and catechins with 16% acetonitrile (pH 2.6). Finally this was purified by liquid/liquid extraction with ethyl acetate to isolate anthocyanins (Pinelo et al., 2006). This was a mixture of all five anthocyanins, and their acylated derivatives normally found in grapes, and the majority was malvidin-3-glucoside.

The extract was incubated with pig intestinal contents at 37°C under an anaerobic atmosphere for up to six hours. The incubation was then tested for anthocyanins as well as any new substances by an LC-MS Method, using electrospray ionization, and SIM mode for quantification with positive polarity.

All anthocyanins were metabolized below limit of detection after six hours in the active intestinal contents, and some chemical degradation of all the anthocyanins was observed in the inactive incubations as well.

The metabolites that were formed included syringic acid. This was also found in small amounts in inactive samples. The major metabolites were 2,4,6-trihydroxybenzaldehyde, 3, and 3-O-methylgallate, 1. The latter required synthesis that was accomplished by protecting two phenoxyl hydroxyl groups of methylgallate with sodium tetraborate, and methylating the 3 position with dimethyl sulfate (Scheline, 1966). Its structure was confirmed by NMR spectroscopy.

![Malvidin-3-glucoside Metabolism](image)

The activity of these metabolites, plus gallic acid, observed as an anthocyanin metabolite by others, was measured by screening with caco-2 cells (human colon...
cancer cells). The tests were to measure their effects on cell proliferation, cytotoxicity, and apoptosis.

The metabolites showed limited toxicity, but clearly suppressed cell proliferation. On the other hand, these metabolites did induce apoptosis at 140 μM. Future studies will address the mechanism by which these effects could occur, including cell cycle analysis, transcription factors and DNA fragmentation. One follow up study showed that the active substances had a very transient existence in the incubation media, breaking down in a few hours. This suggested that the activity might be due to these substances acting as pro-oxidants, releasing hydrogen peroxide that would, in a

![Cell Proliferation Dose Response Curves](image1)

![Cytotoxicity of Treatments](image2)

![Comparing Induction of Apoptosis at 140 μM](image3)
Fenton-type reaction, oxidize any enzymes present, possibly causing the effects seen above.

**Conclusions**

Consumption of cabernet sauvignon grape anthocyanins could produce metabolites 3-O-methylgallic acid, syringic acid, and 2,4,6-trihydroxybenzaldehyde from bacterial metabolism in the gut. 3-O-methylgallic acid, gallic acid, and 2,4,6-trihydroxybenzaldehyde are promising candidates for affecting development of colon cancer treatments at low concentrations.

**References**


