EFFECT OF RADIOTHERAPY COMBINED WITH CAPSAICIN AND FRAXETIN AS ENHANCED THERAPY ON COLORECTAL CANCER CELL LINE IN VITRO

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by

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"In the name of Allah, the Most Gracious, the Most Merciful"

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LIST OF SYMBOLS

MV	Mega electron volt
Gy	Gray
eV	Electron volt
H^+	hydrogen ions
OH^-	hydroxide ions
℃	Degree Celsius
µl	Microliter
µm	Micrometer (1×10^{-6} m)
µm	Microgram
µl	Micromile
g	gram
T	Time
CO_2	Carbon Dioxide

LIST OF ABBREVIATIONS

AKI	Acute kidney injury
CDDP	Cisplatin
CKI	Chronic kidney injury
CRC	Colorectal cancer
CTV	Clinical target volume
EBRT	External beam radiotherapy
ELF	Extremely low frequency
EMR	Electromagnetic radiation
EMS	Electromagnetic spectrum
ER	Endoplasmic reticulum
FXT	Fraxetin
GTV	Gross tumor volume
IGRT	Image-guided radiotherapy
LINAC	Linear accelerator
MAPK	Mitogen-activated protein kinase
NSCL	Non-small cell lung cancer
OAR	Organs at risk
PCD	Programmable cell death
PRV	Planning risk volumes
PTV	Planning target volume
PUFAs	Polyunsaturated fatty acids
Ros	Radiation oncologists
ROS	Reactive oxygen species

RT	Radiotherapy
RTs	Radiation therapists
SGNs	Spiral ganglion neurons
SSD	Source surface area
TPS	Treatment planning system
UV	Ultraviolet
Vis	Ultraviolet-Visible

KESAN TERAPI SINARAN YANG DIGABUNGKAN DENGAN KAPSAISIN DAN FRAXETIN SEBAGAI TERAPI DIPERTINGKAT TERHADAP GARIS SEL KANSER KOLOREKTUM SECARA IN VITRO

ABSTRAK

Kanser kolorektal adalah kanser kedua paling biasa pada wanita dan ketiga pada lelaki, menyumbang 9.7% daripada semua kejadian kanser di seluruh dunia, dengan kira-kira 814,000 kes pada lelaki dan 664,000 kes pada wanita. Kanser kolorektal adalah penyakit penuaan di mana lebih daripada 90% berlaku pada individu yang berumur sekurang-kurangnya 50 tahun. Sebagai contoh, kejadian adalah 50 kali lebih besar pada umur enam puluh hingga lapan puluh tahun berbanding umur empat puluh tahun atau lebih muda. Dari tahun 1997 hingga 2004, insiden keseluruhan dan kematian akibat kanser kolorektal masing-masing menurun sebanyak 2.6% dan 4.7%. Kemoterapi adjuvant dan radioterapi kekal sebagai asas dalam pengurusan kanser kolorektal, dari segi kemandirian bebas penyakit dan manfaat kemandirian keseluruhan. Dalam kajian kami, ia bertujuan untuk mengkaji kesan Capsaicin dan Fraxetin, bahan aktif lada cili dan Fraxinus, yang telah mengetahui aktiviti antikanser, dan cisplatin sebagai kemoterapi sahaja atau gabungan dengan radioterapi, pada garis sel kanser kolorektal dan manusia semula jadi. fibroblas kulit (HDFn). Fitokimia yang boleh bertindak sebagai antioksidan telah digunakan oleh orang dalam rawatan terhadap kanser sepanjang sejarah manusia kerana ketoksikannya yang rendah dan mudah didapati. Selain memberi perlindungan terhadap kanser, ia juga didapati membunuh sel-sel kanser. Garisan sel telah ditanam dalam kultur dalam kelalang kultur konvensional dalam medium DMEM pada 37 ° C dan 5% CO_2. Apabila sel-sel adalah 70-80% konfluen,

perubahan morfologi diperiksa di bawah mikroskop terbalik. Sel-sel telah disalurkan ke dalam 96 plat mikro, dan selepas laluan, dos radioterapi yang berbeza (1, 3 dan 5 Gy), capsaicin dan Fraxetin (100 μM/ml) dan cisplatin 20 μg/mL telah digunakan pada sel. Selepas pentadbiran, kesan sitotoksik dan kadar percambahan/percambahan sel dianalisis dengan kaedah MTT. Untuk berbuat demikian, sel CaCo-2 dan HDFn telah dirawat dengan gabungan radioterapi + capsaicin, + Fraxetin, atau + cisplatin. Tambahan pula, sel telah dirawat terlebih dahulu dengan capsaicin, Fraxetin dan dan cisplatin sahaja dan terdedah kepada pelbagai dos terapi sinaran sahaja untuk tempoh 48 jam dan 24 jam. Kesan capsaicin dan Fraxetin bersama-sama dengan penyinaran pada pertumbuhan sel, pengedaran kitaran sel, apoptosis, telah diperiksa. Rawatan penyinaran gabungan dengan capsaicin dan Fraxetin telah mengurangkan aktiviti pertumbuhan dengan ketara berbanding dengan rawatan penyinaran sahaja rawatan sahaja. Peratusan kadar apoptosis sel CaCo-2 dan HDFn meningkat apabila sel dirawat dengan penyinaran dalam kombinasi dengan cisplatin berbanding dengan radioterapi sahaja, tidak seperti cisplatin sahaja. Data kami menunjukkan bahawa capsaicin dan Fraxetin meningkatkan radiosensitiviti dalam sel-sel kanser kolon, dan kesan gabungan capsaicin dan Fraxetin dengan radiasi menunjukkan kesan selular yang lebih besar daripada rawatan tunggal, menunjukkan bahawa gabungan capsaicin dan Fraxetin dan radiasi berpotensi untuk menjadi strategi klinikal untuk rawatan kanser.

