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Review

Therapeutic Properties of Flavonoids in Treatment of Cancer through Autophagic Modulation: A Systematic Review

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ABSTRACT Cancers have high morbidity and mortality rates worldwide. Current anticancer therapies have demonstrated specific signaling pathways as a target in the involvement of carcinogenesis. Autophagy is a quality control system for proteins and plays a fundamental role in cancer carcinogenesis, exerting an anticarcinogenic role in normal cells and can inhibit the transformation of malignant cells. Therefore, drugs aimed at autophagy can function as antitumor agents. Flavonoids are a class of polyphenolic secondary metabolites commonly found in plants and, consequently, consumed in diets. In this review, the systematic search strategy was used, which included the search for descriptors "flavonoids" AND "mTOR pathway" AND "cancer" AND "autophagy", in the electronic databases of PubMed, Cochrane Library, Web of Science and Scopus, from January 2011 to January 2021. The current literature demonstrates that flavonoids have anticarcinogenic properties, including inhibition of cell proliferation, induction of apoptosis, autophagy, necrosis, cell cycle arrest, senescence, impaired cell migration, invasion, tumor angiogenesis and reduced resistance to multiple drugs in tumor cells. We demonstrate the available evidence on the roles of flavonoids and autophagy in cancer progression and inhibition. (Registration No. CRD42021243071 at PROSPERO)

KEYWORDS anti-cancer effects, apoptosis, cell proliferation, cell survival, flavonoids

Currently, cancer is the second leading cause of death in the world, and it was estimated to occupy the first place in the coming decades, especially in poor and developing countries. This pathology is characterized, among other factors, by the disordered growth of cells, through cell cycle alterations.[1] It is a disease of multiple origins, which can be caused by lifestyle, environmental factors, genetic influence, and the aging process of the organism.[2]

Because it is a disease related to the cell cycle, processes that can damage cellular structures are factors favorable to neoplastic development. In this sense, flavonoids—phytocomposites that have varied actions in plants, such as protection against ultra violet rays, protection against pathogenic organisms and antioxidant action—have been studied. Due to these properties, flavonoids have been evaluated for their therapeutic potential in the treatment of cancer, for having an antioxidant function, acting in the control of cell proliferation and in blocking neoplastic formation by mechanisms that regulate enzymes of the carcinogenic metabolic pathway. In addition, some of these compounds can inhibit the cyclooxygenase (COX) and lipoxygenase pathways, and important inflammatory markers, showing an anti-inflammatory property,[3] demonstrating their potential for maintaining cellular homeostasis, thus decreasing the chances of cell damage and the consequent neoplastic development. Studies demonstrate the therapeutic use of flavonoids to improve the prognosis, as well as prophylactic action regarding the development of breast cancer and thyroid cancer.[4,5]

It is also worth mentioning that one of the natural

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processes of the cell to oncogenesis is autophagy, a catabolic process that consists of the formation of phagosomes to digest parts of the cell, usually in situations of stress or accumulation of defective organelles.[6] Despite being a widely researched disease, there is still little knowledge about the mechanism by which flavonoids play an anti-cancer function through autophagic regulation. In this sense, the objective of this study is to carry out a systematic review, analyzing the relation of therapeutic properties in the treatment of cancer through its autophagic modulation, verifying the therapeutic potential of flavonoids in cancer.

METHODS

Search Strategy

This systematic review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Guideline, registered at PROSPERO with No. CRD42021243071. The survey was conducted from April 2020 to March 2021 using the PubMed, Cochrane Library, Web of Science and Scopus data platforms. We tried to identify all studies that involved the following combinations of descriptors: "autophagy" AND "cancer" AND "flavonoids" AND "mTOR pathway". The research was restricted to the last 10 years (January 2011 to January 2021).

The PubMed, Web of Science and Scopus platforms were chosen because they have attached several journals that cover our areas of interest, namely: cell biology, molecular biology, oncology, medicine. The Cochrane Library platform, in turn, was chosen in order to better support our knowledge of the subject with a possible review on the topic or something similar.

At the beginning, the articles were selected by reading their titles and abstracts. In the second stage of the screening process, the articles were submitted to the inclusion and exclusion criteria, as described below and were reviewed by three authors. The articles that generated disagreement were revised again until a consensus was reached. Afterwards, the articles were read in full.

