Antigenotoxic and Physiological Properties of Anthocyanin-Containing Fruit Extracts in Intestinal Epithelial Cells.

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Summary

In-vitro aronia extracts show biological activity exerting protective effects at concentrations of approximately 25 μg anthocyanins/ml against DNA damage induced by the oxidant H$_2$O$_2$. The extracts are also potent inhibitors of hormone-induced changes in cellular metabolism, at least in transformed human colonic tumor cells. The precise nature of these inhibitory actions needs to be elucidated in further studies. Also it is not clear to which extent these protective activities are actually due to the anthocyanin components of these complex extracts. According to a detailed first analysis, the extracts also contain other phenolic compounds which may be contributing to the biological activities. Therefore, ongoing studies are directed at elucidating analogous activities by purified fractions and by individual components of these extracts, including chemically pure anthocyanidins and anthocyanins.

Introduction

Anthocyanins and their aglycones, the anthocyanidins, belong to the flavonoid family of plant polyphenolic compounds. As water soluble plant pigments the anthocyanins are particularly present in high concentrations in various berries and grapes in the human diet. Comprehensive reviews of the chemical and plant physiological aspects of anthocyanins were recently published (1, 2). Flavonoids in general have long been regarded as health promoting, protective substances with respect to their antioxidant, antiallergenic, antiinflammatory, antiviral, antimutagenic and anticarcinogenic properties (3). Several mg or more of mixed flavonoids per day are contained in a typical Western diet (4, 5). This amount could have marked physiological or pharmacological effects, provided these compounds are significantly absorbed from the gastrointestinal tract. On account of the structural similarity of protective flavonoids and anthocyanidins, the latter category of compounds are also considered to have antigenotoxic / antioxidative activities, although they have been much less investigated (6). Primarily they have been implicated in contributing to postulated health effects by red wine in some regions of France (7, 8, 9).

In order to elucidate the protective properties of naturally occurring anthocyanidins, we are presently investigating complex extracts from various edible red and blue berries for antigenotoxic/antioxidative properties and for their potential to interfere with cellular hormonal signalling pathways. These effects may either contribute to preventing initiation processes of carcinogenesis, or they may lead to modulation of proliferation involved in tumor progression (10).
Materials and Methods

The studies were performed with primary isolated human colonic cells obtained from biopsy samples and with cells of a human colonic tumor cell line, HT29 clone 19A (11). This report will focus on the activities of crude anthocyanin-containing extracts from the aronia fruit (Aronia melanocarpa; Wild GmbH, Heidelberg, Germany).

Extracts of Aronia melanocarpa:

As is shown in Table 1, the extracts of the black chokeberry fruit (aronia) are reported to contain mainly glycosides of cyanidin (1, 12). Our crude fruit extracts contained approximately 2% pure anthocyanins, the exact composition of which are presently being elucidated (in progress). For the in vitro studies, the extracts were diluted to yield an approximate concentration of 25 µg anthocyanidins/ml in the assays for antigenotoxicity and 6.25 µg anthocyanidins/ml in the experiments determining interference with cellular hormonal signalling pathways.

Table 1. Anthocyanins in Aronia melanocarpa Elliot
Black chokeberry, North America

<table>
<thead>
<tr>
<th>Anthocyanin Glycoside</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>cyanidin 3-galactoside</td>
<td>(64.5%)</td>
</tr>
<tr>
<td>cyanidin 3-arabinoside</td>
<td>(28.9%)</td>
</tr>
<tr>
<td>cyanidin 3-glucoside</td>
<td>(2.4%)</td>
</tr>
<tr>
<td>cyanidin 3-xyloside</td>
<td>(4.2%)</td>
</tr>
</tbody>
</table>

according to Oszmianski and Sapis (12)

Determination of DNA damage in colon cells with the COMET assay

Antigenotoxic effects were studied with the single cell microgel electrophoresis (COMET) assay in isolated human colonic cells obtained from biopsy samples (13). Oxidative DNA damage was induced by incubation of the preparation with hydrogen peroxide (H₂O₂).

Cytosensor Microphysiometer measurements

The interaction between peptide hormones (insulin, neurotensin) and anthocyanin-extracts was assessed in HT29 clone 19a cells with a Microphysiometer CytoSensor® (Molecular Devices, Munich, Germany). This physiometer, based on silicon technology allows the sensitive detection of hydrogen ions in the extracellular medium. Cells will increase the acidification of extracellular medium as a result of increased energy metabolism or of receptor ligand interactions (14).