EFFECT OF RADIOTHERAPY COMBINED WITH CAPSAICIN AND FRAXETIN AS ENHANCED THERAPY ON COLORECTAL CANCER CELL LINE IN VITRO

ABSTRACT

Colorectal cancer is the second most common cancer in women and third in men, accounting for 9.7 % of all cancers incidence globally, with about 814,000 cases in men and 664,000 cases in women. Colorectal cancer is a disease of ageing where more than 90% occurs in individuals at least 50 years old. For instance, the incidence is 50 times greater in sixty to eighty year olds than in forty year olds or younger. From 1997 to 2004, overall incidence and death due to colorectal cancer decreased by 2.6% and 4.7% respectively. Adjuvant chemotherapy and radiotherapy remain cornerstones in the management of colorectal cancer, in terms of disease-free survival and overall survival benefit. In our study, it was aimed to examine the effects of Capsaicin and Fraxetin, the active ingredients of chili peppers and Fraxinus, which has known anticancer activities, and cisplatin as chemotherapy alone or combination with radiotherapy, on the colorectal cancer cells line and natural human dermal fibroblasts (HDFn). Phytochemicals that can act as antioxidants have been used by people in treatment against cancer throughout human history because of their low toxicity and the ease in availability. Besides providing protection against cancer, they are also found to kill cancer cells. Cells lines were grown in culture in conventional culture flasks in DMEM medium at 37 °C and 5% CO_2. When the cells were 70-80% confluent, morphological changes were examined under an inverted microscope. The cells were passaged into 96 microplates, and after passage, different doses of radiotherapy (1, 3 and 5 Gy), capsaicin and Fraxetin (100 μM/ml) and

cisplatin 20 µg/mL were applied to the cells. After administration, cytotoxic effect and proliferation rates/cell proliferation were analyzed by the MTT method. To do so, CaCo-2 and HDFn cells were treated with combination of radiotherapy + capsaicin, + Fraxetin, or + cisplatin. Furthermore, cells were pretreated with capsaicin, Fraxetin and and cisplatin alone and exposed to various doses of radiation therapy alone, for period of 48 h and 24 h. The effects of capsaicin and Fraxetin along with irradiation on cell growth, cell cycle distribution, apoptosis, were examined. Combination irradiation treatment with capsaicin and Fraxetin significantly decreased growth activity compared with irradiation treatment alone. The percentage of CaCo-2 and HDFn cells apoptotic rate was increased when cells were treated with irradiation in combination with cisplatin compared with radiotherapy alone, unlike cisplatin alone. Data demonstrated that capsaicin and Fraxetin enhanced radiosensitivity in colon cancer cells, and the combination effects of capsaicin and Fraxetin with radiation showed greater cellular effects than that of single treatment, suggesting that the combination of capsaicin and Fraxetin and radiation has the potential to become a clinical strategy for the treatment of cancer.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

According to statistics gathered by the International Agency for Research on Cancer [accessible via the Global Cancer Observatory portal] (McGuire, 2016). Colorectal cancer (CRC) is the third most mutual cancer worldwide in terms of incidence, accounting for 10.2% of the approximately 18.1 million new cancer cases (both sexes, all ages) that were registered in 2018. CRC is the second leading cause of death after lung cancer, accounting for 9.2% of the estimated ~9.5 million deaths each year. The first-line therapy for CRC patients is presently based on a multimodal strategy that permits patients to be classified into risk categories. This is done by taking into account tumor-related features such as the existence of metastases (number and location), stage of tumor growth, probable biochemical indicators, and so on, as well as patient-related aspects such as co-morbidity and prognosis. (Mármol et al., 2017). Depending on the risk group, several therapy techniques may be used. Radiotherapy was first used in CRC treatment as a means of dealing with relapses or oligometastatic states, and it has now become an integral component of postoperative care. The application to colon cancer has limitations: the colon is movable, making the target difficult to pinpoint, and surrounded by dose-limiting structures (small bowel, kidney, and liver). The anatomical structure of the rectum, and the fact that it is placed underneath organs that have a limited tolerance to radiation, better supports the use of radiotherapy for rectal cancer (Agranovich & Berthelet, 2000).

In general, CRC patients may be treated with radiation and chemotherapy before to surgery (neo-adjuvant treatment) or after surgery (adjuvant therapy). There are several complementary techniques for neo-adjuvant therapies, including short-course radiotherapy with a 5 × 5 Gy scheme and long-course radiotherapy with normofractionated irradiation for a total dosage between 45 and 50.4 Gy, with concurrent chemotherapy (Häfner & Debus, 2016).

Chemotherapy is still the most significant adjuvant treatment for colon cancer, although high-risk patients with rectal cancer are also receiving postoperative radiation. Finally, radiation may be utilized to relieve symptoms, especially in the case of colon cancer, whether caused by original lesions or distant metastases. (Agranovich & Berthelet, 2000).

In general, radiotherapeutic concepts are recognized as essential for the primary management of locally advanced rectal cancer (Häfner & Debus, 2016). Despite breakthroughs in treatment options, patients' survival rates remain low, necessitating more study into medication resistance. (Peters, 2020), explore new treatments [including immunotherapy applications] (Golshani & Zhang, 2020), Furthermore, innovative therapy combinations should be developed to capitalize on potential synergy. In this context, preclinical research considerably benefits from the availability of well-characterized in vitro cell and/or tissue systems, which allow for the investigation of the processes underlying the treatment response under controlled laboratory circumstances.

However, current CRC therapy options are complicated and accompanied with hazardous side effects. As a result, researchers are looking for innovative medication options that are low in toxicity to normal/noncancerous cells.

Due to their unique structural composition, wide range of chemical characteristics, and low toxicity, Secondary metabolites found in medicinal plants are regarded an appealing target for screening of novel drug candidates against the dreadful illnesses such as cancer (Zaman et al., 2015).

