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Review

In vitro polyphenol effects on apoptosis: An update of literature data

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ABSTRACT

Polyphenols are secondary plant metabolites which have been studied extensively for their health-promoting properties, and which could also exert pharmacological activities ranging from anti-inflammatory effects, to cytotoxic activity against cancer cells. The main mechanism for programmed cell death is represented by apoptosis, and its dysregulation is involved in the etiopathology of cancer. As such, substances able to induce apoptosis in cancer cells could be used as new anticancer agents. The aim of this paper is to review literature data on the apoptotic effects of polyphenols and the molecular mechanisms through which they induce these effects in cancer cells. In addition, a brief summary of the new delivery forms used to increase the bioavailability, and clinical impact of polyphenols is provided. The studies reported show that many polyphenol rich plant extracts, originating from food and herbal medicine, as well as isolated polyphenols administered individually or in combination, can regulate cell apoptosis primarily through intrinsic and extrinsic mechanisms of action in *in vitro* conditions. Due to these promising results, the use of polyphenols in the treatment of cancer should therefore be deeply investigated. In particular, because of the low number of clinical trials, further studies are required to evaluate the anticancer activity of polyphenols in *in vivo* conditions.

1. Introduction

Thousands of cells in our bodies are undergoing ordered and orchestrated death every second of our life, due to apoptosis or Programmed Cell Death (PCD) [1]. Cell apoptosis is an evolutionarily conserved process, which plays varied and essential roles in tissue homeostasis and organism development [2]. It is also one of the most popular research topics among cell and molecular biologists. Apoptosis is prompted by a variety of stimuli, including hyperthermia, growth-factor or hormone withdrawal, glucocorticoids, oxidative stress, ionizing radiation, and multiple classes of chemotherapeutic agents [3]. It begins with the shrinkage of the cell, followed by the condensation of chromatin and cytoplasm, the fragmentation of DNA in 200 bp multiples, the blebbing of the cell, and finally the formation of apoptotic bodies [4]. During this process, inflammation is not evident; in fact, apoptosis reduces the possible damage caused by the activation of the immune system [5]. The major actors during apoptosis are: Cysteine aspartic acid-specific proteases (caspases), B-cell lymphoma-2 family protein (BCL-2), and Inhibitor of Apoptosis Proteins (IAPs). Caspases are a family of cysteine proteases produced as inactive precursor enzymes (zymogens) which play an essential role in both inflammation and PCD. Apoptotic caspases can be classified as initiation caspases (−2, −8, −9, −10, and −11) or effector caspases (−3, −6, and −7) [6]. The former initiate the proteolitic cascade, whereas the latter contribute to apoptosis by degrading essential proteins involved in cell survival [7]. The BCL-2 gene encodes the production of pro-apoptotic, anti-apoptotic and BH3-only proteins. Their role is to regulate the apoptotic process through the formation of pores on the mitochondrial membrane [7]. The anti-apoptotic proteins (BCL-XL, BCL-W, BLC-B, MCL1 and BCL2A1) are characterized by four BCL-2 homology (BH) domains and their task is to block apoptosis, opposing the pro-apoptotic proteins (BAX, BAK, BOK),which only present three BH domains with the task of promoting apoptotic processes through formation of a membrane pore on the outer mithocondrial membrane [7]. The BH3-only proteins (BAD, BID, PUMA, NOXA, BMF,BMF, BIM, BIK) only present the BH3 domain and are regulated according to several different criteria. Another important role is played by IAPs. This family of proteins contain at least one bacculovirus IAP repeat (BIR) domain, and

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they attend to different cellular processes including apoptosis. X-linked IAP (XIAP) are the most known IAPs, which can bind caspases, inhibiting their activity [8].The mechanism of apoptosis is complex and involves many pathways.

There are three main mechanisms for cell apoptosis comprising of the extrinsic pathway, the intrinsic pathway, and the perforin-granzyme apoptotic pathway [3]. The intrinsic pathway starts within the cell, but can be induced by extra or intracellular stress or damage causing stimuli. These stimuli can prompt the overexpression of specific sensor proteins belonging to the BH-3 only subclass, promoting the inhibition of anti-apoptotic BCL- 2 proteins followed by the assembly of BAK/BAX oligomers [9]. The formation of BAK/BAX oligomers is essential for creating a channel on the outer membrane of the mytochondria. This process allows proteins to move from the intermembrane space of mythocondria to the citosol. One of these proteins is cytochrome c, which induces the activation of caspase-3 and leads to the formation of a quaternary protein complex with ATP, Apoptotic Protease Activating Factor 1 (APAF-1) and caspase-9, called apoptosome [10]. The assembly of the apoptosome allows the caspase-9 to activate other caspases, leading the cell to apoptosis [11].