Results and Discussion

Incubation of the primary human colon cells with or without the crude fruit extracts containing anthocyanins (25 µg/ml) for 5 minutes on ice (4°C) shows a clear cut protective effect of the aronia extract against oxidative DNA damage induced by H₂O₂. A significant reduction of DNA damage is observed in the anthocyanin-pretreated colon cells from all 6 donors (Figure 1.)
Figure 1. Effects of aronia anthocyanin extract (25μg/ml) on DNA damage induced by H₂O₂ (150 μM) in primary human colon cells of 6 individual donors

In the COMET assay the aronia fruit extract clearly had a protective effect on oxidative DNA damage in primary human colon cells. This antioxidant property of the aronia extract may indicate a protective activity of these natural plant extracts in the initiation phase of carcinogenesis.

Modulation of cellular hormonal signalling pathways

The HT29 clone 19A cells express functional receptors for the peptide hormones insulin and neurotensin, respectively. An increased acidification occurred after incubating the cells with either hormone. Insulin acts through transmembrane receptors initializing tyrosine kinase phosphorylation and, in HT 29 clone 19 A cells, induces a significant persistent stimulation of cell metabolism at 10⁻⁷ and 10⁻⁸ M compared to the untreated DMEM control (Figure 2). After removal of insulin the acidification rate (cell metabolism) returns slowly to the control values.

In contrast to the steady-state change of cell metabolism induced by insulin (maximum increase of 10-20%), neurotensin more potently but only transiently stimulated cell metabolism in HT29 clone 19A cells (300-400% increase). Neurotensin is a peptide hormone acting through G-protein receptor coupling on the phospholipase C - inositol-1,4,5-triphosphate - calcium and the diacylglycerol - protein kinase C signalling pathways, respectively.
Figure 2. Concentration and time dependence of insulin-induced acidification in HT29 clone 19A cells (means ± SEM, n = 4)

The stimulation by neurotensin was also observed in the presence of insulin and appears to be additive to the action of insulin, as is shown in Figure 3 (DMEM curve).

Figure 3. Effect of aronia anthocyanin extracts (AA, 6.25μg/ml) on acidification induced by insulin (2 x 10⁻⁷ M) and neurotensin (10⁻⁸ M) in HT29 clone 19A human colonic tumor cells (means ± SEM, n = 3)
Figure 3 also shows that the action of insulin was inhibited after treatment of the cells with aronia extract at low concentrations of 6.25 μg/ml. In addition, the effect of neurotensin on the extracellular acidification rate of HT29 clone 19 A cells was abolished after aronia extract treatment. In this experiment the effects were apparent when concomitantly incubating the extracts with the hormones (Figure 3). The aronia extract also induces a small change of cell metabolism by itself, as is seen in the first peak at 10 minutes, immediately after the beginning of treatment. This induction of metabolism may be caused by one of the major aglycones, cyanidin, as our preliminary results are presently indicating (in progress).

Neurotensin and insulin induce growth and proliferation in the HT29 human colonic tumor cell line and thus may promote tumor development. Neurotensin receptors are only expressed in transformed human colonic cells, but are absent in normal cells, and thus may serve as a biological marker for malignant transformation of colonic cells. The pathophysiological role of neurotensin receptors in transformed colonic cells is unclear, however, proliferation is stimulated by neurotensin. Thus the aronia extracts, containing anthocyanins and other phenolic compounds, may be exerting a suppressive mode of activity by inhibiting the hormone-dependent stimulation of cell metabolism and proliferation. The exact pathways of this inhibitory action and the interaction of anthocyanins with the hormone receptors, particularly of growth related hormones, and the subsequent cellular signalling cascade has to be studied in more detail to fully understand the mechanisms of action of these extracts and their natural ingredients.

Conclusions

In conclusion, crude extracts with red-blue coloured flavonoids (anthocyanins/anthocyanidins) exert direct biological activities in human intestinal cells which may contribute to prevent early events of carcinogenesis. The inhibition of DNA damage and of hormone-stimulated cell proliferation, respectively, may be of potential relevance for tumor development and thus it is certainly very important to investigate these properties in greater detail. In this regard it is also of primary interest to determine the physiological concentrations attained in the intestinal contents and in the circulating blood after the dietary consumption of high amounts of anthocyanin-containing foods. At the moment there are promising observations for potential beneficial health effects of anthocyanins and anthocyanidins, however, we are far from being able to recommend certain dietary intakes of these compounds.

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