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Opioids in Inducing Cell Apoptosis: A Mini Review

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Abstract— Opioids are opium-like drugs which are commonly used as analgesics to treat moderate to severe pain. Apoptosis is a type of programmed cell death to remove unnecessary or damaged cells in an organism. Recently, the ability of opioids to induce apoptosis especially in cancer cell lines has gained the interest of many researchers. This fascinating finding has led to more testing of different kinds of opioids against different kinds of cancer cell lines in the course to search for the potential anticancer drugs. This review provides current information about opioids and apoptosis, and more importantly the compilations of researches over the years on how opioids are related to apoptotic cells death.

Keywords: Apoptosis, heroin, methadone, morphine, opioids

1 INTRODUCTION

The term 'opioid' is often associated with 'analgesic' and 'drug of abuse'. The main purpose of opioids is to reduce moderate to severe pain [1, 2] especially in the situation where common painkiller is ineffective. Nowadays, with the emergence of numerous types of cancers with no right cure, treatment is mainly depending on surgical removal and chemotherapy. Therefore, researchers had started to find alternative approaches to kill cancer cells. One of the possible alternatives is by using opioids to treat cancer patients. Thus, this leads to opioids being tested against cancer cell lines to observe the possible effects. The purpose is to understand the cellular mechanism behind opioid induced apoptosis on cells especially cancer cells. Through the understanding of the mechanism of opioid induced apoptosis, opioids can either be the potential candidate to treat cancer or used as a model to create a new drug that targets the mechanism. This review will focus on three opioids namely morphine, heroin and methadone which are of natural, semi-synthetic and synthetic origins respectively. This review will also reveal the effects of apoptosis induction by these opioids besides providing a basic introduction to opioids and apoptosis.

1.1 Opioids: The Classical Analgesics

The term 'opioids' refers to a variety of substances or drugs that bind to the opioid receptors where the effect of the binding will lead

to numbed senses and reduction of pain [3, 4]. Opioids can be classified according to their origin, chemical structures, or their effects on opioid receptors [5].

There are three major types of opioid receptors which are mu (μ) opioid receptor (MOR), delta (δ) opioid receptor (DOR), and kappa (κ) opioid receptor (KOR) [6]. These three opioid receptors are G-protein coupled receptors [2].

The main use of opioid drugs is for the analgesic purpose [6]. When opioids enter the body, it will travel through the bloodstream to the brain and bind to opioid receptors. Opioid receptors are present in primary afferent neurons, spinal cord, midbrain, and thalamus. These regions are part of the nervous system that is involved in the transmission and control of pain [7].

Figure 1 showed the mechanism of action of opioids that contributes to the analgesic effect of opioids [8, 9].

Other pharmacological actions of opioids drugs include respiratory depression, constipation, euphoria, cough suppression, tolerance, and dependence [4]. Long term consumption of opioid drugs can cause physical dependence and withdrawal effects once the drug is discontinued.

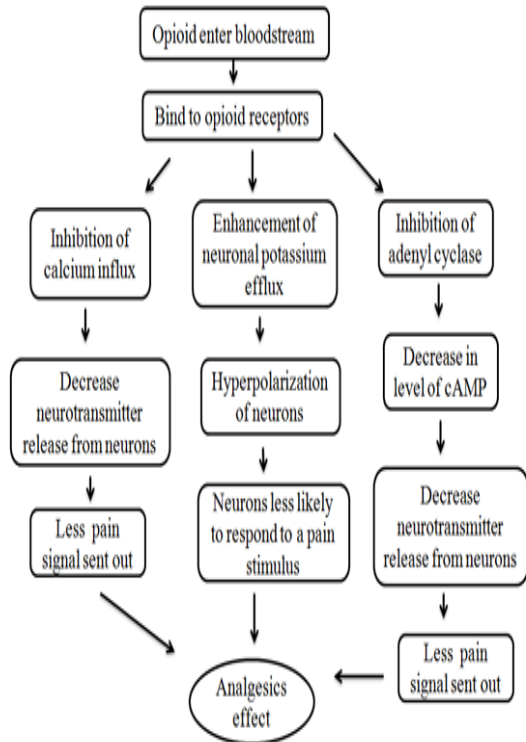


Figure 1: Mechanism of action of opioids that lead to analgesics effects.