Inclusion and Exclusion Criteria

Although studies with flavonoid compounds in the treatment of cancer are already underway, there is a lack of studies on the potential of these compounds, especially with regard to their action in the autophagic pathway. Since there were few studies and none at a clinical level *in vivo* in humans, we chose *in vitro* studies of human or animal cells and *in vitro* studies in animals, as this would be the best way to analyze the molecular/cellular influence of phytocompounds on the pathway autophagic.

In vitro and *in vivo* studies, regardless of language, that evaluated the use of flavonoids as cancer chemotherapy and addressed autophagy were included in this study.

Duplicate studies were identified and excluded. Studies that did not contain or did not specify the control groups were excluded, as they lacked methodological rigor. Review articles and letters to the editor were excluded. Furthermore, articles that did not specify the type of flavonoid used were also excluded.

Data Extraction

After a detailed reading of the studies eligible for inclusion in this systematic review, data extraction was conducted. The data extracted were: year, authors, outcomes, cells studied, time of exposure to flavonoids, flavonoids used, type of cancer and temperature at which cells were maintained.

In addition to the basic data of each article (year, author and outcome), we also extracted other data to better elucidate what each study postulated. The time of exposure to flavonoids, the type of flavonoid used and the type of cancer are crucial factors, because from this information, it is possible to envision the possibility of clinical intervention with flavonoid-based drugs in a specific type of cancer, or even, if it does not have this specification and may influence carcinogenesis as a whole.

RESULTS

Eligible Studies

The search resulted in a total of 184 articles. Of these, 150 were chosen after reading the title and abstract. After removing duplicates, we obtained a total of 97 articles. After this process, the studies were read in their entirety. There were 13 studies left for the complete reading, of which 3 were discarded because they did not have control groups. After reading the studies in full, 10 studies[8-16] met the eligibility criteria

and underwent quality extraction. The procedures and results for choosing the studies are presented in Appendix 1.[7]

Characteristics of Studies

All studies included are observational, carried out on cancer cells (human and animal), of the most diverse types, in the laboratory. Such cells had a flavonoid exposure time of at least 24 h, subjected to a standard temperature of 37 ℃ (98 °F). In relation to the control group, 7 studies defined as control groups those cells not treated with flavonoid compounds[8-14] 2 studies those cells treated with dimethyl sulfoxide[15] and 1 study those incubated cells in phosphated buffered saline (Appendix 2).[16]

Inhibition of Mammalian Target of Rapamycine Pathway

The mammalian target of rapamycine/phosphoinositide-3-kinase/protein kinase B (mTOR/PI3K/AKT) pathway has been described as one of the most important pathways in the regulation of autophagy. The activation of PI3K, stimulates AKT, leading to the activation of mTOR.[17] mTOR has two protein complexes, mTORC1 and mTORC2, with mTORC1 being one of the main regulators of autophagy, since, when activated, it suppresses autophagy and, when inactivated, stimulates autophagy.[18] In this sense, flavonoids have shown ways to inactivate this pathway, thus stimulating autophagy.

One of the ways to interfere in this way is through the inactivation of mTOR. By reading the studies, the following relationship between flavonoids and their actions that inactivate mTOR was established: the compounds sotetsu flavone and pectolinarigenin showed the ability to decrease the phosphorylation of ribosomal protein S6 kinase (p-p70S6K, a marker of the mTORC1 complex), baicalein through the activation of activated protein kinase (AMPK)-Unc-51-like kinase (ULK1), mimulone-C (MML), isoliquiritigenin through CA-mTOR modulation, and silybin by increasing beclin-1.

Another way to interfere with the pathway is through the inactivation of AKT, which also leads to the inactivation of mTOR. Flavonoids that showed this ability and their respective mechanisms are: wongonin, through direct inhibition of ATK; icariin, through the overexpression of ATG5; fisetin, by reducing p-AMPK levels. The following sections will discuss the articles according to the flavonoids used.

Flavonoids Used in the Review

Isolated Mimulone-C Geranyl Flavonoids

Mimulone-C geranyl flavonoids is a flavonoid extracted from *Paulownia tomentosa* fruits, present in some Asian countries, and considerably used in Chinese medicine to treat respiratory diseases. Although its anti-cancer and anti-inflammatory therapeutic functions have been studied, the field is still exceedingly small in the literature, with only one study directly associating it with autophagy.

In the study by An, et al,[8] lung, breast, colorectal and osteosarcoma adenocarcinoma human cells were exposed to varying concentrations of mimulone-C geranyl flavonoids for 24 h. Subsequently, with cell viability counted, a relevant role was noted in the control of tumor cells in a manner dependent on the dose and the time used in the incubation.

