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Andrographolide-Induced Autophagy: Pro-survival or Pro-death Signal

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Abstract
Andrographis paniculata is a traditional medicinal herb with a large number of chemical constituents. The herb is grown widely in areas of India, China and throughout Southeast Asia. The chemical constituents include mainly lactones, diterpenoids, diterpene glycosides, flavonoids and flavonoid glycosides. The major chemical constituents of the herb include the diterpene lactones including Andrographolide (Andro). The major bioactive compound in the extract of Andrographis paniculata is Andro and is principally responsible for its pharmacological activities. Autophagy is a degradation process responsible for the elimination of damaged proteins and organelles. Autophagy has dual roles in cell survival or death. Some studies have indicated the importance of autophagy in cellular adaptation for the internal and external stress and its role in cells protection while others reported that autophagy has an indispensable role in the induction of cell death. Recently, few studies have investigated the effect of Andro on autophagy. The results are contradictory with regard to the effect of Andro on induction or suppression of autophagy. Moreover, the results are also contradictory regarding the survival or death signal that Andro exerts on different cancer cells. Therefore, the aim of the present review paper is to investigate the effect of Andro on autophagy suppression or induction in different cellular settings and the pro-death or pro-survival signal that it exerts which may extend our understanding of new pharmacological agents to overcome relevant diseases.

Keywords: *Autophay; Andrographolide; Pro-survival; Pro-death; Andrographis paniculata*

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I. Introduction

Andrographis paniculata is a traditional well-known medicinal herb grown in different Southeast Asia areas and in particular in India and China. Due to the extreme bitter taste of the herb, it is known as "king of bitters". The Chinese and Indians have used the herb as part of the oriental medicine long time ago due to its therapeutic effects [1, 2]. The major chemical constituents of the herb include lactones, diterpenoids, diterpene glycosides, flavonoids and flavonoid glycosides [3].

The extracts of the whole plant have been reported to have different biological effects including anticancer, anti-inflammatory, anti-allergic, immunostimulatory, antithrombotic, antiviral, hypoglycemic, hepatoprotective, cardiovascular protective activities and hypotensive activities [4-6].

The diterpene lactones are the major chemical constituents of *Andrographis paniculata* including Andro, deoxyandrographolide, 11,12-didehydro-14-deoxyandrograp-holide, neoandro-grapholide, andrographiside, deoxyandrographiside and andropanoside [7]. The major bioactive compound in the extract of *Andrographis paniculata* is Andro and is principally responsible for its pharmacological activities [5].

The cell death can be classified morphologically into four different forms namely: 1) apoptosis, 2) autophagy, 3) necrosis & 4) entosis [8]. Autophagy is a degradation process responsible for the elimination of damaged proteins and organelles where it starts with autophagosome formation that swallow up cytoplasmic materials [9]. Different stressful conditions can induce autophagy including starvation, hypoxia, aging, and environmental toxins [10]. The molecular control of autophagy became more clear after the discovery of Atg genes that regulates autophagy in the yeast *Saccharomyces cerevisiae* [11].

The role of autophagy in cell survival and death is controversial. Some studies have indicated the importance of autophagy in cellular adaptation for the internal and external stress and its role in cells protection [12-14] while others reported that autophagy has an indispensable role in the induction of cell death [15-17].

Recently, few studies have investigated the effect of Andro on autophagy. The results are contradictory with regard to the effect of Andro on induction or suppression of autophagy. Moreover, the results are also contradictory regarding the survival or death signal that Andro exerts on different cancer cells [18, 19].

II. Cytotoxic and cytostatic Potential of Andrographolide

Andrographolide presents a strong candidature as a therapeutic anticancer Pharmacophore as it exhibits a dual property, acting both directly and indirectly on the cancer cells [20]. Different studies have reported the antiproliferative effect of Andro against different cancer cell lines that represent different types of cancers (KB, P388, HeLa, MDA-MB-231, HCT116, HT29, DU145) [2, 5, 21-23]. Moreover, Andro induces different cell death mechanisms including apoptosis and autophagy [18, 21].

