Mechanism of action of isothiocyanates. A review

Mecanismo de acción de los isotiocianatos. Una revisión

Luis Federico Molina-Vargas[1]

ABSTRACT

Isothiocyanates (ITC), originating from the enzymatic degradation of glucosinolates (GSL), which are secondary metabolites of Brassicaceae plants, exhibit anticarcinogenic properties due to their ability to induce cytoprotective genes. The mechanisms of cytotoxic and cytostatic effects of ITC include induction of apoptosis, inhibition of cell cycle progression and inhibition of angiogenesis. Regulation of apoptosis by ITC is accomplished primarily through the mitochondrial release of cytochrome *c*, Bcl-2 family regulation, MAPK signaling and subsequent activation of caspases. A significant portion of the chemopreventive effects of ITC may be associated with the inhibition of the metabolic activation of carcinogens by cytochrome P450 (phase I), along with increased induction of phase II detoxifying enzymes.

Key words: apoptosis, glucosinolates, oxidative stress, phytoalexins, secondary metabolites.

RESUMEN

Los isotiocianatos (ITC) originados a partir de la degradación enzimática de los glucosinolatos (GSL), los cuales son metabolitos secundarios de las plantas Brassicaceae, exhiben propiedades anticarcinogénicas por sus habilidades de inducir genes citoprotectivos. Los mecanismos de efectos citotóxicos y citostáticos de los ITC incluyen inducción de apoptosis, inhibición de la progresión del ciclo celular e inhibición de angiogénesis. La regulación de apoptosis por los ITC se logra principalmente a través de la liberación mitocondrial del citocromo *c*, regulación de la familia Bcl-2, señalización MAPK y subsecuente activación de caspasas. Parte importante de los efectos quimiopreventivos de los ITC puede estar asociada con la inhibición de la activación metabólica de carcinógenos por citocromo P450 (fase I), junto con la mayor inducción de las enzimas de detoxificación de la fase II.

Palabras clave: apoptosis, glucosinolatos, estrés oxidativo, fitoalexinas, metabolitos secundarios.

Introduction

Cruciferae (Brassicaceae), like other plant families, responds to stress using metabolic pathways that involve the biosynthesis of structurally diverse and numerous secondary metabolites such as phytoalexins and phytoanticipins, which are crucial to defense mechanisms (Pedras *et al*., 2009). Included in this type of secondary metabolites are glucosinolates (GSL), which are present in only 15 botanical families of the Capparales order and are very abundant in the Brassicaceae family (Al-Gendy *et al*., 2010). When these plants are incorporated into the soil by the technique known as "biofumigation", for soil pathogen control, it is regarded as a promising and environmentally friendly alternative to chemical fumigation (Galletti *et al*., 2008). The biological approach is based on the release of toxic compounds derived from GSL with the destruction of tissue in the presence of water (Brown and Morra, 1997). The defense induced by the rupture of the GSL is activated by tissue damage and the hydrolysis is initiated by the enzyme myrosinase (MYR) (Grubb and Abel, 2006). The glucosinolate-myrosinase system (GSL-MYR) has become part of an evolved and sophisticated defense mechanism against insect herbivores and pathogens (Andersson *et al*., 2009), in which GSL are hydrolyzed in a variety of components such as isothiocyanates (ITC), nitrites, thiocyanates, epithionitriles and oxazolidines depending on the conditions of pH, metal ions, and other proteinic elements (Vaughn and Berhow, 2005).

GLS, as well as their ITC derivatives, may partly explain the spicy flavor and characteristic odor of Brassicaceae, which are very different from other species of the same family (Pasini *et al*., 2012), and play a significant role in the cancer preventive properties offered by these plants (Zhang *et al*., 2005). Some products of GSL degradation, such as ITC, exhibit the following properties: anticarcinogenic (Yan and Chen, 2007), antimutagenic, antioxidants, antifungal, antibacterial, bioherbicides, and biopesticides (Vig *et al*., 2009). Sulforaphane and erucin, two natural ITC that are very abundant in broccoli and other cruciferous vegetables, strongly inhibit quorum sensing and virulence in *Pseudomonas aeruginosa* (Ganin *et al*., 2013).

Received for publication: 1 February, 2012. Accepted for publication: 29 March, 2013.