1.2 Apoptosis: The Mechanism of Programmed Cell Death

The term apoptosis, also known as programmed cell death, is a genetically controlled process in an organism to eliminate excess or damaged cells [10, 11]. The principle of apoptosis was first described by a German scientist, Carl Vogt in 1842 [12].

Apoptosis is classified into three categories: Type I, Type II, and Type III [13]. Type I apoptosis is the classic, well-known apoptosis. However, there is less information found about Type II, and III apoptosis. Table I showed the characteristics features of Type I, II and III apoptosis.

Studies have revealed that the pathway of apoptosis involved the signaling interaction between various ligands in the cells. Two main apoptotic pathways had been illustrated by researchers: (i) the extrinsic or death receptor pathway, and (ii) the intrinsic or mitochondrial pathway. These two pathways will end up on the same end terminal, which is the execution pathway [14].

Table I: Characteristics features of Type I, II, and III apoptosis.

Types	Characteristics features	
Type I	Caspase dependent	Classic apoptosis features: • Chromatin condensation • Nuclear Fragmentation • Membrane blebbing • Formation of apoptotic bodies
Type II	Caspase independent	Autophagic and double membrane of vacuole
Type III		Absence of chromatin condensation

The extrinsic pathway involves the binding of death receptors to its ligands. When receptor bind to its ligand, cytoplasmic proteins such as Fas-associated death domain protein (FADD) and tumor necrosis factor receptor type 1-associated death domain protein (TRADD) are recruited, and these proteins will associate with procaspase-8, thus leading to the formation of death inducing signaling complex (DISC). Formation of DISC will result in the autocatalytic activation of procaspase 8 to caspase 8 [14, 15]. Activation of caspase 8 will trigger the execution pathway.

The intrinsic pathway, which is also known as mitochondrial pathway, does not involve the binding of receptor and its ligand. Stimuli such as the absence of certain growth factors, hormones, and cytokines to suppress apoptosis, radiation, hypoxia, viral infections, or free radicals are needed to initiate the intrinsic pathway. Two groups of apoptosis-promoting proteins will be released. The first group of proteins released are cytochrome c, second mitochondrial activator of caspases/direct IAP binding protein with low PI (Smac/DIABLO), and serine protease Omi protein A2 (HtrA2/Omi) [14, 16-18]. The interaction between cytochrome c, Apaf 1, and procaspase 9 lead to the activation of caspase 9 whereas Smac/DIABLO and HtrA2/Omi inhibit inhibitors of apoptosis proteins (IAP) activity [14, 17, 19]. On the other hand, the release of the second group of proteins during apoptosis which consists of apoptosis-inducing factor (AIF), endonuclease G, and caspase-activated DNase (CAD) will result in the fragmentation of DNA and condensation of nuclear chromatin [14, 20-22].

Figure 2 showed the extrinsic and intrinsic pathways in apoptosis [14, 23].