The association with autophagy was carried out through protein analysis of light chain 3 (LC3)-Ⅱ, Beclin-1, Atg7 (immunoblotting), with their levels varying considerably during the experiment. Other proteins were also analyzed, such as p53, mTOR, AMPK and p62. p53 was associated with autophagy, reducing its concentration in conjunction with mTOR. AMPK had its levels increased, indicating the beginning of the catabolic process—metabolic stress. However, p62 did not undergo significant oscillation, and was indicated as a marker of autophagic flow. Thus, it can be concluded that there is an increase in autophagy, without an increase in autophagic flow.

Furthermore, *Paulownia tomentosa* derivative shows enormous potential as an adjunctive therapy in cancer. An *in vitro* study concluded that the antioxidant and anti-inflammatory actions of these compounds on AHH-1 cells were able to protect healthy tissue from radiotherapy, by scavenging radicals and inhibiting radiation-induced DNA strands, in addition to increasing levels of glutathione.[19]

Baicalein

A known structure in Chinese herbal medicine, the metabolite extracted from *Scutellaria baicalensis* Georgi has been studied in a considerable way in the last 20 years for its therapeutic characteristics.[12]

Its effects on cancer had been previously studied, but with the study by Aryal, et al,[20] the anti-cancer relationship mediated by autophagy is clarified, when there is activation of the PI3K/AKT/mTOR path-activation of the ULK1 complex.

Furthermore, baicalein showed a high potential to induce cell cycle arrest in prostate and breast cancer cells. After exposure to the flavonoid, treatment with 20 μg/mL was observed to inhibit the growth of more than 90% of PC-3 and MDA-MB-231 cells (prostatic and mammary cells, respectively). Similar results were found in h460 cells from lung cancer, HepG2 cells from hepatocellular cancer, and MiaPaCa cells from pancreatic cancer. This was observed through the downregulation of proteins present in the cell cycle, such as cdc2, cdk2, cdk4 and cyclin D1, in addition to the concomitant increase in p21 proteins. In parallel, the phytocompound also presented itself as an initiator of the autophagic pathway, since it was observed an increase in LC3B in PC-3 and DU145 prostate cancer cells, at the same time it induced cyclin D3 levels, thus demonstrating cell death.[20,21]

Baicalein is a potential phytotherapic relevant in the treatment of tumors, in addition to being a compound with extremely low toxic effects-with actions that indicate a "hunt" for the tumor cells themselves, preserving healthy cells.[21] Its mechanisms have been studied in several types of tumors, including hepatocellular carcinoma,[22] cervical cancer,[23] breast,[24] prostate,[25] among others.

An indisputable differential of the work carried out by Aryal, et al[20] was the connection with autophagy, through analysis emphasized in the autophagic process. Western blotting methods (with measurement of the levels of LC3-Ⅱ and Atg8) and fluorescent microscopy were used by the researchers, in order to probe the possibility of initiation and autophagic flow in the PC3, DU145 and MDA-MB 231 cells. It was found that even at the lowest levels of baicalein, the reduction in tumor growth was notable, although its effectiveness depends on the concentration used.

In addition, the study also worked on cell death caused by autophagy, indicated by the decrease in cyclin D3 inversely proportional to the LC3-Ⅱ present. In other experiments, autophagy is also associated with the activation of AMPK and inhibition of mTOR-a relationship already worked on.

Isoliquiritigenin

Isoliquiritigenin (ISL) is a compound with pharmacological relevance extracted from plant roots such as licorice.[26] Its therapeutic use has a series of studies in the last 20 years in the literature[27] and its studied capabilities include its role in decreasing cancerous cell proliferations[28] via changes in cell viability, in addition to anti-inflammatory effects associated with decreased levels of markers inflammation such as tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-8 and IL-12, among other proinflammatory factors.[15,29]

In the study by Chen, et al,[29] in order to analyze the relationship between ISL and cell death in cystic adenocarcinoma cells associated with autophagy, evidence of the formation of acid vacuoles and increased autophagic flow was found. Through Western blot analysis and immunofluorescence microscopy, the group treated with 20 μmol/L ISL was compared with the control group. As in other studies, it demonstrated increased cleavage of the LC3 protein in LC3-Ⅱ, as well as ATG5 and reduced expression of mTOR-indicating that inactivation of this pathway is associated with increased autophagic levels. It is also shown in the study that autophagy is correlated with apoptosis and that it was not possible to define whether cell death and relevant antitumor activity could be directly and singularly associated with autophagy. In addition, to update the discussion, papers published in 2020[30,31] address the importance of understanding and associating autophagic modulators with other possible chemotherapeutic agents to improve strategies already used.

