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Elucidating the Mechanism of Action of Crocin, a Therapeutic Agent for Cancer and Inflammation

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Abstract—*Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Chronic inflammation favors selection of additional features in initiated cells that may promote their transition to malignant tumors ('promotion'). Acute inflammation might hamper the process and is used therapeutically to inhibit tumor formation. Crocin, a natural compound with anti-tumor and anti-inflammatory properties was selected. Its Action on tumor cells, and mast cells is analyzed thereby showing the correlation between Cancer and Chronic Inflammation. Computational methods show that Crocin binds effectively to TLR2 receptor. Experimental studies using MTT Assay show that Crocin inhibits cell viability at 1mM dose.*

Keywords*: Cancer, Inflammation, YAC-1 cells, TLR2, Crocin, Effective Dose.*

INTRODUCTION:

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer results from the outgrowth of a clonal population of cells from tissue. There are six properties that correlate to cancer development: excessive proliferation, insensitivity to anti-proliferative signals, apoptosis evasion, unlimited potential to replicate, the maintenance of vascularization, and most important, tissue invasion and metastasis.

The inflammatory response is a defense mechanism to protect higher organisms from infection and injury. It localizes and eliminates the injurious agent and removes damaged tissue components so that the body can begin to heal. The response includes changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the damaged tissue site.

Finding therapeutic anti-cancer agents are essential to help with the treatment of this disease. While many drugs do exist already their efficacy is low and they may also be toxic to perfectly healthy cells.

One attractive source of these agents are dietary factors from natural compounds, such as spices, which have shown high cytotoxic effect both on tissue culture and on tumor cells in vivo. Active ingredients (at the molecular level) of various natural sources and their mechanisms of action are less understood. Traditional medicine is key to finding such compounds. Extensive research has identified various molecular targets that can be used not only for the prevention of cancer but also for treatment (Bharat B. Aggarwal, et al, 2002). Limonene and perillyl alcohols can inhibit the proliferation and metastasis of gastric cancer. Flavonoids such as quercetin, genistein or flavopiridol (a synthetic analog of a natural alkaloid rohitukin) have entered late phase clinical trials for several oncological indications (Ferry et al., 1996, Lin et al., 2009, Lazarevic et al., 2011). Crocin is one such compound found to have many biomedical properties, obtained from spice Saffron.

LITERATURE REVIEW:

Cancer and Inflammation:

Carcinoma, sarcoma, melanoma, lymphoma, and leukemia are major types of cancer. Carcinomas originate in the skin, lungs, breasts, pancreas, and other organs and glands. Lymphomas are cancers of lymphocytes. Leukemia is cancer of the blood. It does not usually form solid tumors. Sarcomas are cancers of the connective tissues of the body like bone, muscle, fat, etc:-. They are

uncommon. Melanomas are cancers that arise in the cells that make Melanin. The most common cancers are lung, stomach cancer, liver cancer, colorectal cancer and breast cancer.

Cancer can also be considered as a step-wise development functionally grouped into three phases: initiation, promotion, and progression. Initiation is characterized by genomic changes within the cell such as point mutations, gene deletion and amplification, and chromosomal rearrangements which convert the proto-oncogene to oncogene, leading to irreversible cellular changes. Tumor development is the survival and clonal expansion of the "initiated" cells. Progression is a substantial growth in tumor size and either growth-related or mutually exclusive metastasis.

The five cardinal signs of inflammation are redness, heat, swelling, pain and loss of function Redness is caused by the dilation of small blood vessels in the injured area. Heat results from increased blood flow through the area and is experienced only in parts of the body like skin. Fever is a result of chemical mediators of inflammation and leads to the rise in temperature at the injury. Swelling or edema, is caused by the accumulation of fluid outside the blood vessels. The pain is in part from the injury or edema distortion and in part by certain chemical mediators of inflammation, such as bradykinin, serotonin, and the prostaglandins.

Chemical mediators of inflammation

Chemical factors released upon stimulation bring about the vascular and cellular changes. The chemicals originate from blood plasma, white blood cells, platelets, mast cells, endothelial cells lining the blood vessels, and damaged tissue cells.

One of the best-known chemical mediators is histamine, which triggers vasodilation and increases vascular permeability. Histamine which is stored in granules of circulating basophils and mast cells, is released immediately when these cells are injured. Prostaglandins increase the effects of other compounds that promote vascular permeability. Anti-inflammatory drugs, such as aspirin, are effective in part because they inhibit Cyclooxygenase, an enzyme involved in prostaglandin synthesis.