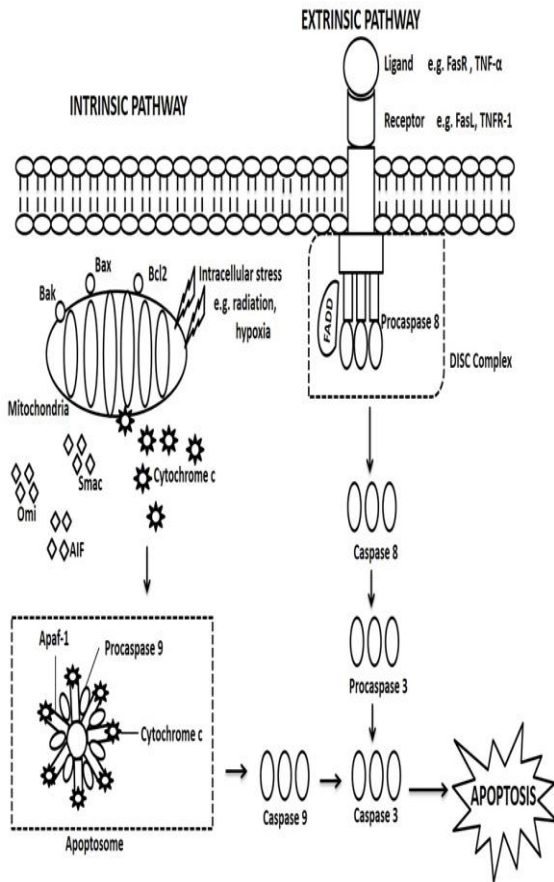


Figure 2: Extrinsic and intrinsic pathway in apoptosis (Adapted from Beesoo R. *et al.*, 2014).

Table II showed the comparison between the extrinsic and intrinsic pathway of apoptosis. Replacement of old or damaged cells with new cells is one of the functions of apoptosis. Nevertheless, dysregulated apoptosis which is either too little or too much of apoptosis can be problematic. If the occurrence of apoptosis is too low, it can cause the survival of abnormal cells to be prolonged which may lead to cancers and autoimmune disease. Meanwhile, too much or uncontrolled apoptosis will result in loss of normal cells which may give rise to ischemic injury and neurodegenerative disease [10, 14].

Apoptosis induces various changes in the morphology of cells. Cell shrinkage, intact cell membrane, blebbing, and retained cytoplasm in apoptotic bodies are some of the features that can be observed when cells are undergoing apoptosis [14, 27, 28]. Apoptosis of cell does not cause inflammation as the contents of the cells are well enclosed in the apoptotic bodies. Necrosis is another form of cell death besides

apoptosis. Morphologically, when cells die through necrosis, the cells gain in volume, organelles will swell, the plasma membrane is ruptured, and consequently, the intracellular contents will be lost [29]. Table III showed the comparison between apoptosis and necrosis.

Table II: Similarities and differences between extrinsic and intrinsic pathway(14, 24-26).

	Extrinsic Pathway	Intrinsic Pathway
Involved	<ul style="list-style-type: none"> Transmembrane receptor-mediated interactions 	<ul style="list-style-type: none"> Intracellular signals by non-receptor mediated stimuli Mitochondrial initiated events
Sources of stimuli that trigger apoptosis	<ul style="list-style-type: none"> External Binding of death receptor to ligand, e.g. FasL/FasR, TNF- α /TNFR1, Apo3L/DR3	<ul style="list-style-type: none"> Internal DNA damage, cytokine deprivation, endoplasmic reticulum (ER) stress, physical and chemical injuries (ultraviolet radiation, heat shock, hypoxia)
Complex formed	<ul style="list-style-type: none"> Death Inducing signaling complex (DISC) death receptor + FADD + procaspase 8	<ul style="list-style-type: none"> Apoptosome Apaf 1 + cytochrome c + procaspase 9
Initiator caspases	Caspase 8, 10	Caspase 9
Involvement of Bcl-2 proteins	No	Yes
Inhibitor protein	Cellular FLICE (FADD-like IL-1 β -converting enzyme)-inhibitory protein (c-FLIP)	Inhibitor of apoptosis protein (IAP)
1. Both pathway involved activation of caspases in initiating apoptosis 2. Both pathway end with the same end terminal which is the execution pathway where caspase 3 and 7 were activated.		

Table III: Differences between apoptosis and necrosis.