[1] Technical Direction of Plant Protection, Instituto Colombiano Agropecuario (ICA). Bogota (Colombia). lufemol@yahoo.com

The pesticide effect of ITC is associated with dose (Yuan *et al*., 2008) and the type of ITC, which was corroborated by Molina (2008) by inhibiting the mycelial growth of *Rhizoctonia solani* using allyl-ITC (AITC), benzyl-ITC (BITC), phenyl-ITC (PITC), phenethyl-ITC (PEITC) and methyl-ITC (MITC) *in vitro*. An inhibitory effect was observed in the development of the mycelium with PEITC and BITC for six and 20 d respectively, while for AITC, PITC and MITC this effect lasted for one year after applying the treatments, suggesting that the mechanism of action involved is different depending on the type of ITC. Since no specific mechanism of action of ITC in *R. solani,* or other pathogens, is known, it is necessary to research the associated mechanism of action that has been elucidated in different animal models, which is the purpose of this review. ITC are potent chemopreventive agents that favorably alter carcinogen metabolism in mammals by inhibiting the metabolic activation of carcinogens and/or by inducing carcinogen detoxification enzymes (Gross *et al*., 2000). In fact, ITC are usually considered as GLS degradation products more biologically active (Gimsing and Kirkegaard, 2006), whose main characteristic is volatility (Oliviero *et al*., 2012).

Carcinogenesis

Carcinogenesis is a molecular process induced by genetic and epigenetic changes that interrupts pathways that control cell proliferation, apoptosis, differentiation and senescence. It is generally divided into the stages of initiation, promotion and progression (Fimognari and Hrelia, 2007). Initiation is defined as an event that usually results from mutagenic DNA damage by physical, chemical or viral exposure. Promotion is characterized by transforming an initiated cell to a preneoplastic cell population due to epigenetic alterations in cells by chronic exposure to tumor promoters such as growth factors, hormones or ultraviolet radiation. Progression is regarded as the final stage of carcinogenesis, which converts preneoplastic cells to an invasive and metastatic cell population (Keum *et al*., 2004). The principal carcinogen agents are exogenous or metabolically generated by reactive oxygen species (ROS) and by electrophilic derivatives of the environment and normal oxidative processes *in vivo*. The increased levels of ROS are involved in the initiation and promotion of tumors and may ultimately lead to carcinogenesis (Yang Liu, 2009).

Chemoprevention is defined as the use of natural or synthetic agents that reverse, inhibit or prevent the development of cancer (Fimognari and Hrelia, 2007). Virtually all dietary and environmental carcinogens undergo a metabolic process after entering the human body. This enzymatic process occurs mainly through oxidation and to a lesser extent reduction and hydrolysis, which make chemical molecules more hydrophilic. This physiological event, called phase I metabolism, is mainly catalyzed by cytochrome P450 (CYP). Consequently, procarcinogens are usually converted into highly reactive intermediates that can bind to critical macromolecules such as DNA, RNA and proteins. A second group of enzymes, known as phase II enzymes, which can combine intermediate reactives with endogenous cofactors, leads to the generation of water-soluble products that can be readily excreted in bile or urine (Keum *et al*., 2004). Some phytochemicals found in fruits and vegetables can inhibit the activation of carcinogens, maintaining the balance between phase I enzymes that activate carcinogenesis and detoxifying enzymes of phase II, which may play a protective role against cell damage with xenobiotic agents (Yang Liu, 2009), a class of chemical compounds outside the body of living organisms and resistant to environmental degradation (Belgiorno *et al*., 2007).

The enzymes of phase I consist mainly of the superfamily of cytochrome P450 (CYP) and microsomal enzymes. Conjugators or metabolizing enzymes of phase II consist of several enzyme superfamilies including sulfotransferases (SULT) and UDP-glucuronosyltransferases (UGT), DT-diaphorase or NAD (P) H oxidases: quinone oxidoreductase (NQO) or NAD (P) H: menadione reductase (NMO), epoxide hydrolase (EPH), glutathione S-transferase (GST) and N-acetyltransferases (NAT) (Xu *et al*., 2005). Induction of phase II enzymes, such as GST and quinone reductase (QR), generally leads to protection of tissues and cells against endogenous and/or exogenous carcinogenic intermediates. ARE/EpRE (anti-oxidant response element / electrophile responsive element) found in the 5' region of these phase II genes may play an important role in mediating their induction by xenobiotics including chemopreventive agents (Kong *et al.* 2001). Both the induction of detoxifying enzymes of phase II (GST and QR) and the elevation of glutathione (GSH) are the principal strategies to protect cells from a variety of endogenous and exogenous toxic compounds such as ROS and some chemical carcinogens (Ye and Zhang, 2001).

GSL and the anticancer effect

Cruciferous consumption is more strongly associated with protection against cancer than consumption of vegetables in general (Blazevic and Mastelic, 2009). Among the most studied bioactive compounds in cruciferous associated with

protection against cancer are GSL (Fimognari and Hrelia, 2007); and their hydrolysis products especially receive a lot of attention due to their potential anticarcinogenic effect (Van Eylen *et al*., 2009), considered as very potent inducers of phase II detoxifying enzymes, such as GST, while reducing phase I enzymes such as CYP (Zhang *et al*. 1992; Seow *et al*., 2002). GSH conjugation by GST is an important step in the metabolism and subsequent detoxification of carcinogens, including metabolizing ITC, hydrolysis products of GSL, resulting from the formation of conjugates of N-acetylcysteine (Seow *et al*., 2002).