ISL-induced autophagy was measured through LC3 levels by Western blot. As a result, it was observed that LC3 levels were higher in ISL-treated cells than the control group, in addition to increasing Atg5 levels. Thus, cell viability was demonstrated after 48 h of exposure. Another point studied was the effect of 3-methyladenine (3-MA, an autophagy inhibitor) on induced ISL cells. Decreased cell viability resulting from 48 h of exposure to ISL was rescued by treatment with 3-MA. Thus, although autophagy is already known as a modality of cell death, its defensive role against cancer remains uncertain for the author.[30,31]

Despite the published reviews presenting

numerous *in vitro* and *in vivo* studies that address ISL's antitumor, antioxidative, and anti-inflammatory actions,[27] we have not found relevant clinical studies ensuring the pharmaceutical use of ISL, since work is still needed on possible biological side-effects prior to its large-scale administration.[26]

Wogonin

The flavonoid wogonin, extracted from *Scutellaria baicalensis*, has remarkable inflammatory suppressive actions, through a decrease in the levels of inflammatory markers such as cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), among others.[32] It has also been associated with the treatment of tumors, partly linked to the inhibition of inflammation caused by carcinoma, but in addition also decreasing carcinogenic and angiogenic factors *in vitro*.[33] *In vivo* study[28] also showed favorable results, with tumor reduction, but it lacks further studies on its pharmacological use.

The work of Chow, et al[34] brought a vision of autophagy, related to apoptosis, with an anticancer effect in human cells affected by nasopharyngeal carcinoma. The induction of autophagy was performed by inhibiting the mTOR pathway, with the formation of vacuoles conferred by electromicroscopy. In addition, a comparison with the control group without substance use showed changes in LC3-Ⅱ concentrations, indicating progression of the autophagic process. Thus, it is understood that there is a biological potential in the use of the substance to be explored.

Icariin

Icariin is a natural flavonoid found in *Epimedium grandiflorum* with widespread use in alternative medicines for its therapeutic potential, with studies linking it even to the treatment of psychic illnesses.[35] Its anticancer activities have been studied and published in the literature, linking it to breast cancer,[36] colon cancer,[37] ovarian cancer,[11] among others.

A curious fact to be addressed about the substance is that icariin was widely used for its apoptosis-inducing autophagy inhibition properties, with the purpose of reducing established cancers.[11,37] Its relationship with autophagic modulation becomes clear in the study by Huang, et al,[38] in which the induction of autophagy is performed by the substance via PI3K/mTOR inhibition. The study, conducted on human cervical cancer cells, aimed to evaluate the potential of flavonoid in reducing tumors *in vitro*. It was found that there was an increase in the levels of LC3-Ⅱ and a decrease in p62, which indicates that there was an increase in the autophagic flow. Furthermore, the levels of proteins present in the PI3K/AKT/mTOR pathway were also analyzed, measuring the suppression of the pathway. It was understood, then, that the relevance of Icariin, which was already being evaluated in the treatment of other diseases, may also emerge in research associated with tumor reduction, requiring more research for confirmation and *in vivo* analysis.

In a study conducted on SKVCR cells that were treated with icariin,[11] it was observed that these flavonoids promote cell cycle arrest, while promoting the G_0/G_1 phases of the cycle. The results also showed that treatment with cisplatin + incariin induced apoptosis and autophagy in SCKVCR cells, compared to those treated with cisplatin alone. to the expression of protein-associated autophagy, including proteins such as Beclin-1, ATG5, p62 and AKT. Thus, it is concluded that icariin increases the chemosensitivity of SKVCR cells by suppressing the autophagic pathway and by activating the AKT/mTOR pathway. Furthermore, the study also pointed to greater activation of the AKT/mTOR pathway in cells treated with cisplatin + icariin compared to those treated with cisplatin alone. Activation of this pathway led to the inhibition of autophagy and the resensitization of cells to cisplatin.