Capsaicin is a significant component of chili peppers that gives them a spicy flavor. Numerous in vitro studies have revealed the anti-cancer effects of capsaicin in several cancer models (Ip et al., 2012a; Ito et al., 2004a; Lee et al., 2015), Although various research claimed that capsaicin had possible cancer-promoting properties (Agrawal et al., 1986; Hwang et al., 2010). Our study previously established that capsaicin caused apoptosis and inhibited cell proliferation via NSAID-activated gene-1 (NAG-1) and β-catenin/TCF4-mediated pathways in human colon cancer cells (Lee et al., 2010a, 2012). Recently, we evaluated the anti-cancer mechanisms by which capsaicin led to apoptosis and growth arrest while inhibiting metastasis and angiogenesis (Clark & Lee, 2016). Cao S et al. evaluated the anticancer mechanisms of capsaicin based on the various cancer kinds (Cao et al., 2015). However, most of published searches have used in vitro cell culture methods, and there has been no in vivo trial to examine the effectiveness of capsaicin in a colon cancer model.

Fraxetin is a plant-derived coumarin reported to have antibacterial, neuroprotective, anti-inflammatory, and anti-dysenteric properties. It is primarily isolated from Fraxinus bungeana A.DC (Zhao et al., 2020). Recently, several studies have demonstrated that fraxetin suppresses tumor growth and metastasis in multiple cancer types (Liu et al., 2017a; Sánchez-Reus et al., 2005). Although studies have reported its anticancer properties in lung and breast cancer, little is known about colon cancer, the most frequent type of cancer (Lee et al., 2022a).

The human cell line Caco-2 derived from a colon adenocarcinoma. Caco-2 cells have been extensively adopted as a model for the intestinal epithelial barrier, due to their capacity to differentiate and generate a functional polarized monolayer when cultivated on a porous membrane (Verhoeckx et al., 2015a). Many studies have employed this experimental model to test the interaction, absorption, and cellular transit of medicines and dietary components, but the Caco-2 response to radiation has received less attention. However, particularly in comparison to other colorectal cancer cell lines, Caco-2 exhibit peculiar features, among which are: their poorly aggressive tumor phenotype allows studying mechanisms at work at an early stage of cancer progression, as well as using radiation as a probe to gain molecular understanding; their p53 null status (Liu & Bodmer, 2006a), given the well-recognized role of this gene in altering the responses to cancer therapeutic agents (Bunz et al., 1999a), provides the opportunity to concentrate on p53-independent pathways that may also play a major role in therapy response. This calls for more research to identify and quantify some of the unknowns in Caco-2's reaction to radiation. Recent research on this cell line has focused on its response to various doses of X-rays from a standard radiotherapy accelerator, utilizing Caco-2 cells alone or co-cultured with peripheral blood mononuclear cells (PBMCs) from healthy donors (Borsci et al., 2020a; Morini et al., 2017a).

1.2 Problem statement

Colorectal cancer is the third most prevalent cancer diagnosed globally, with an anticipated incidence of 1.93 million by 2020 (Morgan et al., 2023). It is also the second major cause of cancer-related death, with 0.94 million cases expected in 2020 (Morgan et al., 2023). Surgery, chemotherapy, and radiation have been advanced as

the primary therapeutic options for colorectal cancer. Although the control of illness is possible through the developed methods, there is still room for improvement of overall survival. The survival of colorectal cancer patients is mainly affected by metachronous metastasis. The 5-year overall survival for Stage IV metastatic cases is 10.5–28.1%, which is significantly lower than Stage III (73.2%) (Wang et al., 2020; Wolpin et al., 2007). There are several techniques to cancer treatment, but they are all aggressive and have a multitude of side effects that reduces the patient's of age (Shah & Gil, 2009). The major treatment options for colorectal cancer include surgery, radiation, chemotherapy, or a combination of these therapies (Pedruzzi et al., 2008). Radiotherapy is recommended as a treatment for colorectal cancer, particularly in advanced instances, either alone or in combination (Pedruzzi et al., 2008; Tobias et al., 2010).

Chemotherapy has been established as the standard of treatment for patients with Stage II or above colorectal cancer, for survival benefits (Wolpin et al., 2007). The hunt for innovative cancer treatments should balance patient tolerance of side effects with possible toxicity (Cabrera et al., 2013). lant-derived products are excellent sources for discovery and development of new anticancer agents since the 60's, with the first researches on epipodophyllotoxin and its derivatives as cytotoxic agents, until nowadays, with the study of vinca alkaloids, vinblastine, vincristine, epipodophyllotoxin and taxanes, which are natural sources of drugs with activity against cancer (Srivastava et al., 2005). Several researches have been undertaken employing plant-derived compounds, which are promising sources for developing novel anticancer medicines. Tokgun et al. 10 observed that therapy with plant derivatives had fewer negative effects than synthetic medicines. As a result, the hunt for derivatives of natural substances to cure cancer seems promising. Recent research

using plant extracts from diverse places have emerged demonstrating the cytotoxic effects in cell lines of different cancer types, such as breast, esophageal, stomach, lung, and others (Liu et al., 2012; Song et al., 2013; Tokgun et al., 2012).

The study seeks to investigate the adjunctive application of using radiotherapy and chemotherapy for the treatment of colorectal cancer in vitro. The study will aim to determine the effect of different doses of x-ray (1, 3 and 5 Gy) on the Colorectal Cancer cell line and comparing it with the effect on the Dermal Fibroblast Normal, the effect of the extracts (Capsaicin and Fraxetin) on the viability of Colorectal Cancer cell line and normal cell line, the effect of X-ray at different doses with of a Capsaicin and Fraxetin instead of the chemotherapy and investigate the effect of the chemical drug Cisplatin on the viability of Colorectal Cancer cells line and Dermal Fibroblast Normal.

The findings of this study could have significant implications for the treatment of colorectal cancer and pave the way for the development of more effective and targeted therapies. The study could also contribute to a better understanding of the biophysical mechanisms underlying the use of radiotherapy conjunction with chemotherapy and the efficacy of plant extract in the treatment of colorectal cancer.