Effect of ITC

ITC, phytochemicals with promising cancer preventive potential, can have advantages and disadvantages in the modulation of cellular oxidative stress. While ITC transcriptionally stimulate many antioxidant enzymes and non-enzymatic proteins, resulting in greater protection against oxidative stress, they also directly alkylate and reduce thiol cellular groups, damage mitochondria and elevate ROS, leading to cellular stress (Zhang *et al*., 2005). There is dose dependence in these responses: generally, induction of cytoprotective genes and inhibition of CYP activity occur at relatively low concentrations of phytochemicals, while activation of cell cycle arrest and apoptosis occur at high levels (Hayes *et al*., 2008). These paradoxical effects appear to occur in parallel: the exposure of cells to an ITC quickly leads to a sharp increase in stress, which is followed by a delayed but lasting increase in cellular protection against oxidants and carcinogens. However, while the ITC-induced stress can lead to oxidative damage, it has become increasingly clear that much of the chemoprotective activity of ITC is derived from the cell response to stress generated by these compounds (Zhang *et al*., 2005).

The mechanisms of cytotoxic and cytostatic effects of ITC include induction of apoptosis, inhibition of cell cycle progression and inhibition of angiogenesis. The regulation of apoptosis by ITC is achieved primarily through mitochondrial cytochrome *c* release, regulation of Bcl-2 family, MAPK (mitogen-activated protein kinases) signaling and subsequent activation of caspases, a family of cysteine proteases responsible for the initiation and execution of apoptosis (Traka and Mithen, 2009). The most important regulators that have been characterized include caspases in apoptosis, the TNF receptor family (Tumor Necrosis Factor), adapter proteins and Bcl-2 family, among others (Boedefeld *et al*., 2003).

AITC and PITC are known to inhibit TNF, a protein involved in a type of apoptosis known as extrinsic apoptosis (Thejass and Kuttan, 2007), while BITC and PEITC, aromatic ITC more hydrophobic than AITC (Tang and Zhang, 2005), induce a type of apoptosis dependent on mitochondrial cytochrome *c* release (Miyoshi *et al*., 2004, Nakamura *et al*., 2002) known as intrinsic apoptosis (Shawn *et al*., 2005). PEITC and BITC are more potent inducers of apoptosis than AITC and sulforaphane (SFN) (Tang and Zhang, 2005).

It appears that a major part of the ITC chemopreventive effects may be associated with the inhibition of the metabolic activation of carcinogens by CYP (Phase I), together with the strong induction of phase II detoxifying enzymes and cellular defensive enzymes. The induction of cellular enzymes of phase II are largely mediated by ARE (antioxidant responsive element) which is regulated by the transcription factor Nrf2 (Keum *et al*., 2004). The enzyme-inducing potential is connected with the total intracellular concentrations (Zhang and Talalay, 1998) and with the formation of ROS (Nakamura *et al*., 2000). Since GST enzymes metabolize ITC, the beneficial effect of ITC is therefore partly dependent on the presence or absence of the activity of GST (Seow *et al*., 2002). Additionally, ITC may act as anticarcinogenic agents through more than one path. ITC and other inducers of phase II enzymes can act as suppressors during the post-initiation of carcinogenesis by apoptosis (Bonnesen, *et al*., 2001; Kirlin *et al*., 1999). Some studies have shown that BITC, for example, may increase resistance to apoptosis and promote carcinogenesis when administered in the post-initiation phase (Seow *et al*., 2002).

ITC blocking activity, in particular in SFN, has received significant attention, mainly on the induction of phase II enzymes via gene expression directed by ARE. Targets directed by ARE include the reductase NAD (P) H: quinone (NQO1), heme oxygenase 1 (HO1) and gamma-glutamylcysteine synthetase (c-GCS), a rate limiting enzyme in the synthesis of GSH. The regulation of these genes involved in detoxification of carcinogens and oxidants is mediated by the nuclear factor Nrf2 (nuclear factor E2-related factor), a member of the NF-E2 family which binds to ARE sites as a cis activation element in the 5' region of the genes in most phase II enzymes (Clarke *et al*., 2008). Recent studies suggest that Nrf2-mediated signaling, which controls the expression of several genes responsible for detoxification of carcinogens and protection against oxidative stress, is regulated by ITC such as SFN (Yeh and Yen, 2009). Transcriptional activation of phase II enzymes is mainly mediated by ARE/EpRE (electrophile responsive element)

located upstream of the genes involved. Through experiments with EpRE, Ye and Zhang (2001) demonstrated that AITC, BITC, PEITC and SFN elevate levels of GST, QR and GHS. The correlation between the ITC inducing activity and intracellular accumulation levels showed that PEITC is accumulated at much lower levels than SFN (ITC control) and that the accumulated components are also more rapidly removed.