	Apoptosis	Necrosis
Cell	Shrink	Swell
Cell membrane	Remain intact	Disrupted
Cytoplasm	Retained in apoptotic bodies	Cytoplasm released
Inflammation	Absent	Present
Caspase activation	Present	Absent

2. The Opioids: Morphine, Heroin, Methadone

2.1 Morphine

Morphine was discovered and isolated from the opium poppy plant by a German pharmacist known as Friedrich Serturmer in 1804 [30-32]. Morphine makes up for 10-17% of the latex collected from the opium poppy plant [33]. Some other constituents that can be found in the latex are codeine (0.7 - 4 %), thebaine (0.5 – 2%), and some agents that do not interact with opioid receptors such as papaverine (0.5 – 1%) and noscapine (2 – 9 %) [33]. Morphine was named as morphium, which is after Morpheus, the Greek god of dreams. Morphine with the molecular formula of $C_{17}H_{19}NO_3$ has the molecular weight of 285.34 g/mol. The common street names for morphine are Dreamer, Emsel, First Line, Mister Blue, Morf, God's Drug, Morpho, and Unkie. Morphine can be prescribed in the form of oral solutions, immediate and sustained release tablets and capsules, suppositories, and injectable preparations [3]. Morphine can bind with either μ , δ or κ opioid receptor but among those three opioid receptors, it has highest affinity towards μ opioid receptor [34, 35]. Morphine is used to relieve moderate to severe pain [36]. Morphine has a half-life of 4 - 6 hours, and it is metabolized mostly in the liver [37]. Biotransformation of morphine is mainly by hepatic glucuronidation into the biologically active, morphine-6-glucuronide (M6G) and the inactive form, morphine-3-glucuronide (M3G) [36-38]. Mostly, morphine and its metabolite are excreted by the kidney through urine. It is listed in the Schedule II drugs by the Controlled Substances Act (CSA).

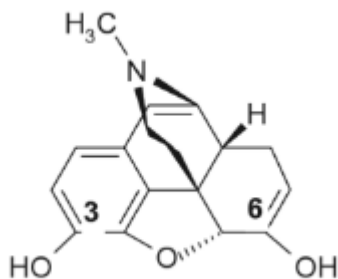


Figure 3: Structural formula of morphine (Adapted from Human Drug Metabolism: An Introduction by Michael D.Coleman).

Studies on Effect of Morphine in Inducing Apoptosis in cancer cell lines

A study had been conducted using morphine and methadone to test against two types of human lung cancer cells: non-SCLC NCI-H157 and SCLC NCI-N417 cell lines. One μ M of either morphine or methadone alone was able to cause morphological changes that matches with the characteristics of apoptotic cells [39]. The apoptotic effect induced by methadone and morphine on human lung cancer cells was further shown through the formation of characteristic DNA ladder pattern of the extracted DNA from the treated cells on an agarose gel stained with ethidium bromide. The findings of this study led to the conclusion that opioids induce apoptosis in human lung cancer cells [39].

Morphine promoted apoptosis in Jurkat cells [40]. The apoptosis induced by morphine is Fas-mediated [41, 42]. Transfection of Jurkat cells was done with pcDNA3-GFP or pcDNA3-GFP/ dnFADD expression vectors to check whether FADD is needed in morphine induced apoptosis. Fas-associated death domain (FADD) is an adapter protein that is recruited when death receptor bound to its ligand and associated with procaspase 8 to form death inducing signalling complex (DISC) which will eventually lead to activation of caspase 8. The dnFADD is the dominant –negative form of FADD where it was unable to activate caspase 8 but able to interact with other death domain containing protein [43]. Flow cytometry was carried out to identify the apoptotic cells. The result showed that pcDNA3-GFP transfected Jurkat cells had more apoptotic cells when treated with morphine concentration of 10 μ M (14%) and 30 μ M (25%) compared to dnFADD transfected Jurkat cells treated with morphine showed only 4% of apoptotic cells in the sample at respective concentrations. The result obtained from this study lead to the conclusion that apoptosis induced by morphine is dependent on FADD [41]. Other findings in the same study showed that p53 pro-apoptotic protein is involved in the morphine induced cell apoptosis. RNAi knockdown of p53 in Jurkat cells followed by morphine treatment had a lower number of cells that undergo apoptosis compared to the normal Jurkat cells treated with morphine. Another finding obtained in this study showed that Jurkat cells treated with P13K inhibitor, LY294002 followed by treatment with morphine resulted in