Plasma contains four interrelated systems of proteins that generate various mediators of inflammation. Activated complement proteins act as chemotactic factors for neutrophils, increase vascular permeability, and stimulate the release of histamine from mast cells. The kinin system, most important being Bradykinin, produces substances that increase vascular permeability. The coagulation system converts the fibrinogen into fibrin. The fibrinolytic system contributes to inflammation by forming plasmin, which breaks down fibrin into products that affect vascular permeability.

The relationship between cancer and inflammation is paradoxical as in some cases inflammation has been found to have repressed tumors, while in other cases, inflammation itself has triggered cancer initiation. The response of the body to a cancer is not a unique mechanism but is related to inflammation and wound healing.

Positive effect of Inflammation on Cancer Progression:

The theory that chronic inflammation results in cancer progression has evidential proof. The inflammatory responses necessary for enabling an immune reaction may set the stage for promoting neoplastic disease. The theory that cancer originates at sites of chronic inflammation, was first postulated by Virchow, based on his hypothesis that some classes of irritants causing inflammation also enhance cell proliferation (David H: Rudolf Virchow, 1988). When tissues are injured or are exposed to chemical irritants, damaged cells are removed by the induction of cell death pathways, while cell proliferation is enhanced to facilitate tissue regeneration in an attempt to re-establish tissue homeostasis. Proliferation and inflammation resolve only after inflammatory agents are removed or upon complete tissue repair.

When insulting agents persist over time, sustained cycles of cell proliferation and death in environments rich in inflammatory cells and their bioactive products may increase risk of cancer and tumor progression (Coussens LM, et al, 2002). While sporadic or inherited genetic mutations in critical genes regulating cell cycle, programmed cell death, differentiation and adhesion do initiate tumorogenesis, chronic inflammation favors selection of additional features in initiated cells that may promote their transition to malignant tumors ('promotion').

In addition, Viruses and bacteria can cause chronic inflammation through oncogene activation. However this is not always true. For example, Helicobacter pylori, a bacterium is one of the main contributors to gastric cancer. Yet this microorganism neither carries oncogenes nor inactivates tumour-suppressor proteins. Furthermore, there are several non-infectious causes of chronic inflammation (such as cigarette smoke, asbestos, silica and others) that also increase the risk of developing cancer.

The long-term use of Non-Steroidal Anti-inflammatory Drugs (NSAIDs), such as aspirin, as well as natural compounds, such as ginseng extract, green-tea extract, resveratrol and curcumin reduces the incidence of cancers of the colon, lungs, stomach, oesophagus and ovaries, as well as Hodgkin's lymphoma. Taking this into consideration, it is evident that in most cases Chronic Inflammation causes or aggravates Cancer.

Negative effect of Inflammation on Cancer Progression:

According to the immunosurveillance hypothesis of Lewis Thomas and F. Macfarlane Burnet, which was initially proposed by Paul Ehrlich, in 1909, the innate and adaptive immune systems collaborate to reduce tumour incidence by recognizing tumourspecific neo-antigens as non-self. Acute inflammatory response might exert a protective role on tumor development by contributing to tumor surveillance. Moreover, leukocyte infiltrate that characterize acute inflammation might also contribute to destruction of tumor cells. It was demonstrated that artificial infection with inflammation- causing bacteria induced often dramatic regressions of otherwise incurable cancers (A. Sgambato, et al, 2010). NK cells, undeniably play a vital function in constraining tumor development (Dunn GP, et al, 2004).

Crocin:

Crocin is a carotenoid chemical compound that is the glycated form of saffron constituents. It is a diester formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin. As a pure chemical compound, it has a deep red color and forms crystals with a melting point of 186 °C. It forms an orange solution in water.

Anti-Cancer Effects: The effects of four constituents of saffron (crocin, crocetin, picrocrocin and safranal) on the HeLa cells of cervix carcinoma were investigated. The apoptosis inhibition were mainly attributed to crocin (ID50 = 3μM). Crocin showed the strongest inhibitory effect. Therefore, crocin may be considered as the most important anti-cancer constituents of saffron (Escribano, et al, 1996).

Crocin and di glucosyl crocetin have inhibitory effects at different doses on the primary expression of tumor antigens in the adenovirus infected cells (Molnar, et al, 1999).Sugars play a major role in the potential toxicity effect of the crocin, since crocetin (which is deglycosylated derivative of crocin) could not inhibit cell growth, even at high doses (Morjani,et al,1990).

It seems that glucose addition to the crocin structure may increase its antitumor properties. It may be possible because of the ability of glucose to interact with DNA structure. Saffron isolated crocin and dimethyl crocetin were not mutagenic (Salomi,et al,1991).