BITC rapidly and continuously accumulates within the cells (intracellular concentrations above 300 mM) and alters mitochondrial function. The production of ROS and the resulting change in the cellular redox potential may be part of the signal transduction pathway during apoptosis (Miyoshi *et al*., 2004). ITC enter cells and are converted to their respective GST conjugates, which ultimately leads to reduced intracellular GSH. Additionally, free ITC also react with intracellular proteins with thiocarbamoylation reactions. It is believed that reducing cellular GSH and protein thiocarbamoylation activate signal transduction pathways and apoptotic mechanisms (Satyan *et al*., 2006). ITC accumulate in cells with intracellular levels reaching the millimolar range, as dithiocarbamates (DTC) derived from intracellular GSH conjugation, which is a determinant factor in the accumulation. Inducing activity of almost all phase II enzyme inducers are related to their chemical reactivity with sulfhydryl groups. Both ITC and their DTC derivatives may change the reaction with sulfhydryl groups (Ye and Zhang, 2001). *In vivo*, ITC are conjugated with GSH and then sequentially metabolized in mercuric acid. These metabolites are collectively designated DTC (Shapiro *et al*., 2001).

ITC and oxidative stress

Oxidative stress, the cellular imbalance between the production and the elimination of ROS, is thought to be the basis of the pathogenesis of various diseases. ITC may act indirectly, increasing the antioxidant capacity of animal cells by inducing the activity of phase II enzymes or by increasing levels of GSH (Traka and Mithen, 2009). BITC induces expression of GSTP1 (GST isoenzyme) to mediate intracellular ROI (reactive oxygen intermediates) with short periods of time (1 h) that are sufficient to elevate GST activity; and ROI production activities are highly correlated with the induction of GST, whereas the antioxidant GSH inhibits them (Miyoshi *et al*., 2004). AITC and PITC inhibit specific angiogenesis by tumors through the significant reduction of the production of nitric oxide (NO) and TNF-α (Tumor necrosis factor-α) (Thejass and Kuttan, 2007). ITC, upon oxidation, are converted into their corresponding isocyanate chromosome aberration causing mutations and cancer. These events could be correlated with the potential of the ITC with intracellular ROI production, although the cytotoxicity of high ITC doses can be inhibited by the antioxidant N-acetylcysteine (Miyoshi *et al*., 2004).

ITC and apoptosis

Apoptosis or programmed cell death is an active process whereby dysfunctional cells are removed to maintain normal functioning of the tissues. Depending on the stimulus, apoptosis can be initiated by the mitochondrial pathway intrinsically or extrinsically, or receptor mediated in response to exogenous stimuli. The main executors of apoptosis are caspases (cysteine aspartyl protease) that can be subdivided into two groups: initiator caspases which initiate apoptosis by activating a second group, downstream effectors or executing caspases. Several endogenous inhibitors, including anti-apoptotic members of the Bcl-2 family, inhibit the activation of caspases (Shawn *et al*., 2005). Bcl-2 is a prototypical antiapoptotic protein, a member of the class of molecules classified under the Bcl-2 family, has the ability to form heterodimers which constitutes the basis of the model that involves the prototypical antiapoptotic Bcl-2 factor and is the prototypical proapoptotic Bax member. Under this model, the cell susceptibility to apoptosis depends on the relative amounts of Bcl-2/Bax heterodimers, Bax/Bax homodimers, and Bcl-2/Bcl-2 homodimers. An excess of proapoptotic homodimers (Bax) will result in apoptosis, while an excess of Bcl-2 homodimers results in cell survival.

Another theory related to the inherent ability of these family members to interact involves the creation of a cluster of molecules including cytochrome *c*, caspases and adapter proteins. The functions of Bcl-2 include the creation of membrane channels, the regulation of caspase activity and inhibiting the output of mitochondrial cytochrome *c* (Boedefeld *et al*., 2003). MAPK, important components of cell signaling, converts various extracellular signals into intracellular responses through phosphorylation cascades. Three different MAP kinase cascades have been identified that are parallel and correspond to: ERK (extracellular signal-regulated kinases), JNK (c-Jun NH 2-terminal kinases) and p38 (Kong *et al*., 2001; Yeh and Yen, 2009), which along with MAPK belong to a superfamily of serine/threonine kinases, which play a role in carcinogenesis and cancer development (Clarke *et al*., 2008); and which when activated can phosphorylate many transcription factors, ultimately generating changes in the expression of genes (Kong *et al*., 2001). Given the fact that MAPK are activated

by a wide range of factors, these signaling cascades may serve as common mechanisms that integrate signaling pathways to control cellular responses to various extracellular stimuli, including pharmacological and xenobiotics agents (Kong *et al*., 2001; Yeh and Yen, 2009).