1.3 Objective of Study

The main objective of this study is to evaluate the individual and combined effects of X-ray radiation, plant extracts (Capsaicin and Fraxetin), and chemotherapy (cisplatin) on a colorectal cancer cell line.

i. To evaluate the cytotoxic effects of X-ray radiation (1, 3, and 5 Gy) and cisplatin chemotherapy on CRC cells and normal dermal fibroblasts.

ii. To assess the anti-proliferative and apoptosis-inducing properties of capsaicin and fraxetin in CRC cells, compared to normal cells.

iii. To determine whether combining X-ray radiation with capsaicin or fraxetin enhances therapeutic efficacy while reducing toxicity to normal cells. Key Changes:

1.4 Scope of study

The potential effect of radiotherapy combined with capsaicin and fraxetin plant extracts on colorectal cancer cell lines represents an intriguing and relatively novel area of research in oncology. The study involves combining radiotherapy, a conventional treatment for cancer, with capsaicin and fraxetin plant extracts. This combination approach suggests a novel strategy for enhancing the efficacy of radiotherapy and potentially reducing its side effects. Capsaicin, found in chili peppers, and fraxetin, derived from various plant species such as ash trees, possess bioactive properties that have been studied for their potential anticancer effects. Investigating these natural compounds as adjunctive therapies in cancer treatment adds originality to the study, especially in the context of colorectal cancer.

This study focuses on evaluating the effects of combination therapy on colorectal cancer cell lines in vitro. In vitro studies are essential for elucidating the underlying mechanisms of action and assessing the initial efficacy of potential

treatments before advancing to more complex in vivo and clinical studies. Colorectal cancer is one of the most prevalent and deadly cancers worldwide. Targeting this specific type of cancer with a novel combination therapy approach underscores the significance of the research and its potential clinical implications. While there is existing research on the individual effects of radiotherapy and the plant extracts on cancer cells, there may be limited prior studies investigating their combined effects specifically on colorectal cancer cell lines. Therefore, this study fills a gap in the literature by exploring the synergistic or additive effects of these treatments in a specific cancer context. Overall, the originality of the study lies in its innovative combination therapy approach using natural compounds alongside conventional treatment modalities, its focus on a specific cancer type, and its contribution to the growing body of research aimed at improving treatment outcomes for colorectal cancer patients.

1.5 Thesis outlines

This thesis consists of five chapters. the present chapter highlights the key points of the research. Chapter 1 describes the research background, motivation and problem statement, objectives of the research and thesis outlines. Chapter 2 presents a literature review of the studies performed in the fields related to this thesis. Chapter 3 describes the sampling of the research, the equipment used, experimental setup, and methodology of the data analysis. Chapter 4 discusses the results and procedures of this study, together with the statistical analysis. Chapter 5 conclusions of this research and provides suggestions for possible future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Radiotherapy is considered one of the most common cancer treatments, which involves the use of high-energy particles or waves, such as ionizing radiation in its various forms (X-ray, gamma rays, particles, and protons) to kill cancer cells that don't spread to other parts of the body while slowing their growth and dividing and reducing their number. It reduces the chance of cancer coming back after treatment or surgery and shrinks the tumor before or after surgery (Gianfaldoni et al., 2017).

Despite improved treatment regimens, overall cure rates have remained relatively stable, and, most critically, individual patient responses to radiochemotherapy or irradiation therapy alone vary widely and cannot be predicted. It is consequently of special therapeutic importance to implement new models for predicting the individual response to radiation therapy (Koch et al., 2021).

In the beginning, radiotherapy was utilized to treat cancer at low kilovolt energies with limited penetration powers. Nowadays, MV X-ray technology is frequently used in clinical settings (Baskar et al., 2012a). where Radiation therapy utilizing an X-ray beam is now used to treat cancer, A significant area of cancer treatment now involves the use of medical LINAC, Radiation therapy can be administered either internally (teletherapy) or externally (alone or in combination with other therapies like surgery, chemotherapy and brachytherapy) (Skowronek, 2017).

Radiation can be administered in a variety of ways, for the same malignancy (a patient may occasionally receive many radiation treatments), in fact, several medications are known to act as radiosensitizers, increasing the sensitivity of cancer cells to radiation (Gong et al., 2021). Has indicated that intracellular DNA is damaged by radiation by inducing micro-cracks. These cracks stop the growth of cancer cells and often lead to their division and demise. Radiation can also destroy nearby normal cells, but most of them recover and resume their normal functions (Ahmed et al., 2020).

2.2 Cancer therapies

Conventional cancer treatments includes chemotherapy, surgery, and radiotherapy, which are the three most commonly used (Mroz et al., 2011). Each therapy has limits and efficacy, and depending on the stage of the tumor, a combination of therapies may be required. As technology advanced, new cancer treatment methods emerged, including immunotherapy, targeted therapy, radiofrequency ablation, and hormone therapy. These methods were developed to increase the therapeutic index in order to treat cancer more specifically and, ideally, without side effects for patients (Peidaee et al., 2014).

2.2.1 Surgery

Surgery is one of the oldest, most successful, and most widely used methods of removing solid malignant tumors. Misconceptions about cancer, such as the fact that it is an incurable illness with a documented recurrence of malignant tumors and associated consequences, along with rudimentary cancer surgical practices, have slowed progress in the development of cancer treatments (Peidaee et al., 2014). To date, technological advancement have produced helpful instruments, including fiber

optic, laparoscopic, and horoscopic technology, to facilitate the surgical process and reduce the level of invasiveness in cancer removal surgery. Some novel treatments, such as cryosurgery or cryotherapy, employ liquid nitrogen spray to cryopreserve irregularly behaving cells, lasers to substitute scalpels to grasp cancer growing tissue, or even cautery excision of cancer. Despite the fact that these new approaches for cancer surgery lessen side effects, certain issues remained after the surgery, such as organ or part of the body damage or deformity, infections from the surgical process, blood loss, and other illnesses, including pneumonia (Peidaee et al., 2014).