an increase in apoptotic cells compared to Jurkat cells treated with morphine alone suggesting that inactivation of P13K promotes morphine induced apoptosis.

Studies on Effect of Morphine in Inducing Apoptosis in non-cancer cell lines

Morphine induced apoptosis of human endothelial cells through nitric oxide and reactive oxygen species (ROS) pathway [35]. The purpose of the study done by Hsiao *et al.*, (2009) was to investigate the effects of morphine on vascular endothelial cells and the possible mechanism involved. Human umbilical vein endothelial cells (HUVECs) were used in this study. The result showed that HUVECs with morphine treatment showed apoptosis in the manner of dose and time dependent [35]. Intracellular ROS was generated, and there was a reduction of mitochondrial membrane potential after HUVECs was treated with morphine. The nitric oxide contents increased significantly in morphine treated HUVECs compared to the PBS treated HUVECs. Through western blot analysis, the expression of pro-apoptotic proteins such as Bak and Bax were overexpressed with the increase in the concentration of morphine used.

Chen *et al.* (2008) studied the effect of morphine on neurons of rat [44]. The study showed that 5 days morphine treatment of primary rat neurons at a concentration of 1 μ M decreased the Hsp 70 expression in total and activated Bax levels [44]. The result of this study suggested that there is involvement of opioid receptors in the effects of morphine treatment on neurons of rat, which can be blocked by treatment with naloxone, the opioid antagonist.

2.2 Heroin

Heroin is available as a white or brown powder or black sticky substance known as "black tar heroin" [45]. Heroin with a molecular formula of $C_{21}H_{23}NO_5$ has a molecular weight of 369.411 g/mol. According to Dr. Daniel Ciccarone from University of California, San Francisco, who conducted research on heroin, the origin of heroin can be traced based on the colour of heroin powder. Heroin from Colombia, Pakistan and Afghanistan is in the form of brown powder; white powder heroin comes from Southeast Asia while "black tar" heroin comes from Mexico [46]. It is derived from morphine, which is extracted from

the seedpod of the opium poppy plant. Heroin which has the chemical name of diacetylmorphine is either known as Black Tar, Horse, Negra, Smack, Thunder, Hell Dust, or Big H on the street. People normally took heroin either through injection, smoking, or sniffing. It has a half-life of around 5 – 7 minutes [47]. The presence of two acetyl groups in heroin makes it much more lipophilic than morphine. Therefore, it travels faster to the central nervous system [47, 48]. Heroin can be hydrolysed by esterases of hepatic, plasma, and central nervous system origin. Examples of esterases involved in the hydrolysis of heroin are butyrylcholinesterase (BChE), humancarboxylesterase I (hCEI), and acetylcholinesterases where plasma butyrylcholinesterase act as the primary enzyme [5, 47]. The products obtained from hydrolysis of heroin are 6-monoacetylmorphine (6-MAM), which are the active metabolites and its inactive one, 3-MAM [5]. Heroin is the most rapid acting drug among opiates, and it is also highly addictive [3]. It is listed in the Schedule I drugs by the Controlled Substances Act (CSA).