Miyoshi *et al*. (2004), with experiments using epithelial rat liver cells, showed that BITC synergistically modifies the antiapoptotic function of Bcl-2 through the phosphorylation catalyzed by JNK and induces apoptosis dependent on mitochondrial death via the activation of caspases 9 and 3, but they did not observe significant changes in the expression or degradation of Bcl-2 and Bax as suggested by Chen *et al*. (1998), who reported the importance of protein expression of the Bcl-2 family during apoptosis induced by components related to ITC.

Mitochondria are damaged by ITC intracellular metabolites that can accumulate in the cells and mitochondrial damage induced by ITC leads to cytochrome *c* release, caspase-9 activation and apoptosis. Similarly, the action of ITC in mitochondria involves Bcl-2 family member proteins, which influence the integrity of the membrane. The ability of AITC, BITC, PEITC and SFN to activate caspase 9 is strongly correlated with their ability to cause transmembrane potential loss and release of cytochrome *c*, depending on dose and time of exposure (Tang and Zhang, 2005). BITC induces cytochrome *c* release from mitochondria (Nakamura *et al*., 2002), while Bcl-2/Bax decreased interaction with BITC treatments. Bcl-2 appears to lose the ability of phosphorylated binding with Bax, generating the enhanced susceptibility of the cells to apoptosis. Bcl-2 phosphorylation, induced by BITC, could dissociate the interaction between Bcl-2 and Bax, resulting in the induction of apoptosis triggered by the signal of mitochondrial death (Miyoshi *et al*., 2004). BITC and PEITC stimulate phosphorylation of Bcl-2 at a dose of 7.5 mmol L^{-1} but in high concentrations (15-30 mmol L^{-1}) cause more damage to the mitochondria (Tang and Zhang, 2005). Treatments with excessive BITC concentrations result in severe cytotoxicity without the formation of the DNA ladder (DNA fragmentation into nucleosome units and chromatin condensation are characteristics of apoptosis). BITC induces ROS production in a dose dependent manner. These phenomena may lead to oxidative cell death by necrosis and therefore damage the cells (Miyoshi *et al*., 2004). PEITC inhibits several CYP enzymes involved in the metabolic activation of carcinogens and sensitizes cells to apoptosis by sustained activation of JNK and the stress-activated protein kinases pathway (Satyan *et al*., 2006). The effect in Bak and Bcl-2 is highly dependent on the concentration of ITC. ITC can cause dissociation of Bcl-XL with Bak and Bax in the mitochondria and cause damage. Exposure of cells to ITC, including BITC and PEITC, also result in a rapid increase of ROS at the cellular level, dose dependent. Mitochondria are the principal sites of ROS production, which could result in the breaking of the respiratory chain. The aromatic ITC, BITC and PEITC are hydrophobic and powerful agents, causing damage in mitochondria, whereas AITC and SN are more soluble and show less potential for damage to the membrane. The high lipid solubility facilitates the release of membrane components. Mitochondrial damage is caused by the principal intracellular metabolites of ITC (their GSH conjugates). The increased phosphorylation of Bcl-2, mitochondrial translocation of Bak and disruption of the association of Bcl-xL with Bak and Bax in mitochondria occur in ITC treatments, which contributes to mitochondrial damage and apoptosis (Tang and Zhang, 2005).

ITC and the cell cycle

When DNA is damaged, cell cycle arrest occurs through the activation of cellular control points such as the phases G1/S or G2/M until the errors are corrected in the DNA. However, if DNA damage is enormous and irreparable, apoptosis is initiated instead of cell cycle arrest. Thus, both cell cycle arrest and apoptosis are closely linked as cellular protection mechanisms (Keum *et al.,* 2004). The orderly progression of mammalian cell division through phases G1, S, G2, and M is governed by a set of proteins called cyclins, which exert their effects by binding and activating a series of kinases dependent on specific cyclins (CDK, cyclin dependent kinases). This process is modulated by inhibiting proteins such as p21/WAF-1, p16/INK41 and p27/Kip-1, collectively referred to as CDK inhibitors (Keum *et al*., 2004). The ITC ability to regulate the cell cycle and inhibit proliferation contributes to the chemopreventive ability and is mediated through indirect orientation of molecules and CDK inhibitors (Traka and Mithen, 2009). Visanji *et al*. (2004) showed that PEITC and BITC arrest cell cycle in the G2 phase of the Caco-2 colon cell, inducing $p21^{waf1/cip1}$ expression. The progression through the G1/S checkpoint of the cell cycle is regulated by the CDK family, whose activity is in turn controlled by CDK inhibitors, including $p21^{waf1/cip1}$ (Bott *et al*., 2005). The arrest of the G2/M phase by BITC in human pancreatic cancer cells are also associated with the activation of Chk2 (checkpoint kinase 2), whereas expression of other regulatory proteins of the G2/M phase, including cyclin B1, Cdc2 (Cell division cycle 2) and Cdc25C (cell division cycle 25C) were sub-regulated (Zhang *et al*., 2006). AITC treatments targeted accumulation of