The cytostatic effect of Andro was also shown by several studies. The studies have shown that Andro can induce cell cycle arrest at the different phases of the cell cycle [5, 21, 22].

III. Role of Andro in autophagy

The role of autophagy in cancer is rather complex. Andro was shown by different studies that it is able to induce autophagy [18, 21, 24], while others have shown that Andro suppresses autophagy [19, 25]. Suppression of autophagy has been increasingly recognized as a novel cancer therapeutic approach [19]. Furthermore, the results of Andro effects on autophagy was shown to be controversial in various *in vitro* or *in vivo* models. Some studies reported that Andro induced autophagy while others demonstrated that Andro inhibited autophagy by employing distinct molecular mechanisms [18, 19, 21].

Andro induced autophagic-cell death in some cell lines [18] while cell survival in others [21]. Liu et al. reported that Andro induce cell death in MG-63 and U-2OS cell lines through autophagy but not apoptosis [26]. In contrast, Alzaharna et al. have shown that Andro induced protective-autophagy and this encouraged survival in HeLa cells [21].

Andro-suppressed autophagy augments cells death

It was shown that Andro treatment has led to the suppression of autophagic flux at the maturation and degradation stage by impairing autophagosome-lysosome fusion in various cancer cell lines, namely HCT116, HeLa and MCF7 cells. Moreover, the induction of autophagy by cisplatin serves as a prosurvival mechanism and decreased cisplatin-induced apoptosis while the addition of Andro decreased autophagy and augmented the effect of cisplatin through a p53-independent process [19].

In another study Tan et al. investigated the modulatory effects of Andro on reactive oxygen species (ROS) and autophagy in human bronchial epithelial BEAS-2B cells after the exposure to cigarette smoke extract (CSE). The exposure of BEAS-2B cells to 2% of CSE increased the levels of autophagic markers p62 and LC3B-II. The treatment of BEAS-2B cells with Andro alone increased p62 and p-p62 (S349) but not LC3B-II. While, the treatment with Andro and CSE increased the LC3B-II level and decreased the oxidative stress by increasing the total antioxidant capacity, through upregulation of nuclear Nrf2 via the p62-Nrf2 positive feedback loop. The mechanism involve the impairment of autophagosome fusion with lysosome by Andro, which led to moderate increase in apoptosis indicated by the increase in cleaved caspase 3/7 and annexin V levels. [25].

Andro-induced autophagy augments cells death

Various studies have shown that Andro and Andro analogues can increase the death in different cell lines via the induction of autophagy. Chen et al. investigated the cytotoxic effect of Andro on human liver cancer cell lines (Huh-7, QGY-7703 and Bel-7402) and explored the cell death mechanism. The results indicated that Andro induced cell death distinct from apoptosis and through the induction of autophagy. The mechanism of cell death was further elucidated and found to be via the disruption of mitochondrial transmembrane potential (MMP) and elevation of ROS. The results further indicated that cyclophilin D plays an important role in mediating Andro-induced cytotoxicity [18].

In another study, Kumar et al. investigated the effect of the Andro analogue (AG–4) on the human leukemic U937 cells. They illustrated that AG-4 induced autophagy in U937 cells while the pre-treatment with 3-MA or autophagy 5 (Atg 5) siRNA suppressed the cytotoxic effect of AG–4 implying the pro-death role of autophagy. Furthermore, the results indicated that apoptosis and autophagy acted as partners in the context of AG–4 mediated action. The mechanism was shown to be via the inhibition of AG–4 to PI3K/Akt/mTOR pathway which plays an important role in AG–4 induced apoptosis and autophagy signifying its crucial role in its mechanism of action [27].