Effect on Mast cells: Crocin inhibited the increase of thymus and activation-regulated chemokines, interleukin (IL)-4, and IL-13 on the dorsal skin of mice. Increase in epidermal thickness and dermal inflammatory cells (eosinophil and mast cells) infiltrations observed on the back skin of atopic dermatitis control mice were inhibited in a dose-dependent manner by topical application of crocin in atopic dermatitis treatment mice (Sung YY, et al, 2018). Crocin suppressed the paw edema and neutrophil recruitment induced by histamine. Crocin produces an anti-edematous effect on histamine-induced local paw edema. In addition, crocin may affect the histamine H1 receptors function. Provided an evidence of anti-edematous effect of crocin on histamine-induced paw edema in rats. It is well known that histamine is synthesized and released by different cells including basophils, mast cells, platelets (Esmaeal Tamaddonfard, et al, 2012)

RESULTS AND DISCUSSION:

1. Computational Techniques were used to elucidate the molecular target for Saffron. The structure of the ligand (Crocin) was obtained from PubChem website and the structure of the protein cell receptors was obtained from Protein Data Bank database. To identify potential binding conformations of Protein and Compounds, an automated molecular-docking procedure using the web-based SwissDock program (Grosdidier A, et al, 2011) was used. The docking was performed using the 'Accurate' parameter at otherwise default parameters, with no region of interest defined (blind docking). The targets are arranged in the order of increasing deltaG.

Table 1: Crocin and TLR Results

S.I No.	Toll- Like Receptors	Energy	deltaG (k cal per mol)	No.of H bonds (total)
1.	TLR2	36.2926	-10.286027	3
2.	TLR8	55.1202	-10.170127	6
3.	TLR9	63.3923	-9.938695	5
4.	TLR4 Chain C	40.9195	-9.786728	1
5.	TLR3	39.2454	-9.731238	6
6.	TLR7	48.3553	-9.232778	2

7.	TLR1	44.3567	-9.033196	1
8.	TLR6	54.2386	-8.618517	5
9.	TLR4 Chain A	60.4987	-8.241035	4

Table 2: Crocin and TCR Results

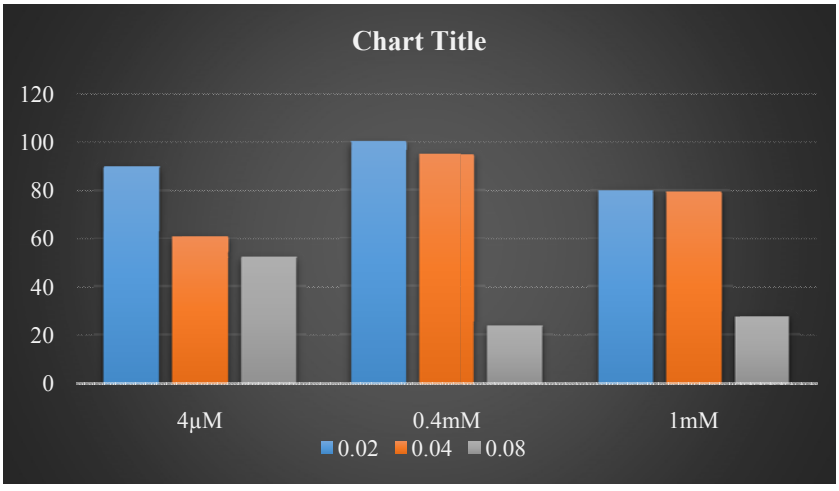
S.I No.	T cell Receptors	Energy	deltaG (k per mol)	cal	No.of H bonds (total)
1.	TCR alpha chain	49.0603	-9.630586		4
2.	TCR gamma chain	50.3796	-8.851549		1

Table 3: Crocin and FCER1 Results

S.I No.	FCER1 Receptors	Energy	DeltaG(k per mol)	cal	No.of H bonds (total)
1.	FCER1 alpha chain	56.9816	-9.480946		3
2.	FCER1 gamma chain	59.2805	-8.703989		6

As displayed in Table 1, it can be seen that Crocin TLR2 has the least binding energy (ΔG), therefore it will have better stability and is the best molecular target among all other Toll Like Receptors. Crocin interacts with chain alpha of T cell Receptors more than gamma chain. Crocin also binds better to the alpha chain of FCER1 Receptor of mast cells as indicated by the Binding Energy.

2. The effect of Crocin on T cell proliferation and viability was studied by conducting MTT assay of YAC-1 cells with Crocin. It was found that Crocin inhibited the proliferation of cells at a higher cell concentration and at a dose of 0.04mM and 1 Mm.



Graph 1: YAC-1 cell viability after incubation with Crocin at different doses, increasing cell viability, 48 hrs incubation

From the graph it is observed that cell viability is least at the highest cell concentration (0.08×10^5). The most effective dose is 1 mM of Crocin. Further studies can be done to understand the working of Crocin on a molecular level.

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