Fisetin

Fisetin is a well-studied flavonoid that is present in apples, kiwis, grapes, strawberries, onions, among other foods.[39] Its anti-inflammatory (with modulation of inflammatory mediators), antioxidants and anti-cancer properties have been studied in the literature. In the review conducted by Kashyap, et al,[16] the section "anticancer effects of fisetin" brings associations of tumor reduction with induction of apoptosis, cell cycle arrest, suppression of metastasis and angiogenesis in addition to anti-inflammatory and antioxidant effects. There is, however, low bioavailability of this substance when ingested low absorption and solubility, it is interesting that its increase is carried out when associated with other substances, such as caffeine.[16]

The study by Jia, et al[40] found that after treatment with fisetin, autophagic levels were relevant, but there was no apparent association with traditional

AMPK, but with other regulatory proteins. A possible explanation for this is that, instead of using the AKT/mTOR pathway, fisetin induces mitochondrial stress which, in turn, leads to autophagy. This was verified by increasing the expressions of protein kinase R like endoplasmic reticulum kinase (PERK), activating transcription factor (ATF) 4 and ATF6. Through immunofluorescence, it was verified that mitophagy was greater in cells treated with fisetin. Via Western blotting and microscopic immunofluorescence analysis, they analyzed PANC-1 cells treated with fisetin in varying concentrations and intervals. Prior to treatment, chloroquine (known as an autophagy inhibitor) was used for purposes of better accuracy in the analysis. The protein levels of LC3 were modulated, and AMPK was increased, in addition to the differentiated expression of autophagic proteins. It was then concluded that the modulation of autophagy in cancers can go beyond the known mTOR/PI3K/AKT pathway and have differentiated initiations as the stresses suffered by the cells in question. The study presents fisetin as a cytoprotective substance and interesting drug potential.

Pectolinarigenin

Pectolinarigenin (PEC) is a metabolite of pectolinarin, a flavonoid present in plants of the genus *Cirsium*, more present in some Asian countries.[41] The anti-inflammatory, antioxidant, anti-tumorigenic and metastasis reduction mechanisms are not yet fully elucidated, but they have a potential for success for possible *in vivo* therapies.[12,41]

In gastric cancer cells AGS and MKN28, scientists from Korean institutes found that the cancer inhibitory effect was attributed to three fronts: cell apoptosis, autophagy, and cell cycle arrest. Regarding the cell cycle, the AGS and MKN28 cells were treated with concentrations of 50 and 100 μmol/L of PEC for 24 h and, later, the DNA was analyzed by flow cytometry, verifying that there was an accumulation of the G_2/M phases. of the cell cycle in cells. Subsequently, an analysis via Western blot showed that there was depletion of the expression of CDK1 and CDC25C proteins at those concentrations. There were no significant changes in p53 and p21 levels in both cells. Autophagy analysis was performed using Western blot, which showed increased levels of two types of LC3 (LC3-Ⅱ/LC3-Ⅰ) and decreased levels of Beclin-1 in both cells. Finally, PEC also decreased PI3K and ATK expressions, corroborating autophagic activity.[12,41]

The study by Lee, et al[42] included a control group, using different concentrations of the substance in question, they verified the formation of acidic organelles (autophagosomes) by means of AO (dye) and in protein analysis, increased concentrations of LC3-Ⅱ and decreased p62. The literature associates the regulatory effects of PEC with the inhibition of the PI3K/AKT/mTOR pathway,[43] since studies have noted a considerable decrease in its concentrations after the use of the flavonoid. In the study by Lee, et al,[42] the result was not different: the concentrations of PI3K, AKT and the active form of mTOR after 24 h of incubation reduced.

Silibinin

Silibinin is a substrate of the plant *Silybum marianum*, also known as thistle. It has been commercialized in the pharmacy industry since 1970 for the treatment of liver diseases and for the hepatoprotective effect. In addition, it has antioxidant and anti-inflammatory effects.[44] Its bioavailability, however, is low, due to its low intestinal absorption. Thus, its use is associated with other substances, increasing its tissue penetration capabilities and effective action.[45]

In the work conducted by Li, et al,[46] silibinin was applied to RCC ACHN and 786-O cells from kidney cancer. It was noticed that there was an increase in this induction in nutritional deficit, in addition to the process dependence on reactive oxygen species. Using LC-3 as an autophagic marker, AMPK was found to be involved in autophagic regulation through silibinin. Furthermore, it was also verified that the autophagic activation induced by silibinin may contribute to stop the metastasis in RCC cells *in vitro*.