Furthermore, Hsieh et al. studied the effect of the Andro analogue dehydroandrographolide (DA) on oral cancer cell lines (SAS & OECM-1). The results showed that DA induced autophagic cell death that was inhibited by autophagy inhibitors. Additionally, the results show that DA induced autophagy and increased cell death by decreasing the p53 expression. DA-induced autophagy was triggered by an activation of JNK1/2 and an inhibition of Akt and p38. DA was also tested *in vivo* and where it suppressed the tumor formation in the oral carcinoma xenograft model effectively [28].

Moreover, Liu et al. reported the anticancer effect of Andro on osteosarcoma cells (MG-63 & U-2OS cell lines). The results indicate that Andro induced cell death was via autophagy and not apoptosis. Andro suppressed PI3K/Akt/mTOR and enhanced JNK signaling pathways. 3-MA and Beclin-1 siRNA could reverse the cytotoxic effects of AG. In addition, Andro was able to inhibit the invasion and metastasis of osteosarcoma, which could could be reversed with Beclin-1 siRNA [26].

Additionally, Zhang et al. investigated the radiosensitizing effects of Andro in human ovarian SKOV3 xenografts. The results indicated that Andro induced autophagy and apoptosis when combined with radiation on xenografts compared with Andro alone or radiation only treatment. The results indicated an increase in the Bax/Bcl-2 protein ratio and pp53 expression after exposure to combination treatment. Andro acts as a strong radiosensitizer in human ovarian SKOV3 xenografts *in vivo* [24].

Andro-induced autophagy as a prosurvival mechanism

Andro can also act as a prosurvival mechanism that protect the treated cells by reducing the induction of cell death mechanisms in various cell lines. It was reported that Andro induced a protective ROS-dependent autophagy. Andro-induced autophagy was via the activation of p53 where the use of pifithrin-α (PFT-α) or the flavonoid Taxifolin decreased it and enhanced the cells death. The results also show that While the activation of JNK was involved in the cell death of HeLa cells but not in the induction of autophagy. The results also showed that MOMP increase led to the increase in AIF and cytochrome c release from mitochondria which consequently increased caspase-dependent and independent cell death [21].

In another study, the results indicated that Andro induced early upstream autophagy in the breast cancer cell lines MDA-MB-231 and MCF7. The results showed that Andro-induced autophagy has a pro-survival role where the use of autophagy inhibitors increased the Ando-induced apoptosis. The induction of autophagy in MDA-MB-231 was shown to be via inhibition of mTORC1 signaling pathway. In contrast, the induction of autophagy in MCF7 developed through mTOR-independent mechanism. The results also showed that the induction of autophagy in MDA-MB-231 is Beclin1 independent. [29].

In an additional study, Du et al. reported that hypoxia induced autophagy while reduced cell apoptosis in Mouse cortical astrocytes C8-D1A. The upregulation of the proteins S-100B, JNK and ATG5 were involved in the induction of autophagy. Andro induced autophagy gave a survival advantage to hypoxia-injured astrocytes and reduced apoptosis [30].

Furthermore, Gu et al. results show that Andro activates autophagy and rescued the neuronal PC12 cells from death induced by Aβ1–42. The treatment also restored abnormal changes in nuclear morphology, lactate dehydrogenase, malondialdehyde, intracellular ROS and MMP. The results also revealed that mechanism involved Nrf2-mediated p62 signaling pathway [31].

In a recent study, Jiang et al. reported that Andro can induce autophagy and this caused a skin flap survival. The results showed that Andro induced autophagy by activating the PI3K/Akt signaling pathway. Moreover, it was found that Andro enhanced the viability of random skin flaps by enhancing angiogenesis, inhibiting apoptosis, and reducing oxidative stress. The results also revealed that Andro- upregulated angiogenesis was via stimulating the expression level of the VEGF, Cadherin5, and MMP9 [32].

