Indicaxanthin Bioactivity in Health and Disease

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Indicaxanthin is a dietary, highly bioavailable phytochemical from cactus pear fruit, with nutraceutical potential. Studies in healthy, transformed cells and whole organisms suggest health-promoting activities, particularly in counteracting inflammation and regulating mechanisms that control cell growth and longevity. In vitro evidence to date does not provide a unified picture of the molecular mechanisms mediating the action of Indicaxanthin; rather different mechanisms have emerged in relation to different stimuli and conditions in both healthy and transformed cells. Many of the activities appear to be geared toward restoring cellular redox homeostasis, correcting dysfunction generated by oxidative stress, and modulating signaling pathways that control vital processes in healthy cells; other activities, apparently independent of cellular redox balance, have also been observed.

betalains human bioavailability antioxidative pro-oxidant activity

1. Indicaxanthin, Redox Balance and Oxidative Stress in Healthy Cells

Phytochemicals are recognized as valuable in maintaining cellular redox homeostasis. To what extent is the redox chemistry of Indicaxanthin involved in its effects on cells?

Data so far show that Indicaxanthin is stable and does not interfere with the physiological redox state of healthy cells, in the absence of pro-oxidant stimuli. This was observed with various cell lines incubated with amounts in a fairly wide range of concentrations, including postprandial blood levels (5–10 μM) up to more than one order of magnitude in excess, for time intervals from 6 h to 72 h [1][2][3][4][5][6]. On the other hand, the extent and nature of the pro-oxidant insult has appeared to be decisive for its response and activity.

2. Indicaxanthin Activity in Organ Bath

An experimental approach using a mouse ileum preparation showed that, as a component of an aqueous extract of the cactus pear fruit, Indicaxanthin exerted antispasmodic effects on intestinal motility [7]. It is interesting that vitamin C, to the extent of its content in the fruit extract, potentiated the effect of purified Indicaxanthin. Protection of integrity and stability of the phytochemical by ascorbic acid are known in solution under a variety of conditions [8]. Functional interactions between these bioactive dietary components in a complex biological environment may deserve deeper investigation.

On a mechanistic perspective, the myorelaxant effect was independent of neurotransmitter release and ascribed to direct action of Indicaxanthin on the smooth muscle cell, in particular the inhibition of a phosphodiesterase isoform [9]. This caused an increase of intracellular cAMP, a second messenger associated with smooth muscle inhibitory effects [10], including gastrointestinal smooth muscle relaxation [11] (**Table 1**). Among 11 different isozymes of the PDE family, the PDE-3 and -4 are expressed in the ileal smooth muscle [12][13]. Dynamic interactions between Indicaxanthin and PDE-4 have recently been described by a molecular modelling approach. The ability to cross cell membranes [14][15] and reach and accumulate in the cytoplasm could allow Indicaxanthin to target the enzyme. More intriguing though still unexplored possibility is that Indicaxanthin located at the membrane [16][17] would interact with, or cause alteration of the lipid bilayer surrounding the exclusive and large trans-membrane NH-terminal of PDE-3, a segment involved in inhibiting the enzyme activity [12][18].

Table 1. Bioactivities of Indicaxanthin in cells and tissues.

Cells	Experimental Design	Effect	Key Molecular Mechanisms	Ref.
RBCs (human)	Exposure to cumene hydroperoxide	Antioxidant	Radical-scavenging ↑ Resistance to oxidative hemolysis	[19]
	Exposure to toxic oxysterols	Antioxidant Anti-eriptotic	↓ ROS production, glutathione depletion, PGE2 release, and Ca^{2+} entry	[2]
β–Thalassemia RBCs (from patients)	Exposure to cumene hydroperoxide	Antioxidant	Perferryl-Hb reduction ↑ Resistance to oxidative hemolysis ↓ Vit E and GSH depletion ↓ Lipid and hemoglobin oxidation	[14]
HUVEC (human umbilical vein endothelial)	Exposure to oxidized human LDL	Anti-inflammatory	↓ ROS formation ↓ NF-κB transcriptional activity ↓ expression of adhesion molecules (ICAM-1; VCAM-1; ELAM-1)	[3]
	Exposure to TNFα	Anti-inflammatory	Preserved activity of cholesterol efflux system ABC-A1 ↓ expression of ICAM-1	[20]
RAW 264.7 (murine macrophages)	Exposure to LPS	Anti-inflammatory Pro-oxidant	↓ NF-κB activation ↑ lipid hydroperoxides ↑ HNE formation Modulation of	[4]