prostate cancer cells in the G2/M phase with the down-regulation of Cdk1 (cyclin dependent kinase 1), Cdc25B and Cdc25C proteins (Xiao *et al*., 2003; Tang *et al*., 2006). SFN caused G2/M phase arrest and increased the fraction of apoptotic cells in a time and dose dependent manner, with necrosis after prolonged exposure to high doses. SFN altered cell cycle progression and induced apoptosis, increasing the expression of p53 and Bax and negatively affected the expression of Bcl-2 (Fimognari *et al*., 2002). The ITC BITC, PEITC and SFN are electrophiles that bind covalently to certain cysteine residues in tubulin, protein constituent of microtubules, inducing changes in the formation and collapse of the same, which contribute to cell cycle arrest in G2/M and apoptosis (Mi *et al*., 2009). PEITC cells suppressed the proliferation of PC-3 (prostate cancer cells) in a dose dependent manner, causing arrest in the G2/M phase cell cycle and apoptosis by reducing the level of Cdk1 and Cdc25C. Interestingly, PITC, which is structurally similar to PEITC, does not have the same effects. These results indicate that even a subtle change in the structure of the ITC can have a significant impact on its biological activity (Xiao *et al.* 2004).

Conclusions

The fungistatic effect of several ITC in the *in vitro* assay performed by Molina (2008), with differences in inhibition of mycelial growth of *R. solani*, suggests several mechanisms of action dependent on dose and ITC type. While the fungistatic effect produced by benzyl-ITC (BITC) and phenethyl-ITC (PEITC) lasted 6 and 20 d, respectively, in allyl-ITC (AITC), methyl-ITC (MITC) and phenyl-ITC (PITC) the inhibitory effect lasted up to one year after the treatments were applied. It is evident that the response is dose dependent, since, generally, cytoprotective gene induction and inhibition of CYP activity occur at relatively low ITC concentrations, while activation of cell cycle arrest and apoptosis occur at high doses.

The mechanisms of cytotoxic and cytostatic ITC effects include induction of apoptosis, inhibition of cell cycle progression and inhibition of angiogenesis. The regulation of apoptosis by ITC is achieved primarily through mitochondrial cytochrome *c* release, regulation of the Bcl-2 family, MAPK signaling and subsequent activation of caspases, responsible for the initiation and execution of apoptosis. Specifically, AITC and PITC inhibit TNF (extrinsic apoptosis), generating a mycelial inhibition for several months, while BITC and PEITC induce a cytochrome *c* release-dependent type of apoptosis from mitochondria (intrinsic apoptosis) that generates a mycelial inhibition that lasts only a few days. The differences in the fungistatic effect of ITC are possibly due to the type of apoptosis induced.

Literatute cited

Al-Gendy, A.A., O.D. El-Gindi, Al.S. Hafez, and A.M. Ateya. 2010. Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). Food Chem. 118, 519-524.

Andersson, D., R. Chakrabarty, S. Bejai, J. Zhang, L. Rask, and J. Meijer. 2009. Myrosinases from root and leaves of Arabidopsis thaliana have different catalytic properties. Phytochemistry 70(11-12), 1345-1354.

Belgiorno, V., L. Rizzo, D. Fatta, C.D. Rocca, G. Lofrano, A. Nikolaou, V. Naddeo, and S. Meric. 2007. Review on endocrine disrupting-emerging compounds in urban wastewater: occurrence and removal by photocatalysis and ultrasonic irradiation for wastewater reuse. Desalination 215, 166-176.

Blazevic, I. and J. Mastelic. 2009. Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.). Food Chem. 113, 96-102.

Boedefeld, W.M., K.I. Bland, and M.J. Heslin. 2003. Recent insights into angiogenesis, apoptosis, invasion, and metastasis in colorectal carcinoma. Ann. Surg. Oncol. 10(8), 839-851.

Bonnesen, C., I.M. Eggleston, and J.D. Hayes. 2001. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. Canc. Res. 61, 6120-6130.

Bott, S.R.J., M. Arya, R.S. Kirby, and M. Williamson. 2005. p21WAF1/CIP1 gene is inactivated in metastatic prostatic cancer cell lines by promoter methylation. Prost. Cancer Prost. Dis. 8, 321-326.

Brown, P.D. and M.J. Morra. 1997. Control in soil borne plant pests using glucosinolate-containing plants. Adv. Agron. 61, 167-231.

Chen, Y.R., W. Wang, A. Kong, and T. Tan. 1998. Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. J. Biol. Chem. 273, 1769-1775.

Clarke, J.D., R.H. Dashwood, and E. Ho. 2008. Multi-targeted prevention of cancer by sulforaphane. Cancer Letters 269, 291-304.

Fimognari, C., M. Nusse, R. Cesari, R. Iori, G. Cantelli-Forti, and P. Hrelia. 2002. Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. Carcinogenesis 23, 581-586.