Sotetsu Flavone

Sotetsu flavone is a flavonoid found in nature in the form of *Cycas revoluta* that has shown effective results in the treatment of lung,[14] gastric cancers,[13] and has even been studied for the treatment of corona virus disease 2019.[47] This flavonoid, despite its demonstrated phytotherapic actions, does not have much material available in the literature.

An increase in LC3 levels and a decrease in p62 levels were observed, demonstrating that there was an autophagic flow. To confirm that autophagy was indeed

induced by sotetsu flavone, the researchers detected the distribution of LC3 dots. Using immunofluorescence, it was revealed that LC3 points were increased in treated cells to the detriment of the control group. In addition, there was also increased expression of beclin-1, Atg5 and Atg7 in A549 non-small cell lung cancer cells. To analyze the mTOR pathway, Western blotting was used to verify the expression levels of the phosphorylation of PI3k, Akt, mTOR, Raptor and p70S6K. Inhibition of the PI3K/Akt/mTOR pathway was found in cells treated with sotetsu flavone. Thus, the authors point out that the induction of autophagy induced by sotetsu flavone occurs precisely by the inhibition of the aforementioned pathway.[46]

In the background, sotetsu flavone was associated in the research to the treatment of non-small cell lung cancers, with triple effects in tumor reduction: cell cycle arrest, apoptosis, and autophagy. Its capabilities include suppression of mechanisms necessary for carcinogenesis and cancer establishment, such as cell proliferation and invasion. In the study conducted by Wang, et al,[46] no evidence of toxic effects in the use of the substance was found, and the results show a cytoprotective effect—increasing the viability of chemotherapy. The measurement of autophagy was performed by indicated methods: western blotting (evaluation of LC3-Ⅱ, p62, Beclin 1, Atg5 and Atg7) and electron microscopy. There was an increase in the autophagic flow and formation of vesicles inversely proportional to the activities of the mTOR/AKT/PI3K pathway. Finally, the results proved to be valid for further studies associating them with chemotherapeutics among other drugs for tumor suppression *in vivo*.[48]

Oroxylin A

Formed as a metabolite of baicalein, oroxylin A is yet another flavonoid of high phytotherapic relevance.[49] There are published associations of the substance with the suppression of cancer via apoptosis,[50] and the study by Zou, et al[51] was the only one found that also addresses autophagy.

The cells used in the analysis were Hep62 and SMMC7721 from hepatocellular carcinoma, treated with fixed concentration of oroxylin A for different periods, with an autophagy inhibition control group. In results, it was confirmed that the substance has tumor suppressor effect, in which better results were found in the groups with longer treatments (48 h). Through fluorescent microscopy and Western blotting analysis, the induction of autophagy in the studied cells was confirmed, proven by the formation of autophagosomes and the analysis of autophagy biological marker proteins (such as LC3 and Beclin-1). Autophagy induction was related to Beclin-1 expression, proving the need for protein in the progression of the initiation of the process.[51]

DISCUSSION

Types of Cancer and Their Impact on Health System

Gallbladder Cancer

Gallbladder cancer (GBC) is a relatively rare type of cancer, having the highest incidence recorded in Delhi, India (21.5 per 100,000), and the lowest recorded in Northern Europe, USA, Canada and Singapore, in addition to affecting women up to 6 times more than men.[52] GBC is a silent cancer, often being diagnosed in an advanced stage, with less than 10% of cases subject to dryness. Approximately 1/3 of diagnoses are made accidentally during a cholecystectomy, and it is found in 1%–2% of cholecystectomies performed.[52-54]

Because it is an extremely silent cancer, GBC has an almost always poor prognosis. When accidentally diagnosed during other tests, the survival prognosis is, on average, 26.5 months, and when diagnosed by suspicion, after presenting some symptoms, the survival time drops to 9.2 months on average.[53]

In health systems, the main difficulty is the delay in the presentation of symptoms, which conditions the discovery of cancer by chance, and in cases where symptoms are present, the lack of a specific cell marker that can be used to identify the disease of GBC.[53] In this way, flavonoids with modulatory action on autophagy pathways could be used to improve the prognosis of these patients, without representing great costs to the already overloaded health system.

Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma (HNSCC) is a broad group of neoplasms that occur in the upper part of the digestive and respiratory tract, as the name implies, and originates from mucosal epithelial cells.[55] Unlike GBC, HNSCC has a better prognosis, with rates of up to 64.5% survival for at least 5 years, being recorded in South Korea.[55]

The major impediment to HNSCC is the presence of cancer stem cells, which have self-renewal abilities, high proliferation capacity, high migration capacity, drug resistance, resistance to chemo- and radiotherapies, and differentiation capacity.[56] These cells do not have specific cell markers known that can be used to identify and specifically target these cells, making it difficult to completely dry out the HNSCC, prolonging the time of coexistence with the disease.[56]

Unlike GBC, HNSCC is easy to identify, however, there is still a long delay for its diagnosis, which ends up being done already in advanced stages, due to the low presentation of symptoms.[57] This delay in diagnosis and the prolongation of the time of living with the HNSCC translates into high expenses with medication and treatments for the various health systems, in addition to an increase in the queues for treatment.

Oral Cancer

Despite being a subtype of head and neck cancer, oral cancer has a much higher prevalence, being the most common neoplasm in the head and neck region.[58] It accounts for 80,000 new cancer cases recorded annually, 95% of which are HNSCC subtypes.[58-60]

The main factor to consider for oral cancer is its high incidence, directly related to the consumption of tobacco, alcohol, and contamination by human papilloma virus (HPV), its main risk factors.[58,61] Even though it is an easily identifiable cancer, which usually occurs early, making it treatable, oral cancer is an extremely preventable cancer, which generates a financial impact on health systems that would not have to occur. In this way, flavonoids could be used not only to treat cancer, but also to prevent it, reducing not only health system expenses, but also the occupation of vacancies for consultations, biopsies, and the treatment itself.

Cancer and Autophagy

Macroautophagy, or just autophagy, is characterized by the physiological process of cellular self-digestion, in which cell structures are directed to lysosomal degradation through acid hydrolases.[24] Initially described by de Duve C and Wattiaux R,[62] the catabolic process plays an indispensable role in homeostasis, recycling materials that are damaged or that could become toxic, acting as a quality control of the microenvironment.[63]

The process is initiated by stress, and may have nutritional and metabolic causes, among others. Its onset can be stimulated by the lack of energy in the form of ATP and activation of the AMP kinase enzyme, as well as some metabolic alteration causing the deactivation of mTORC1, which tonically inhibits the formation of pre-initial ULK1 complexes. The formation of ULK1 encourages the activation of Beclin-1 and the Ⅲ PI3K complex, associated with the endoplasmic reticulum.[30,64]

Thus, self-digestion has been undergoing a series of analysis for its proven therapeutic actions.[64] It is also understood that there is an ambiguous relationship of the autophagic stimulus in relation to the studied diseases, since it can improve prognosis in cases of initial cancers, as well as worsen prognosis in advanced and stable cancers. This relationship can be attributed to the levels of oscillating p62 in cancers since the catabolic process decreases its concentrations.[65] With that, it becomes evident that the modulation (stimulus or inhibition) of the amount of autophagy that the body performs is an interesting therapeutic factor in the treatment of tumors.

However, measuring the amount of autophagy is not an easy task, and the mechanisms that currently exist have associated flaws. The fourth edition of the guidelines for the analysis of quantitative studies of autophagy, updated in 2021, suggests that at least two methods of analysis be used, the most common being the verification of proteins by means of Western blotting (autophagic genes, LC3-Ⅱ, mTOR and AMPK) and the formation of acidic vesicles with the presence of lysosomal proteases by means of electron or fluorescent microscopy.[66]

Flavonoids and Cancer

Natural compounds used in alternative medicines have been around for longer than any of the common pharmaceutical drugs today. Flavonoids represent about 60% of the group of natural polyphenols, with a chemical structure that has at least one aromatic ring associated with functional hydroxyl groups.[67,68] They can be divided into 6 subgroups according to their chemical conformation and are found in several foods of plant origin.[69] Plant-based or plant-rich diets have already been associated in the literature with better prognosis in the treatment of diseases such as

cancer, other inflammatory diseases and infections.[70] Flavonoids appear in this branch as a regulator of the immune system, anticancer, antiinflammatory, anti-metastatic, antioxidant associated with both cancer treatment and prevention.[68,71]

Flavonoids, Cancer and Autophagy

Through their gene modulations and effects on the cell cycle, polyphenol compounds have been studied affecting normal body autophagic regulation. The presence of these substances in the body can cause a decrease in inflammation marker proteins, as well as pro-apoptotic proteins and autophagy markers.