Cells	Experimental Design	Effect	Key Molecular Mechanisms	Ref.
			prostaglandin biosynthetic pathway: ↓ mPGES-1 expression ↑ COX2 and MPGDS expression ↑ Synthesis of proresolvin cyclopentenone	
Differentiated human Caco-2 (colorectal adenocarcinoma)	Exposure to IL-1β	Anti-inflammatory	↓ active NOX-1 assembly ↓ ROS generation ↓ NF-κB activation ↓ COX2 and NOS expression	[15]
	Exposure to a mixture of TNFα, IL-1 β, IFNγ	Anti-inflammatory	↑ release of IL6, IL8, PGE2, NO ↓ expression of inflammatory IL6, IL8, COX2, NOS, NOX-1 ↑ expression of glutamate-cysteine ligase catalytic subunit (GCLC) and glutathione peroxidase-1 (GPX-1)	[21]
THP-1 (human monocyte/macrophages)	exposure to 7-keto-cholesterol	Anti-apoptotic	↓ NOX-4 basal activity ↓ NOX-4 over-expression ↓ NF-κB activation ↓ intracellular Ca^{2+} ↓ mitochondrial apoptotic pathway	[1]
OECS (olfactory ensheating nerve)	Exposure to amyloid beta	Anti-apoptotic and cell regenerating	↓ $O_2^{\bullet-}$ and ROS production ↓ transglutaminase 2 expression ↓ caspase 3 expression ↑ nestin and cyclin-D1	[22]
Tissues				
Mouse ileum muscle [23][24]	Recording of spontaneous or carbachol- or KCl-evoked mechanical activity in organ bath	Spasmolytic	Interference with pathways regulating intracellular Ca^{2+} release ↓ Phosphodiesterase activity ↑ cAMP [5][25] [6]	[7] [9]

city and a treat this thin have r [26], and in a mouse melanoma model as well.

Cells	Experimental Design	Effect	Key Molecular Mechanisms	Ref.
			Vitamin C potentiated the Indicaxanthin effect[29]	[27][28]

↑ increase; ↓ decrease with respect to relevant experiments in the absence of Indicaxanthin.

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ecognized
cause for cancer initiation [30]. Subsequently, cancer cells adapt to high levels of ROS by up-regulation of antioxidant systems, to limit oxidative damage and escape apoptosis. Then, while targeting ROS and ROS signaling with antioxidants is a potential strategy to prevent cancer and/or in the precancerous lesion stage, it may be inappropriate or even detrimental at an advanced stage. Instead, at this point, very high levels of ROS would be required to promote cell death pathways [31][32], or agents that abrogate the antioxidant systems or target unique biochemical features of cancer cells [33][34][35][36]. In this scenario, phytochemicals shown to inhibit proliferation in several tumor cell lines have been associated with several activities: they can act as ROS scavengers; modulate redox-dependent pathways and transcription factors that control gene expression and apoptotic death; activate epigenetic mechanisms; elicit anti-oxidative effects by induction of enzymes involved in antioxidant defence; be pro-oxidant or induce ROS production to kill cancer cells [24][34][37][38]. As for the Indicaxanthin, its antiproliferative effects seemed to be the result of different activities. The in vitro studies have shown that concentrations 5 to 20 times higher than those measured in human plasma after dietary ingestion are required to be effective. The findings are reported in this section, with emphasis on the substantiated mechanisms of action. Based on the known properties of the molecule, additional or alternative mechanistic approaches are also proposed for future research.

Added to proliferating Caco-2 colon adenocarcinoma cells, Indicaxanthin caused a concentration-dependent growth arrest with apoptosis at 48 h, and IC_{50} of 115 μM [5]. The anti-proliferative activity was not associated with changes in the cellular redox balance. Indicaxanthin did not scavenge ROS, nor did it change their intracellular level, nor the level of total thiols [5]. Somewhat intriguingly, this could implicate that Indicaxanthin did not affect the activity of NOX-1, the main source of ROS in colon cancer cells [39], which instead occurred in the differentiated normal-like Caco-2 cells, when submitted to the inflammatory agent IL1-β [15]. Lipid composition and biophysical properties of cancer cell membranes are quite different from those of healthy cells [40][41], it would be meaningful to find out whether and to what extent Indicaxanthin interacts with the membranes of colon cancer or other tumor cells.

Aberrant methylation patterns, i.e., global DNA hypo-methylation contributing to chromosomal instability, but site-specific CpG promoter island hyper-methylation with silencing of tumour suppressor genes, characterize tumor cells, and have been extensively investigated in colorectal cancer [42]. The studies of Naselli et al. [5][25] revealed that Indicaxanthin was able to modulate the epigenome-regulated gene expression by affecting DNA methylation at multiple levels. Treatment of Caco-2 cells with Indicaxanthin (50 μM) increased the global DNA methylation and re-activated the expression of the onco-suppressor $p16^{INK4a}$ gene inducing demethylation of its promoter region [5][25] thus driving cell arrest and apoptosis [43][44][45]. Anti-proliferative effects of Indicaxanthin were also observed in other colon cancer cell lines such as LOVO-1, HCT-116, DLD-1, but not in HT-29, with differences in magnitude of effects, and Caco-2 cells as the most responsive [25]. In addition to $p16^{INK4a}$ Indicaxanthin induced demethylation in the promoters of other onco-suppressor genes (GATA4; ESR1), but left others unchanged (SFRP1; HPP1). The

basal methylation seemed to be determinant, being the promoters with the highest methylation levels (SFRP1, HPP1) unaffected [25].