Andro-induced autophagy attenuates inflammation

Different studies reported the anti-inflammatory effect of Andro-induced autophagy. It was reported by one study that Andro can trigger mitophagy in macrophages, leading to a reversed MMP collapse, which in turn inactivated the (NLR family, pyrin domain containing 3) NLRP3 inflammasome. The mechanism underlying the activity of Andro involved the inactivation of the NLRP3 inflammasome via induction of mitophagy and activation of PIK3CA-AKT1-MTOR-RPS6KB1 signaling pathway. They also found that Andro attenuated the NLRP3 inflammasome and recovered murine models from colitis and colitis-associated cancer [33].

In another study by Geng et al., they explored the antidepressant potential of Andro in chronic unpredictable mild stress (CUMS), a reliable model for modeling depression in rodents. They found that 5 mg/kg Andro treatment induce autophagy which led to an improved depressive-like behavior. They also reported the anti-inflammatory effect of Andro which involved the decrease in expression of different pro-inflammatory mediators and cytokines (NO, COX-2, iNOS, IL-1β, IL-6 and TNF-α) NF-κB signaling and NLRP3 inflammasome assembly in the prefrontal cortex [34].

IV. Conclusions

Autophagy plays a controversial role in cell survival and death. Some reported that autophagy plays a key role in cellular adaptation for the inside and outside stress and therefore considered as cytoprotective [12-14] while others reported the importance of autophagy in cell death [15-17]. Different studies have shown controversy effects of Andro on autophagy (Table 1). Some have shown that Andro suppresses autophagy and this led to augmentation of cell death and Andro was used in combination with conventional anticancer drugs

that induce autophagic survival mechanism where the inhibition of autophagy by Andro has increased cell death.

On the other hand, other studies have shown that Andro induced cell death distinct from apoptosis and through the induction of autophagy via different pathways [18]. Additionally, induction of autophagy by Andro was able to inhibit the invasion and metastasis of cancer [26]. Moreover, Andro was shown to induce a pro-survival mechanism in several studies and this leads to the inhibition of cell death where the inhibition of autophagy was able to augments the cell death. Recently, Andro was also shown to act as an anti-inflammatory agent via the induction of autophagy. This recovered murine models from colitis and colitis-associated cancer via attenuation of NLRP3 inflammasome assembly. Further research is needed for more understanding of the action of Andro in different cell lines and in vivo studies regarding the effect on autophagy and the role of autophagy in survival or death and the involved molecular mechanisms. This may further deepen the role of Andro and may lead to future use as a combined therapy for enhancement of cell death or protection of cells from death.

Table 1: Studies supporting the regulation of autophagy by Andro or Andro analogues.