2.2.2 Chemotherapy

The term "chemotherapy" was coined by the German scientist Paul Ehrlich, who was particularly interested in alkylating chemicals and later described the chemical treatment of sickness (Arruebo et al., 2011). Chemotherapy, one of the most widely used conventional methods of cancer treatment, frequently employs medicine. These chemotherapy drugs are classified into several categories based on their mechanism of action, including alkylating agents, antimetabolites, antitumor antibiotics, inhibitors of topoisomerase, miotic's, proteases, and tyrosine kinases, hormonal drugs, and plant alkaloids (Le et al., 2017). Chemotherapy drugs typically target the genome at various periods of the cell cycle during growth or division. For example, alkylating agents bind to DNA in the nucleus to prevent cell division; antimetabolites agents were used to replace the normal building blocks needed for RNA and DNA replication with active substances; antitumor antibiotics would interfere with DNA structure/enzyme and prevent DNA from uncoiling or replication; and other cancer treatments include topoisomerase inhibitors, mitotic inhibitors, and cortisteroids (Huang et al., 2017).

2.2.2(a) Cisplatin

Cisplatin is a very strong broad-spectrum anticancer medication that is often used to treat solid malignancies, including CRC. CRC is treated with a multimodality strategy that includes surgical tumor excision followed by chemotherapy or radiation (Griso et al., 2022; Hassanen et al., 2021). Despite its high effectiveness, cisplatin's usage is limited due to cumulative nephrotoxicity (Curry & McCormick, 2022). Furthermore, refined cycles of cisplatin chemotherapy have resulted in drug resistance, either acquired or intrinsic, resulting in a marked reduction in its clinical efficacy and relapse of the illness (Griso et al., 2022). To beat adverse effects and medication resistance, cisplatin-based combinatorial regimens are being investigated as a first line of treatment (Basma et al., 2005). The combination of cytotoxic medicines in chemotherapy serves as the foundation for advanced CRC treatment. (Van Cutsem et al., 2011). The recent decade has seen an increase in interest in studying cisplatin's synergistic anti-cancer effects (Wang et al., 2010). Every malignancy, even cancer of the same kind, has unique biochemical properties. Nevertheless, to date, there is no report on the synergistic effect of cisplatin and on CRC (Islam et al., 2023).

Due to its special properties cisplatin has been advocated as a radiosensitiser to achieve synergistic effects with radiation therapy. Lower doses are often used in multiday low dose schedules together with radiation therapy with rather limited systemic side effects (Marcu et al., 2003). There should be a differentiation between concurrent and induction chemotherapy. Concomitant or contemporaneous chemoradiation with cisplatin has been shown to help more advanced instances of uterine cervical cancer (Grigsby & Herzog, 2001) as well as lung cancer (Bartelink et al., 2002). Even induction or neoadjuvant chemotherapy improves survival in non-

small cell lung cancer (NSCL). Adelstein et al. performed a randomised phase III trial in stage 3 and 4 head and neck cancer patients with resectable disease, adding concurrent chemotherapy with cisplatin and 5-FU to definitive radiation. They found increased disease clearance, increased disease free interval and increased primary site preservation at long-term follow up (Adelstein et al., 2000; Milas et al., 2003). The improved survival observed at early follow up was lost at the long-term follow up. This was attributed to the general state of health of the population with head and neck cancer, in which other causes of death are common. In a randomised and stratified phase III trial Al Sarraf et al. compared chemoradiation with cisplatin and 5-FU to radiation alone in advanced nasopharyngeal carcinoma. Their conclusion was that chemoradiation produced superior overall survival and progression free survival (Jingu et al., 2020).

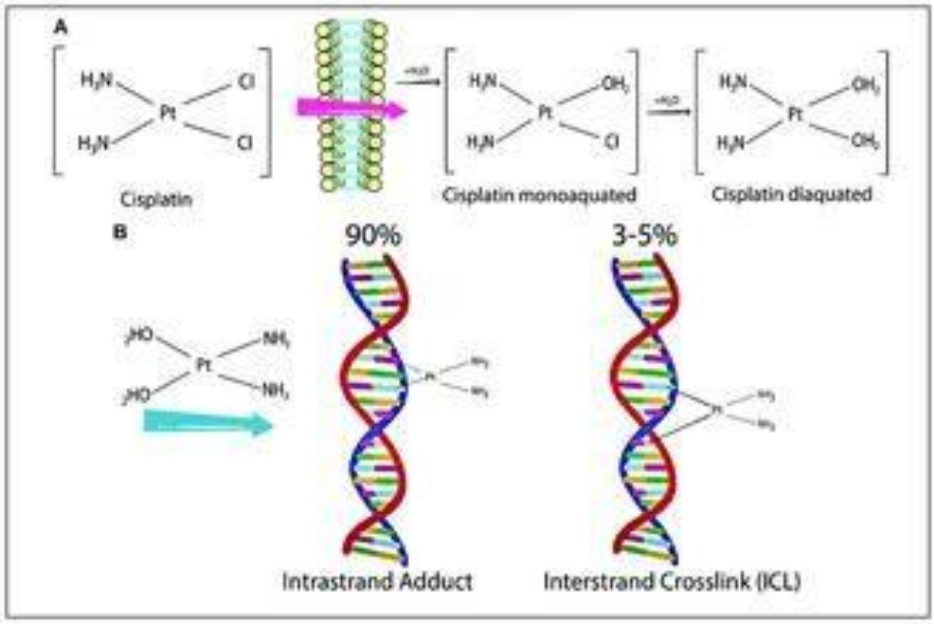

Figure 2.1 Cisplatin activation and formation of cisplatin-DNA adducts

Source: Adapted from Rocha et al., (2018).

2.2.2(a)(i) Mechanism of action of cisplatin

Cisplatin is usually administered to the patients through intravenous route. As it is neutral by nature, it can easily diffuse into cells by passive diffusion (Sedletska et al., 2005). However, it can enter into the cells by active transport as well (Byfield & Calabro-Jones, 1981). Several transporters, including the Na^+, K^+-ATPase, copper transporter-1 (CTR1) and organic cationic transporters SLC22 family of proteins have been implicated in facilitating the entry of cisplatin into the cells (Basu & Krishnamurthy, 2010).