Despite the already studied relationship with autophagy, flavonoids have still been the target of few studies in the triple relationship with cancer and autophagy, showing a need in the area.

Through literature review, it was observed that, *in vitro*, flavonoids can lead to repression of the AMPK/mTOR pathway, leading to autophagy and, thus, halting neoplastic cell growth, something common in all studies included in this systematic review. In addition to the AMPK/mTOR proteins, Zou, et al[51] noted the presence of baicalein-1, which is also an initiator of the autophagic pathway. An, et al[8] used MML to treat cells of various types of cancer, observing, in addition to the AMPK/mTOR pathway, an increase in Atg7 levels. Similar to Atg7, the increase in Atg8 levels was observed by Aryal, et al,[20] a constituent of the autophagic pathway after exposure of cells from different types of cancer to baicalein. The involvement in the autophagic pathway is also present in the study by Chen, et al,[29] since the ISL phytocompound was shown to be able to induce the formation of acid vacuoles. In this sense, Chen, et al,[29] Chow, et al,[34] and Lee, at al[42] observed that, respectively, wogonin and pectolinarigenin also induce the formation of vacuoles. In addition to the mechanisms elucidated, Huang, et al[38] showed that the apoptosis characteristic of cancer cells also induces autophagy, highlighting this process as a mechanism for retarding neoplastic growth. Among the studies analyzed, only Jia, et al[40] reiterated that despite the flavonoid fisetin being able to induce autophagy, this process seems not to be related to the AMPK/mTOR pathway, but with proteins not yet known to it, which confronts the study by Li, et al[46] in which exposure of cancer cells to silibinin elucidatively inhibits the AMPK/mTOR pathway and activates autophagy. In addition to the therapeutic potential, at the *in vitro* level, Wang, et al[14] stressed that the exposure of cancer cells to sotetsu flavone did not generate toxic effects.

Factors That Can Influence Results

Exposure Time

In the studies used, the vast majority of researchers exposed the cell culture for up to 48 h to flavonoids, with the exception of Jia, et al.[40] This low exposure time is not compatible with a supposed treatment using flavonoids, thus, the results may be different when increasing the exposure time of cultures. In addition, in the hypothesis of using flavonoids as medication, this exposure would not be continuous as in cell cultures, but interspersed, with concentration peaks as they are used, and it is also not clear whether this intercalated exposure can change the results obtained.

Human Testing, Ethnicity, Sex and Age

The studies used did not carry out experiments on humans, which disregards the great complexity of the organism, capable of modulating the performance of flavonoids for better or worse, in addition to preventing reports of side effects in humans. In addition, it is not yet known whether the patient's ethnicity has the potential to change the results obtained with flavonoids, especially considering that all studies come from Asian countries and regions, where historically the consumption of plants and medicinal herbs has been used. it is much more intense and usual. More trivial factors such as sex and age could not be analyzed either, so it is also unclear whether factors inherent to them, such as hormone production, can alter the results.

Proper Management

In addition, the management of flavonoids in the studies, in a controlled laboratory environment, was ideal, however, as a hypothesis of potential treatment, the management of flavonoids would not be as controlled, but more coarse. It is not clear whether the process of producing an eventual medication could lead to worse results.

Conclusions

In general, it was observed that the repression of the mTOR/AMPK pathway and the consequent activation of the autophagic pathway is favored by the

exposure of flavonoids, demonstrating the potential to delay the proliferation of cancer cells. With the modulation of autophagy via flavonoids, effects of oncogene degradation, combating local inflammation, tumor growth suppression, increased cytotoxicity of chemotherapeutic agents and even disease prevention were observed.[67,72-75]

However, the studies used were largely *in vitro*, thus, *in vivo* studies are lacking to corroborate the results obtained. Another limitation found was the low number of scientific productions that studied the subject, and the fact that all of them were laboratory, not clinical, making it difficult to understand the chemotherapeutic potential of flavonoids. Thus, the chemotherapeutic potential of flavonoids for the treatment of cancer is undeniable, but more in-depth studies, *in vivo*, in humans of different genders, ages and ethnicities, and clinical studies are needed to further consolidate this chemotherapeutic potential.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Silva DTR contributed to the conception, project administration and supervision. Silva GVS, Lopes ALVFG, and Viali IC contributed to the formal analysis, investigation, and methodology. Haag FB and Bizuti MR supervised the methodology. Lima LML contributed to the writing and translation of the original draft. Bizuti MR, Silva DTR and Haag FB revised the final work.

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