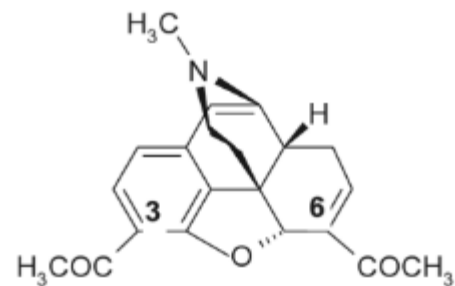


Figure 4: Structural formula of heroin (Adapted from Human Drug Metabolism: An Introduction by Michael D.Coleman).

Studies on Effect of Heroin in Inducing Apoptosis in non-cancer cell lines

Heroin was found to induce apoptosis of cerebellar granule cells (CGCs) whereby these cells made up of more than 90% of the cerebellum neurons [49, 50]. The studies by Tan *et al.*, (2003) showed that heroin induced apoptosis of CGCs. The mature neurons were treated with 10 μ g/ml of heroin for various time frames (12, 24 and 48 hours). The results reflected that the longer the treatment period with heroin, the higher the percentage of apoptosis of CGCs. The study also showed that Bim, a proapoptotic protein was upregulated in CGCs

when treated with heroin and shRNA knockdown of Bim resulted in the significant prevention of heroin induced apoptosis. Meanwhile, the pathway that was activated in the heroin induced apoptosis of CGCs is the c-Jun N-terminal kinase JNK/ c-Jun pathway [50].

2.3 Methadone

Methadone, also known as Dolophine, Methadose, and many other names, is a synthetic opioid that exists more than 70 years. The first synthesis of methadone was in 1939 at the pharmaceutical laboratories of the I.G. Farbenkonzern, a subsidiary of the Farbwerke Hoechst, Frankfurt am Main, Germany [51]. It is a long acting μ opioid receptor agonist [52]. Methadone which is available as a hydrochloride salt on the market with a pH range of 3-6.5 in a solution [53, 54] is the racemic mixture of two stereoisomers which are L-methadone and D-methadone [55, 56]. The molecular weight of methadone ($C_{21}H_{27}NO$) is 309.45 g/mol while the molecular weight of methadone hydrochloride ($C_{21}H_{28}ClNO$) is 345.91 g/mol. Methadone is metabolized by the liver [55, 57]. Kidneys are the main organ that eliminates methadone and its metabolites [55]. Methadone hydrochloride is used for maintenance treatment of heroin addicts [58]. In addition, it is also used as analgesics in the management of cancer pain [59]. It is listed in the Schedule II drugs by the Controlled Substances Act (CSA).

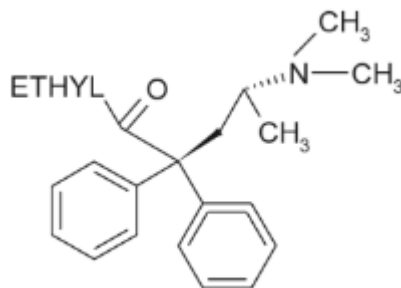


Figure 5: Structural formula of methadone (Adapted from Human Drug Metabolism: An Introduction by Michael D.Coleman).

Studies on Effect of Methadone in Inducing Apoptosis in cancer cell lines

A study done by Friesen *et al.* (2008) showed that D, L-methadone hydrochloride killed leukemia cells through apoptosis. Besides that, this drug

which is commonly used in the maintenance treatment for opioid addiction is also shown to be able to induce death in doxorubicin resistance, multi-drug resistance, and apoptosis resistance leukemia cells [60]. Interestingly, peripheral blood lymphocytes which were isolated from the blood of healthy person were not killed after treatment with D, L-methadone hydrochloride. In the same study, two leukemia cell lines which were human lymphoblastic T cell line CCRF-CEM and human myeloid leukemia cell line HL-60 were incubated with several concentrations of D, L-methadone hydrochloride for 24 and 48 hours before apoptosis was quantified by using flow cytometry. With the methadone concentration of 10, 15, 20, and 30 $\mu\text{mol/l}$, the percentage of specific apoptosis for both cell lines increases with the increase of the concentration of drug [60]. Western blot was used to determine the role of caspases in methadone induced apoptosis. The result obtained showed that there were cleavages of caspase 3 and PARP in both leukemia cell lines used. Furthermore, the importance of the role of caspases was proven when methadone induced apoptosis was almost completely inhibited when CCRF-CEM and HL-60 leukemia cells were pre-incubated with the broad-spectrum inhibitor of caspases, zVAD.fmk. In addition, strong cleavage of caspase 9 and a strong down regulation of XIAP and Bcl-XL were detected in CCRF-CEM and HL-60 leukemia cells.