References	Cell type /animal model used	Effect on cells	Effect on autophagy	Major findings
Zhou et al. (2012)	HCT116, HeLa and MCF7	Andro Induced cell death	Suppression of autophagic flux	Andro impaired the autophagosome-lysosome fusion. Andro decreased autophagy and augmented the effect of cisplatin through a p53-independent process.
Tan et al. (2018)	Bronchial epithelial BEAS-2B		Suppression of autophagic flux	Andro increased the antioxidant activity via the upregulation of the p62-Nrf2 loop and induction of apoptosis through impairment of autophagic flux in bronchial epithelium cells exposed to cigarette smoke extract.
Chen et al. (2012)	Human liver cancer cell lines (Huh-7, QGY-7703 and Bel-7402)		Induced autophagy	Andro induced cell death distinct from apoptosis and through the induction of autophagy via the elevation of ROS and disruption of MMP by cyclophilin D.
Kumar et al. (2015)	U937 cells	Andro analogues Induced cell death	Induced autophagy	Andro analogue analogue (AG–4) inhibited PI3K/Akt/mTOR pathway which led to the induction of apoptosis and autophagy.
Hsieh et al. (2015)	SAS and OECM-1 human oral cancer cell lines		Induced autophagy	The Andro analogue DA induced autophagy and decreased cell viability through the decrease of p53 expression. DA-induced autophagy was triggered by an activation of JNK1/2 and an inhibition of Akt and p38.
Liu et al. (2017)	Osteosarcoma cells; MG-63 & U-2OS cell lines	Induced cell death	Induced autophagy	Andro suppressed PI3K/Akt/mTOR and enhanced JNK signaling pathways.
Zhang et al. (2015)	Human ovarian SKOV3 xenografts		Induced autophagy	Andro acts as a strong radiosensitizer in human ovarian SKOV3 xenografts *in vivo* via increasing the Bax/Bcl-2 protein ratio and pp53 expression.
Alzaharna et al. (2017)	HeLa cells	Induced a prosurvival mechanism	Induced autophagy	p53 was involved in Andro-induced autophagy. JNK was involved in the cell death. MOMP increase led to the increase in AIF and cytochrome c release from mitochondria which consequently increased caspase-dependent and independent cell death
Alqouqa (2017)	Breast cancer cell lines MCF7 and MDA-MB-231		Induced autophagy	Induction of autophagy occurred via inhibition of mTORC1 and Beclin1 independent in MDA-MB-231. MCF7 developed through mTOR-independent mechanism accompanied by upregulation of p-ULK1 (S757), p-AKT (S473), and its downstream p-GSK3β(S9)
Du et al. (2018)	Mouse cortical astrocytes C8-D1A		Induced autophagy	Andro conferred a survival advantage to hypoxia-injured astrocytes by reducing cell apoptosis and promoting autophagy and s100b expression via activation of jnk pathway and regulation of ATG5
Gu et al. (2018)	Neuronal PC12 cells		Induced autophagy	Andro activates autophagy and rescued the neuronal PC12 cells from Aβ1–42-induced cell death. The mechanism involved Nrf2-mediated p62 signaling pathway
Jiang et al. (2021)	Skin flap		Induced autophagy	Andro enhanced the viability of random skin flaps by enhancing angiogenesis, inhibiting apoptosis, and reducing oxidative stress. Andro- upregulated angiogenesis was via stimulating the expression

				level of the VEGF, Cadherin5, and MMP9
Liu et al. (2018)	Human malignant melanoma A375 and C8161 cell lines	Attenuates inflammation	Induced autophagy	The mechanism underlying the activity of Andro involves PIK3CA-AKT1-MTOR-RPS6KB1 pathway mediated mitophagy-dependent inactivation of the NLRP3 inflammasome. Andro attenuated the NLRP3 inflammasome and recovered murine models from colitis and colitis-associated cancer
Geng et al. (2019)	C57BL/6 mice	Attenuates inflammation and stress	Induced autophagy	Anti-inflammatory effect of Andro where it decreased the expression of pro-inflammatory mediators and cytokines (NO, COX-2, iNOS, IL-1β, IL-6 and TNF-α), NF-κB signaling (p-p65, p-IκBα) and NLRP3 inflammasome assembly

AIF: Apoptosis-inducing factor; **ATG5:** autophagy related 5; **$A\beta_{1-42}$:** β-amyloid; **COX-2:** Cyclooxygenase 2; **IL-1β:** Interleukin 1 beta; **IL-6:** Interleukin 6; **iNOS:** Inducible nitric oxide synthase; **Jnk:** c-Jun N-terminal kinase; **MMP:** Mitochondrial transmembrane potential; **MMP9:** Matrix Metallopeptidase 9; **MOMP:** Mitochondrial outer membrane permeabilization; **mTOR:** Mammalian target of rapamycin; **NF-κB:** Nuclear factor Kappa B; **NLRP3:** NOD-, LRR- and pyrin domain-containing protein 3; **NO:** Nitric oxide; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **I3K:** Phosphoinositide 3 kinase; **p-IκBα:** phosphorylated inhibitor of nuclear factor kappa B; **ROS:** Reactive oxygen species; **TNF-α:** Tumor necrosis factor alpha; **VEGF:** Vascular endothelial growth factor.

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