Cisplatin is a relatively inert molecule, but its aquated species are highly reactive. Thus, the conversion of cisplatin into its hydrolysed products $[Pt(NH_3)_2Cl(OH_2)]^+$ and $[Pt(NH_3)_2(OH_2)_2]^{2+}$ is the key factor for activation (Wang & Lippard, 2005). Low chloride concentration inside the cell (4 mM) compared to the blood stream (100 mM) facilitates the aquation process after entry into the cells. However, before binding with DNA, about 90% of cisplatin molecules are deactivated as a result of binding with albumin, glutathione and other plasma proteins that contain the thiol group (Cepeda et al., 2007).

Even though only about 1% of administered cisplatin molecules successfully bind with DNA, cytotoxicity of cisplatin is believed to due to its interaction with nucleophilic N7 positions of guanine and adenine in DNA to form various crosslinks (Eastman, 1987). Binding to the N7 position of guanine is more preferable to that of adenine, because of the formation of a more stable hydrogen bond between a coordinated water molecule and G-O6. Cisplatin can form both monofunctional and bifunctional DNA adducts, but the monofunctional adducts are preferred during the initial DNA-binding step. Monofunctional adducts of cisplatin can distort DNA,

reduce thermal stability and disturb stacking interactions due to local loss in winding (Bursova et al., 2005). Monofunctional adducts can evolve into bifunctional ones. Once bifubctinal adducts are formed, DNA would be further distorted locally that would involve bending and unwinding as applied to the double helix (Trimmer et al., 1998).

Different types of bifunctional adducts can be formed from cisplatin-DNA binding, such as 1,2-intrastrand d(GpG) adduct; 1,2-intrastrand d(ApG) adduct; interstrand adducts linking the two strands of DNA double helix and intrastrand 1,3-d(GpXpG) adducts between purines separated by one or more intervening bases (Bose, 2002). There is also evidence of the formation of protein–DNA cross-links through the coordination of cisplatin with protein and nucleobases (H. Guo et al., 2003). Figure 2.2 describes different types of binding modes of cisplatin-DNA binding.

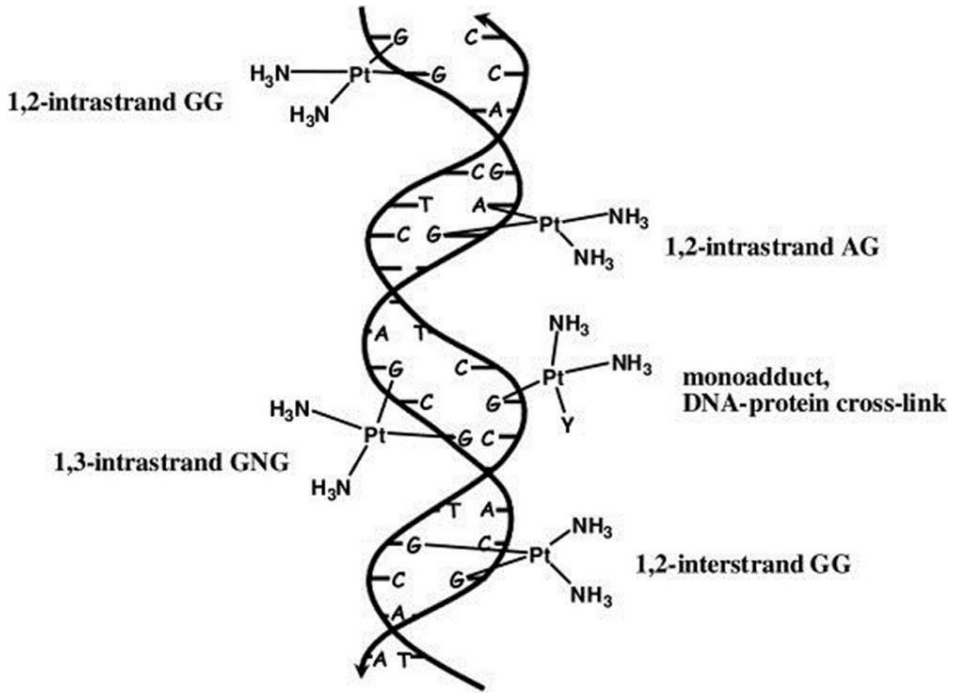

Figure 2.2 Different modes of binding of cisplatin with DNA
Source: Adapted from Pizarro and Sadler (2009).

There is controversy among scientists as to which of the various platinum–DNA adducts might be the more biologically significant in relation to the cytotoxicity of cisplatin. However, evidence from previous studies strongly favours intra-strand adducts as being the lesions responsible for the cytotoxic action (Pinto & Lippard, 1985). It may be noted that over 60% of the adducts formed by cisplatin belong to the categories: 1,2-d(GpG) intrastrand cross-links while about 20% belongs to d(ApG) intrastrand crosslink. These adducts activate various signal-transduction pathways; for example, those involved in DNA-damage recognition and repair, cell-cycle arrest, and apoptosis or necrosis.

2.2.2(a)(ii) Signal transduction pathways responsible for cisplatin induced cell death

Cytotoxicity mediated by cisplatin is directly proportional to the amount of cisplatin bound to DNA (Prestayko, 2013). It is believed that cisplatin induces cell death mainly by apoptosis and to some extent by necrosis (Münger et al., 2001). Apoptosis is likely started or progressed immediately after recognition of DNA damage by more than twenty individual candidate proteins. These proteins are induced by interstrand/intrastrand bifunctional and monofunctional adducts (Bellon et al., 1991). Among these damage recognition proteins, the most prominent ones are HMG1 and HMG2 proteins, hMSH2 or hMutSα component of MMR complex, the transcriptional factor ‘TATA binding protein’ (TBP) and human RNA polymerase I transcription ‘upstream binding factor’ abbreviated as hUBF (Vaisman et al., 1999). However, either a single protein or combinations may be responsible for sensing the damage (Siddik, 2003).

Each of the damage recognition proteins may activate one or more signalling pathways, and thus DNA damage leads towards multiple biological effects. At the same time, both cell-survival and cell-death signals are activated following cisplatin exposure. The relative strength of signals is integrated downstream to determine the ultimate fate of the cell: survival or death. The pathways involved in cisplatin-induced cytotoxicity are summarised in Figure 2.3.