In a study done by Perez-Alvarez *et al.* (2010) on the cytotoxic effects of methadone on human neuroblastoma cell line SH-SY5Y, a high concentration of methadone was required to induce cell death of SH-SY5Y cells where 50% of cell death ≤ 0.5 mM at 24 hours incubation with methadone. A concentration time-dependent of cell death was observed. Results of this study showed that methadone induced necrotic-like cell death in SH-SY5Y cells rather than typical apoptosis and the cell death was induced through an impairment of mitochondrial ATP synthesis [61].

Another study carried out by Friesen *et al.* (2013) showed that D, L-methadone induces cell death in xenograft derived acute lymphoblastic leukemia (ALL) cells depending on opioid receptor expression. Two types of cells were used in their study; cells that have a high amount of opioid receptor and another one was cells with moderate number of opioid receptor. The cells were treated with different concentrations of D, L-

methadone. The result indicated that there was a strong cell death induction in cells with a high amount of opioid receptor while cells with moderate amount of opioid receptor could only be slightly killed when treated with D, L-methadone [62]. In addition, the study found out that treatment with D, L-methadone enhances doxorubicin-uptake and inhibits doxorubicin-efflux. Besides that, another finding from the study showed that induction of apoptosis by D, L-methadone and doxorubicin depends on opioid receptor activation inducing cAMP downregulation. The study also showed that opioid receptor activation using D, L-methadone alone or combination with doxorubicin inhibits the growth of tumor significantly *in vivo* (62).

Following the success in showing D, L-methadone's ability to kill chemo-resistance and radio-resistance leukemia cells and being able to sensitize leukemia cells for doxorubicin treatment, Friesen and her colleagues decided to go further by testing the drug on glioblastoma cells that are unyielding to anti-cancer drugs or radiation. Glioblastoma is a type of malignant cancer arises from astrocytes, the star-shaped cells in the brain. Two types of glioblastoma cell lines were used in this research: A172 and U118MG. The origin of U118MG cell is from a patient with grade IV astrocytoma while A172 cell is from a patient with glioblastoma. These two cell lines were treated with different methadone concentration (0, 1, 3, 10 µg/mL) without, or with fixed concentration of doxorubicin for 120 and 144 hours. The percentage of specific apoptosis was measured by hypodiploid DNA analysis. The result showed combination treatment of methadone and doxorubicin had greatly enhanced the apoptosis of glioblastoma compared to the sole treatment of methadone or doxorubicin [63]. The percentage of specific apoptosis of glioblastoma cells increase with the increase in the concentration of methadone used. This study concluded that co-treatment with methadone had enhanced doxorubicin induced apoptosis in glioblastoma cells [63].

3 CONCLUSION

In summary, opioids induced apoptosis especially in cancer cell lines is an important and interesting finding in the search for the potential anticancer drug. Molecular mechanism of action behind the opioids induced apoptosis is not yet fully

understood. Each opioid may have a different mechanism of action towards certain cancer cells in inducing apoptosis. Therefore, more research should be done to understand the mechanism of action in inducing apoptosis and why certain opioid only induces apoptosis in certain cell lines. This review provides information on the studies that had been carried out on the ability of opioids to induce apoptosis in certain cancer and normal cell lines. But the actual mechanism leading to opioid induced apoptosis and how different opioids affect different cell lines in different manners are still vague.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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