Fimognari, C. and P. Hrelia. 2007. Sulforaphane as a promising molecule for fighting cancer. Mutation Res. 635, 90-104.

Galletti, S., E. Sala, O. Leoni, S. Cinti, and C. Cerato. 2008. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. Biol. Control 45, 319-327.

Ganin, H., J. Rayo, N. Amara, N. Levy, P. Krief, and M. Meijler. 2013. Sulforaphane and erucin, natural isothiocyanates from broccoli, inhibit bacterial quorum sensing. Med. Chem. Commun. 4, 175-179.

Gimsing, A.L. and J.A. Kirkegaard. 2006. Glucosinolate and isothiocyanate concentration in soil following incorporation of *Brassica* biofumigants. Soil Biol. Biochem. 38, 2255-2264.

Gross, H.B., T. Dalebout, C.D. Grubb, and S. Abel. 2000. Functional detection of chemopreventive glucosinolates in *Arabidopsis thaliana*. Plant Sci. 159, 265-272.

Grubb, C.D. and S. Abel. 2006. Glucosinolate metabolism and its control. Trends Plant Sci. 11(2), 89-100.

Hayes, J.D., M.O. Kelleher, and I.M. Eggleston. 2008. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. Eur. J. Nutr. 47(2), 73-88.

Keum, Y.S., W.S. Jeong, and A.N.T. Kong. 2004. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutation Res. 555, 191-202.

Kirlin, W.G., J. Cai, M.J. DeLong, E.J. Patten, and D.P. Jones. 1999. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. J. Nutr. 129, 1827-1835.

Kong, A.N., R. Yu, V. Hebbar, C. Chen, E. Owuor, R. Hu, R. Ee, and S. Mandlekar. 2001. Signal transduction events elicited by cancer prevention compounds. Mutation Res. 480-481, 231-241.

Mi, L., N. Gan, and F. Chung. 2009. Aggresome-like structure induced by isothiocyanates is novel proteasome-dependent degradation machinery. Biochem. Biophys. Res. Commun. 388, 456-462.

Miyoshi, N., K. Uchida, T. Osawa, and Y. Nakamura. 2004. A link between benzyl isothiocyanate-induced cell cycle arrest and apoptosis: involvement of mitogen-activated protein kinases in the Bcl-2 phosphorylation. Cancer Res. 64, 2134-2142.

Molina, L.F. 2008. Método *in vitro* para evaluar la eficacia de los isotiocianatos contra *Rhizoctonia solani*, mediante la determinación de la concentración efectiva media (CE50) calculando el área de crecimiento micelial con el software mapmaker 3.5®. M.Sc. thesis. Faculty of Agronomy, Universidad Nacional de Colombia, Bogota.

Nakamura, Y., H. Ohigashi, S. Masuda, A. Murakami, Y. Morimitsu, Y. Kawamoto, T. Osawa, M. Imagawa, and K. Uchida. 2000. Redox regulation of glutathione S-transferase induction by benzyl isothiocyanate: correlation of enzyme induction with the formation of reactive oxygen intermediates. Cancer Res. 60, 219-225.

Nakamura, Y., M. Kawakami, and A. Yoshihiro. 2002. Involvement of the mitochondrial death pathway in chemopreventive benzyl isothiocyanateinduced apoptosis. J. Boil. Chem. 277, 8492-8499.

Oliviero, T., R. Verkerk, and M. Dekker. 2012. Effect of water content and temperature on glucosinolate degradation kinetics in broccoli (*Brassica oleracea* var. italica). Food Chem. 132, 2037-2045.

Pasini, F., V. Verardo, M.F. Caboni, and L.F. D'Antuono. 2012. Determination of glucosinolates and phenolic compounds in rocket salad by HPLC-DAD–MS: evaluation of *Eruca sativa* Mill. and *Diplotaxis tenuifolia* L. genetic resources. Food Chem. 133, 1025-1033.

Pedras, M.S.C., D.P. Okinyo-Owiti, K. Thoms, and A.M. Adio. 2009. The biosynthetic pathway of crucifer phytoalexins and phytoanticipins: de novo incorporation of deuterated tryptophans and quasi-natural compounds. Phytochem. 70, 1129-1138.

Satyan, K.S., N. Swamy, D.S. Dizon, R. Singh, C.O. Granai, and L. Brard. 2006. Phenethyl isothiocyanate (PEITC) inhibits growth of ovarian cancer cells by inducing apoptosis: Role of caspase and MAPK activation. Gynecol. Oncol. 103, 261-270.

Seow, A., Y. Jian-Min, S. Can-Lan, V. Den Berg, L. Hin-Peng, and Y. Mimi. 2002. Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. Carcinogenesis 23(12), 2055-2061.