Molecular mechanistic explanations have been attempted. The methylation status of DNA results from the cross-talk between the activity of well characterized methylating enzymes (DNMT), DNMT1, DNMT3A, DNMT3B, and of less known proteins working to transform (TET family enzymes) and remove (MBD4, GADD45A) the methyl groups [46][47]. Indicaxanthin treatment (100 μM) was associated with over-expression of at least one of the DNMTs in all cell lines considered, and of TET2, MBD4, and GADD45A in LOVO1, DLD1 and HCT-116 cells [25]. The remarkably increased expression of DNMT3A (but not DNMT1, nor DMT3B) rationalized the increase in global DNA methylation in Caco-2 cells; however, none of the demethylase genes was affected, in contrast with the GATA4 demethylation and reactivation of the $p16^{INK4a}$ gene [5][25]. Ultimately, a cell-independent procedure showing that Indicaxanthin inhibited the total nuclear DNMT, and an in silico computational modelling analysis revealing a stable binding of the phytochemical at the DNMT1 catalytic site [25], suggest that Indicaxanthin could block the DNA methylation process operating at a gene-specific level [48]. Intracellular uptake and accumulation of Indicaxanthin in Caco-2 cells [15][21], as a necessary precondition for this activity have been observed. Redox regulation and signaling may be upstream of epigenetic modulation [43]; direct inhibition of methylating enzymes could explain the activity of Indicaxanthin in proliferating Caco-2 cells where the phytochemical did not appear to alter the redox tone [5].

Epigenetic modifications, potentially reversible, occur early in the process of carcinogenesis, thus representing a target of choice to prevent malignant transformation in cancers with long precancerous stages such as colorectal cancer. Activity at this level may reveal chemopreventive compounds. Phytochemicals in the diet, which can be taken continuously throughout life, can act as natural controllers in a process at the confluence of redox biology and gene-environment interactions. Importantly, Indicaxanthin selectively acted at the level of modified regulatory mechanisms in the transformed cells and did not affect the viability of non-malignant colon cells, either at the IC_{50} concentration or at twice the concentration [5]. Considering the digestive stability and approximate concentration at the intestine after a cactus pear fruit meal [49][50][51], Indicaxanthin appears to be a chemopreventive component of the diet.

Investigation in melanoma cells [6] provided evidence of a concentration-dependent anti-proliferative effect of Indicaxanthin (50–200 μM) in human A375, cell lines, with growth inhibition going from 20% to 56% at 72 h. A comparable response was observed in murine B16/F10 cells, whereas the inhibitory activity in other cell lines (Sk-Mel-28, MALMF) was remarkably lower. Indicaxanthin did not affect the growth of normal human melanocytes under comparable conditions. With regard to molecular mechanisms, growth arrest and apoptotic death were strictly related to the inhibition NF-κB transcription factor and downstream-regulated events, including the expression of two anti-apoptotic proteins, B cell lymphoma gene-2 (Bcl-2) and FLICE inhibitory protein (c-FLIP).

It may be worth noting that NF-κB is subjected to several controls and activating mechanisms [52] finely tuned by oxidants [53][54][55] and that NADPH oxidases [27][28] and superoxide [56] play a main role in maintaining the aberrant NF-κB activation in cancer cells. Whether Indicaxanthin induced redox changes in the melanoma experimental

setup has not been reported, which does not allow confirmation, or refutation, of what has been observed in Caco-2 cells [5], i.e., that Indicaxanthin does not affect NOX activity or ROS level in tumor cells. In addition, the pro-tumorigenic role of NOXes varies with cancer type [27]; in-depth studies on the interaction of NOX with the tumor microenvironment and potential interference by Indicaxanthin should be performed. Other data deserve to be considered. In silico studies have shown that Indicaxanthin can bind and inhibit the active form of human IKKB, the enzyme responsible for the inactivation of IKB-α, the NF-κB inhibitor [57]. Such targeting could offer the advantage of blocking NF-κB directly and would make the action of Indicaxanthin independent of the variety of mechanisms and pathways that activate the NF-κB signaling in a cell- and agent-specific manner [58]. NF-κB has a key role in inflammation, immunity and cancer [59], a reason why new effective and non-toxic compounds to counteract its activity in chronic inflammation, autoimmune diseases, and tumorigenesis are continuously investigated [58]. However, complexities exist for the development of clinically effective inhibitors, as this factor is required to ensure normal immune response and cell survival, i.e., global inhibition of NF-κB function can have serious side effects. Clarifying the aspects and pathways of NF-κB activation and its inhibition by Indicaxanthin in individual diseases will be critical to assessing the potential of the phytochemical as a nontoxic compound to control this factor.