The extrinsic pathway is prompted by the binding of death ligands to the transmembrane death receptors. The most important of these receptors are the tumor necrosis factor (TNF) receptor (TNFR1) and the First Apoptosis Signal (FAS) receptor (CD95). These interact with their respective ligands, TNF-α and Fas Ligand (FasL) [12]. Death receptors can recruit adaptor proteins, also known as the Fas-Associated Death Domain protein (FADD) for CD95 and the TNFR Adaptor Domain (TRADD) for TNFR1. When FasL binds with Fas, its intramembrane domain interacts with FADD, promoting the formation of the Death Inducing Signalling Complex (DISC) [13]. This complex activates caspase-8, an initiator responsible for the consequent activation of a caspase cascade. In some cases, crosstalk can occur between extrinsic and intrinsic pathways, and caspase-8 can mediate the proteolysis of the BH3-only interacting domain agonist (BID) generating truncated BID (tBID). This protein can induce the release of cytochrome c once translocated to the mitochondria, thus activating the intrinsic apoptotic cascade [9].T cytotoxic lymphocytes (CTLs) and natural killer (NK) cells lead the perforin/granzyme pathway; after interaction with their target they release cargo granules, which contain deadly proteins such as perforin and granzyme [14,15]. The Microtubule-Organizing Centre (MTOC) determines the release of these citotoxic granules. The fusion of the granules with the membrane induces the release of perforin and granzymes towards the target. Perforin influences the size of the membrane pore, which then allows the granzymes to pass through the membrane. Granzymes act like caspases, cleaving different substrates, such as BID, caspase-3 and caspase-7, to induce apoptosis [12,14,15].

Defects occurring in any of the steps of these pathways can lead cells towards malignancy, tumor metastasis and drug resistance [1]. As apoptosis is typically disturbed in human cancer, therapeutic targeting of apoptosis represents a promising avenue for the development of novel anticancer therapies [16,17]. To this end, understanding apoptosis in cancer is crucial, both to gain insights into the pathogenesis of cancer and to provide clues on how to treat it [1].

Polyphenols are secondary plant metabolites, widely studied for their health-promoting properties which include antioxidant, anti-inflammatory, antimutagenic and anticancer activities. Many polyphenols possess the potential to regulate cell apoptosis and should represent ideal candidates for cancer treatment. Nevertheless, the unfavorable physico-chemical properties of most of polyphenols (i.e. low water solubility, poor stability at the acidic and alkaline conditions of the stomach and intestine, respectively, and low permeability rate), poor bioavailability, extensive pre-systemic metabolism, and quick excretion, dramatically reduce the use of these compounds as therapeutic agents in cancer treatment. In the last decade, many investigations have been made to overcome this limit through the development of new delivery systems [18].

Thus, the aim of this paper is to review literature data on the basic aspects and some of the molecular mechanisms through which polyphenols induce apoptosis of cancerous cells. Moreover, we also focused on the most recent clinical trials carried out on polyphenols.

Finally, to report an example of polyphenol characterized by poor bioavailability (i.e. curcumin) the most recent studies on new delivery systems leading to an improvement of its bioavailability were reported.

2. Methods

This study consists of an up-to-date review of literature addressing the *in vitro* apoptotic effect of plant extracts rich in polyphenols and polyphenolic substances, originating in food and herbal medicine and tested either as a pure compound or in combination with other polyphenols. Criteria for selecting the material were as follows: a search was conducted on the PubMed database [19] using the keywords “apoptosis AND polyphenols AND *in vitro*”. The results returned 79 papers from 2012 to 2017; of these, 32 papers pertinent to the topic were selected and critically summarized to provide a consistent review. The three main mechanisms for cell apoptosis, and biological effects, pharmacological effects, and phytochemistry of the selected plant extracts in their traditional usage are also discussed.

Moreover, a second search was conducted on the ClinicalTrials.gov database [20], using the keywords “polyphenols” and “cancer”; this returned 37 clinical trials. Of these 37 trials only 19 completed studies were selected; but 7 of them were discarded because were not considerate appropriate for this review [20].

Finally, considering that curcumin is one of the most studied and promising polyphenolic anti-cancer therapeutic agents and that its bioavailability is poor according to the “Nutraceutical Bioavailability Classification Schema” [18]. A third search was conducted on the PubMed database [19] using as keywords “curcumin AND new delivery systems AND cancer” from 2012 to 2017. The results returned 40 papers, among which 20 papers pertinent to the topic that were critically summarized to provide a consistent review.

3. Apoptosis induced by plant extracts rich in polyphenols

In recent years, many studies have evaluated the effects on apoptosis of extracts originating in plant-based foods and herbal medicine. These studies are summarized in Table 1.

Few investigations have focused on pomegranate, *Punica granatum* L. (family *Lythraceae*). Pomegranate is a food plant native to Asia that is now cultivated worldwide as a fruit tree. In recent decades, pomegranate fruit has raised increasing interest from the agrifood industry, which uses it to prepare a refreshing and healthy juice rich in a number of polyphenols, with tannins (i.e. hydrolysable and condensed tannins) and anthocyanins showing highest concentrations. This latter group is responsible for the red color of the juice, but also for health-promoting benefits ranging from *in vivo* antioxidant and inflammatory activities to anti-hyperglycemic effects, which make anthocyanins interesting substances for the prevention of diabetes and its most common complications such as diabetic retinopathy [21,22]. In 2012, Banerjee et al. investigated the potential activity of a pomegranate extract on apoptosis in BT-474 and MDA-MB-231 breast cancer cell lines. The treatment of both cell lines with different concentrations of pomegranate extract (2.5–25 μg/mL) induced apoptotic cell death through the activation of the caspase-3 pathway and the cleaved product of the substrate, Poly (ADP-ribose)-polymerase 1. Moreover, the pomegranate extract induced the suppression of both mRNA and proteins of Sp1, Sp3 and Sp4 genes involved in apoptosis. The extract also decreased Sp-regulated genes involved in cell proliferation, angiogenesis and inflammation (i.e. cyclin D1, BCL-2, survivin VEGF and its receptor (VEGFR-1) and NF-κB, as well as downregulating miR-27a and miRNA-155 in both cell lines) [23]. Chen and his research group have provided another important piece of evidence relating to the effects of pomegranate on apoptosis. In