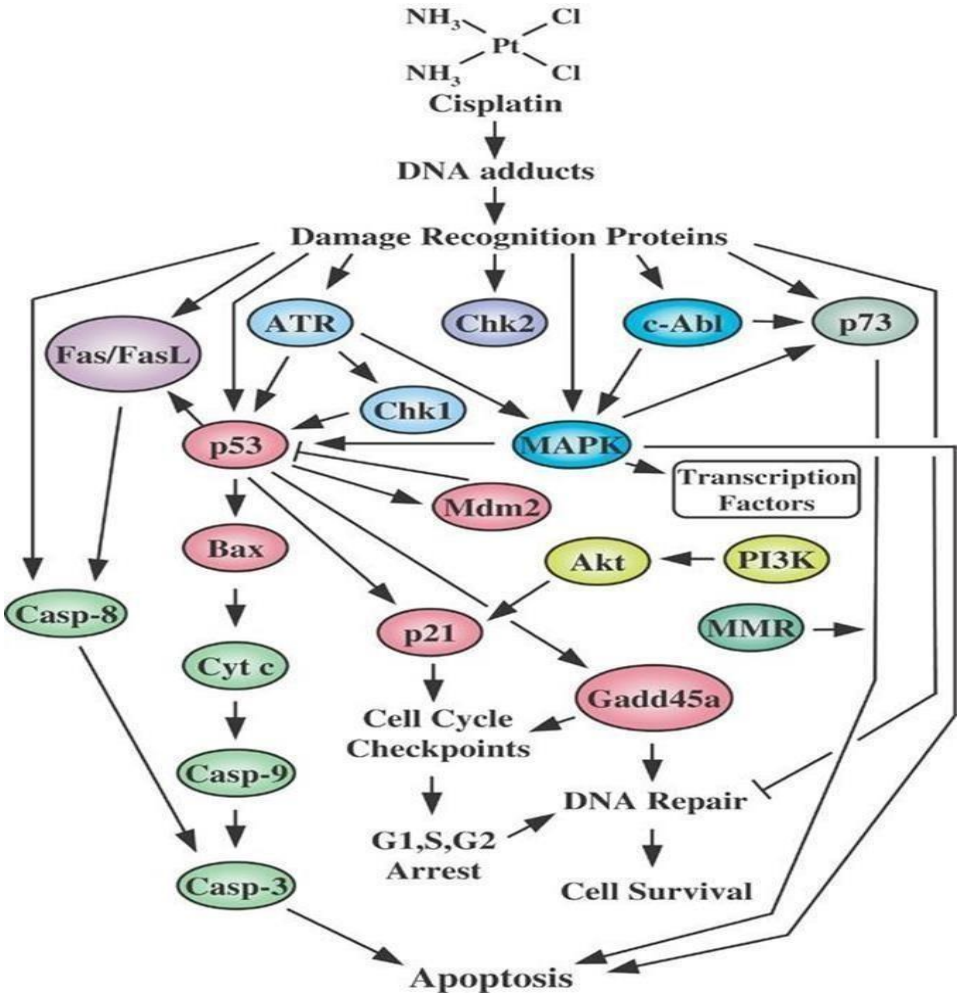

Figure 2.3 Pathways involved in cisplatin-induced cell death

Source: Siddik (2003).

2.2.2(a)(iii) Nephrotoxicity

Cisplatin nephrotoxicity is a dose-limiting adverse effect, meaning that various dosages of cisplatin cause varying degrees of nephrotoxicity and histological alteration. This nephrotoxicity may appear as acute kidney damage (AKI), chronic kidney injury (CKI), or any other clinical renal symptoms (Laplace et al., 2020). Acute kidney damage (AKI) occurs in 25-40% of cisplatin-treated patients, whereas chronic renal impairment of grades 3 or 4 is expected to occur in up to 8.5%. The primary distinction between acute and chronic renal damage is the pace and duration of renal function decrease. (Laplace et al., 2020).

Cisplatin nephrotoxicity occurs in about one-third of individuals who get this medication. Since renal cytotoxicity remains even after hydration and dosage adjustment, proximal tubular epithelial cells are the predominant excretion source for cisplatin. Cisplatin nephrotoxicity can be mitigated or prevented on multiple levels, including cisplatin transport to renal epithelial cells, aggressive hydration, forced diuresis, chelating agents to reduce cell death, blockade of cyclin-dependent kinase, mitogen-activated protein kinase (MAPK), inhibition of p53 activation, antioxidant supplementation, and inflammation blockage (Lash, 2022).

Oxidative stress is one of the primary causes of cisplatin nephrotoxicity. Cisplatin-induced oxidative stress promotes apoptosis, inflammation, mitochondrial damage, and ER stress. As a result, administering antioxidants in this setting may be a viable approach for preventing cisplatin nephrotoxicity. The primary target of cisplatin-induced oxidative stress in mitochondria is mitochondrial thiol proteins, which are damaged, calcium absorption is blocked, and the respiratory chain's

physiological activity to create energy and subsequent cytochrome C release is reduced (Dasari & Tchounwou, 2014).

2.2.2(a)(iv) Neurotoxicity

Neurotoxicity is a frequent, dose-dependent effect of cisplatin. It leads to a reduction in dose, discontinuation of treatment and many times it also lessens cancer survivor's quality life (Reddy et al., 2010). Treatment strategies were used to combat cisplatin-induced neurotoxicity, yet these modalities failed to manage all the signs and symptoms and therefore not efficient in ameliorating these conditions. Pregabalin is used to decrease neuropathy, Duloxetine reduces pain, and Venlafaxine reduces the incidence and albeit some side effects of Cisplatin (Atreya, 2016). Neuroprotectants exhibit weak efficacy for preventing cisplatin-induced neurotoxicity whereas neutraceuticals exhibit potential activity in protecting neurons from damaging effects of chemotherapy (Abhirama et al., 2017; Patil & Balaraman, 2009).