Apart from individual inhibitory effects on cancer cell growth, phytochemicals may exert synergistic effects with other phytochemicals or anticancer drugs [60]. Recent studies in HeLa cells [26] provide further information on the anti-proliferative effect of Indicaxantin and suggest its eventual relevance to enhance chemosensitivity of cisplatin (CDDP). A concentration-dependent antiproliferative activity, related to mitochondrial-dependent apoptosis and arrest of cell cycle, was observed when HeLa cells were treated for 24 h with Indicaxanthin (IC_{50} 149.55 μM) or CDDP (IC_{50} 23.91 μM) alone [26]. Remarkably, when cells were submitted to a combination regimen of Indicaxanthin and CDDP, the Combination Index analysis [61] provided clear evidence of synergism. Moreover, the pre-treatment of HeLa cells with concentrations of Indicaxanthin of nutritional relevance [62], which were ineffective in modifying cell viability per se, potentiated CDDP activity significantly.

Elucidating the mechanism of action of Indicaxanthin in this model, on the one hand would add new knowledge about the potential of the molecule against cancer, but also shed light on the interactions with CDDP, which may serve as a rationale for therapeutic strategies. Apart the formation of adducts with nuclear DNA, the impact of CDDP with mitochondria and mitochondrial genome, followed by ROS generation, has emerged as a cause of cytotoxicity in tumor cells [63][64][65], which was observed in the CDDP-treated HeLa cells. In contrast to CDDP, the apoptotic activity of Indicaxanthin was not related to formation of DCFDA-reactive substances, nor did Indicaxanthin affect key markers of the intrinsic apoptotic pathway, or cell cycle-related proteins or transcription factors. Nevertheless, the CDDP/Indicaxanthin combination resulted in a ROS production and expression of apoptosis-related proteins significantly higher than those observed with CDDP alone.

The importance of elevated production of ROS in the apoptotic action of the combination Indicaxanthin/CDDP was highlighted by the inhibition (over 60%) observed in the presence of NAC [26], however mechanisms through which Indicaxanthin individually promoted apoptosis, or enhanced the prooxidant/apoptotic capacity of CDDP, remain to be clarified. Hypotheses could be made based on previous information. It seems important to emphasize that a substantial anti-proliferative activity of Indicaxanthin in HeLa cells needed concentrations of the order of 10^2 μM

(IC_{50} around 150 μM); on the other hand, nontoxic amounts of the phytochemical (2–10 μM) potentiated the antiproliferative activity of CDDP up to threefold. These findings may suggest distinct activities and possibly molecular mechanisms that complement each other.

Anticancer prodrugs, including phytochemicals, can be activated under high level of oxidants [66], and become pro-oxidant in cancer conditions [67]. Membrane lipids are prone to oxidation in cancer cells, whose environment is rich of ROS [68]. Under these circumstances, pro-oxidant behaviour of Indicaxanthin through activation on lipoperoxides [4][69], can be predicted at the concentrations required to inhibit the HeLa cell growth (50–200 μM). This may eventually promote formation of lipid oxidation breakdown products, including HNE [4], a mediator known to affect cancer cell proliferation [70] and induce apoptosis in HeLa cells [71][72]. Monitoring lipoperoxides and HNE formation and consumption of Indicaxanthin could test this hypothesis.

An activity at the membrane of cancer cells may be considered to explore whether non-cytotoxic Indicaxanthin may facilitate transport/permeation of CDDP. The latter involves interactions of cisplatin with membrane lipids and changes in membrane phase behavior [73], which could be affected by amphipathic Indicaxanthin. Such behavior would be highly relevant to clinical treatment. Facilitating drug entry could effectively decrease the ratio of circulating cisplatin to cellular amount for optimal effect, ultimately reducing the serious side effects of the drug by improving therapeutic efficacy.

The absence of toxicity of elevated amounts of Indicaxanthin observed in various studies [5][6][74][75] suggests safety and potential utility in Combo Therapy to overcome toxicity and drug resistance in cancer treatment [76][77][78][79][80]. CDDP, in particular, has antitumor activity against several types of cancer, which makes it desirable that the activity of their combination be studied in other cancer cells besides HeLa ones, as well as in animal models. In addition, combination of Indicaxanthin with other clinically used anticancer drugs to enhance chemotherapeutic potentiality should be checked.

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