Shawn, L., V. Straszewski-Chavez, M. Abrahams, and G. Mor. 2005. The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. Endocr. Rev. 26(7), 877-897.

Shapiro, T.A., J.W. Fahey, K.L. Wade, K.K. Stephenson, and P. Talalay. 2001. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. Cancer Epidem. Biomark. Prev. 10, 501-508.

Tang, L. and Y. Zhang. 2005. Mitochondria are the primary target in isothiocyanate induced apoptosis in human bladder cancer cells. Mol. Cancer Ther. 5, 1250-1259.

Tang, L., G. Li, L. Song, and Y. Zhang. 2006. The principal urinary metabolites of dietary isothiocyanates, N-acetylcysteine conjugates, elicit the same anti-proliferative response as their parent compounds in human bladder cancer cells. Anti-Cancer Drugs 17, 297-305.

Thejass, P. and G. Kuttan. 2007. Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) inhibit tumour-specific angiogenesis by downregulating nitric oxide (NO) and tumour necrosis factor (TNF) production. Nitric Oxide 16, 247-257.

Traka, M. and R. Mithen. 2009. Glucosinolates, isothiocyanates and human health. Phytochem. Rev. 8, 269-282.

Van Eylen, D., N. Bellostas, B.W. Strobel, I. Oey, M. Hendrickx, A. Van Loey, H. Sørensen, and J.C. Sørensen. 2009. Influence of pressure/temperature treatments on glucosinolate conversion in broccoli (*Brassica oleraceae* L. cv. *Italica*) heads. Food Chem. 112, 646-653.

Vaughn, S.F. and M.A. Berhow. 2005. Glucosinolate hydrolysis products from various plant sources: ph effects, isolation, and purification. Ind. Crop. Prod. 21, 193-202.

Vig, A.P., G. Rampal, T.S. Thind, and S. Arora. 2009. Bio-protective effects of glucosinolates – A review. LWT - Food Sci. Technol. 42, 1561-1572.

Visanji, J.M., S.J. Duthie, L. Pirie, D.G. Thompson, and P.J. Padfield. 2004. Dietary isothiocyanates inhibit Caco-2 cell proliferation and induce G2/M phase cell cycle arrest, DNA damage, and G2/M checkpoint activation. J. Nutr. 134, 3121-3126.

Xiao, D., S.K. Srivastava, K.L. Lew, Y. Zeng, P. Hershberger, C.S. Johnson, D.L. Trump, and S.V. Singh. 2003. Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing G2/M arrest and inducing apoptosis. Carcinogenesis 24, 891-897.

Xiao, D., C.S. Johnson, D.L. Trump, and S.V. Singh. 2004. Proteasome-mediated degradation of cell division cycle 25C and cyclin-dependent kinase 1 in phenethyl isothiocyanateinduced

G2-M-phase cell cycle arrest in PC-3 human prostate cancer cells. Mol. Cancer Ther. 3, 567-575.

Yan, X. and S. Chen. 2007. Regulation of plant glucosinolate metabolism. Planta 226, 1343-1352.

Yang, J. and R.H. Liu. 2009. Induction of phase II enzyme, quinone reductase, in murine hepatoma cells in vitro by grape extracts and selected phytochemicals. Food Chem. 114, 898-904.

Ye, L. and Y. Zhang. 2001. Total intracellular accumulation levels of dietary isothiocyanates determine their activity in elevation of cellular glutathione and induction of Phase 2 detoxyfication enzymes. Carcinogenesis 22(12), 1987-1992.

Yeh, C.T. and G.C. Yen. 2009. Chemopreventive functions of sulforaphane: a potent inducer of antioxidant enzymes and apoptosis. J. Funct. Foods 1, 23-32.

Yuan, P., B.A. Chen, and D.L. Liu. 2008. Anticancer mechanisms and researches of isothiocyanates. Chin. J. Nat. Med. 6(5), 325-332.

Xu, C., Y.-T. Li, and A.-N. Kong. 2005. Induction of phase i, ii and iii drug metabolism/transport by xenobiotics. Arch. Pharm. Res. 28 (3), 249-268.

Zhang, Y., P. Talalay, C.G. Cho, and G.H. Posner. 1992. A major inducer of anticarcinogenic protective enzymes from broccoli – isolation and elucidation of structure. Proc. Natl. Acad. Sci. USA 89, 2399-2403.

Zhang, Y. and P. Talalay. 1998. Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. Cancer Res. 58, 4632-4639.

Zhang, Y., J. Li, and L. Tang. 2005. Cancer-preventive isothiocyanates: dichotomous modulators of oxidative stress. Radic. Biol. Med. 38, 70-77.

Zhang, R., S. Loganathan, I. Humphreys, and S.K. Srivastava. 2006. Benzyl isothiocyanate-induced DNA damage causes G2/M cell cycle arrest and apoptosis in human pancreatic cancer cells. J. Nutr. 136(11), 2728-34.