Neurotoxicity is another well-known dose limiting side effect of cisplatin treatment. It manifests commonly as distal sensory neuropathy, which may be distressing. Again there is a rough correlation to dose and dose intensity, with no known treatment options. Neurotoxicity is commonly irreversible (Quasthoff & Hartung, 2002). The most common and dose-limiting effect of cisplatin is neurotoxicity (Bobylev et al., 2018). Previous research has proposed a variety of pathophysiological pathways to explain the neurotoxicity associated with cisplatin-based therapy, including oxidative stress, inflammation, mitochondrial dysfunction, DNA damage, and apoptosis in nerve cells. According to the study, the brain is the most sensitive to oxidative stress due to its high concentration of polyunsaturated

fatty acids (PUFAs), which are especially prone to reactive oxygen species (ROS) damage (Cui et al., 2004).

2.2.2(a)(v) Ototoxicity

Cisplatin ototoxicity is primarily caused by effects on sensory hair cells, spiral ganglion neurons (SGNs), and secretory and connective tissues (stria vascularis and spiral ligament) in the cochlea (Rybak et al., 2007). Mammalian hair cells and SGNs cannot regenerate, therefore their death is irreversible, resulting in persistent sensorineural hearing loss. Cisplatin-induced ototoxicity causes bilateral, moderate-to-profound high-frequency hearing loss as well as a considerable loss of outer hair cells (OHCs) in the cochlea's basal turn. Although cisplatin predominantly impairs auditory function, inner ear poisoning may also manifest as tinnitus and, in rare cases, ear discomfort or balance disturbance caused by vestibulotoxicity (Prayuenyong et al., 2018, 2021). Ototoxicity is caused by a number of variables, including the patient's age, cumulative cisplatin dosage, and susceptibility (Yu & Gu, 2020). Cisplatin-induced hearing loss affects 40-60% of individuals, with 18% suffering from severe to profound hearing loss and 40% experiencing tinnitus (Knight et al., 2005). Hearing abnormalities in pediatric children might cause difficulty with speech and language. Cisplatin-induced hearing loss may also lead to social isolation, anxiety, and depression, all of which have a negatively impact on overall well-being.

2.2.3 Radiotherapy

Radiotherapy is regarded as an essential component of cancer treatment. External beam radiotherapy (EBRT) is the most often used method of treatment delivery (Jaffray et al., 2013). determining that it is appropriate for the treatment of half of all cancer cases. The optimum clinical result of a curative radiation regimen is complication- and relapse-free survival. A course of radiotherapy is defined as the radiation dosage in Gray (Gy) that will be provided to the tumor over a number of days, or treatment fractions, based on the cancer type and stage (de Boer et al., 2013). To minimise complications, the radiotherapy prescription will also include limits to the dose delivered to surrounding normal tissues and organs at risk (OAR) (van Beek et al., 2010). Individual patient-specific circumstances may necessitate a trade-off between tumor coverage with the recommended dose and exceeding normal tissue and OAR limits. These considerations are given priority throughout the pre-treatment physical planning procedure Prior to starting radiotherapy, a patient will have a planning computed tomography (CT) scan of the area where their cancer is situated (Huang et al., 2015). For this scan, the patient is placed in the right treatment posture utilizing stabilisation and positioning equipment. After being imported into the treatment planning system (TPS), the planning CT is used to locate and identify the size and location of the patient's tumor, as well as the surrounding normal tissues and OAR. International recommendations necessitate the incorporation of tumor margins and some OAR during treatment planning to prevent incomplete tumor irradiation or over-dosing of normal tissues during treatment delivery (Keller et al., 2006; Li et al., 2016). The resulting planning target volume (PTV) and planning risk volumes (PRV) are necessary to account for geometrical uncertainty in treatment delivery. These occur for a variety of causes, including physiological

influences that result in daily variations in tumor and OAR position and shape, as well as radiation equipment precision (Vyas, 2023). The appropriate number of radiation treatment fields, their geometry, and the dose supplied by each field are subsequently computed. Each patient's treatment plan is tailored to their specific radiation prescription, as well as the stage and location of their cancer. The planning CT scan and treatment plan developed from it serve as reference data for all aspects of therapy delivery.

A typical course of curative radiation includes daily treatments for up to 5-7 weeks (25-35 fractions). For treatment to be effective, the tumor must be included in the treatment fields for each treatment fraction; a geographic miss guarantees treatment failure. Image-guided radiation (IGRT) uses on-board imaging technology mounted on radiotherapy treatment units to image a patient's tumor while they are lying on the treatment couch (Korb & Nicholson, 2010). IGRT compares the patient's daily pre-treatment verification photos to reference images from their customized treatment plan. The corresponding tumor and OAR features are then aligned and matched on the verification and reference pictures. The image registration parameters obtained from this process are then used to calculate and apply set-up corrections to the patient's position, guaranteeing proper targeting of the treatment fields. IGRT is commonly performed by radiation therapists (RTs) in collaboration with radiation oncologists (ROs) using a variety of technologies and clinical practices (Oh et al., 2011).

2.3 Electromagnetic radiation (EM)

Electromagnetic radiation (EMR) exists in a variety of energies, known as the radiation-consisting electromagnetic spectrum (EMS). Electromagnetic spectrum (EMS) is a scientific term used to describe the entire available light array. It can be expressed in different ways in terms of energy (unit in eV), wavelength–distance from the height of one wave to the peak of the next (unit in meters) or frequency–the number of waves traveling past one point in a second (Peidaee et al., 2014). The electromagnetic radiation level was classified into two groups, ionizing and non-ionizing, based on the amount of energy to remove electrons and cause the matter to become ionized. When radiation is absorbed by matter, excitation and ionization process would occur. Excitation refers to the motions of atoms or molecules being excited by radiation, or an electron excites from an occupied orbital into another empty but higher-level orbital. Ionization refers to radiation that carries sufficient energy to break a chemical bond by removing an electron from an atom or molecule (Peidaee et al., 2014).

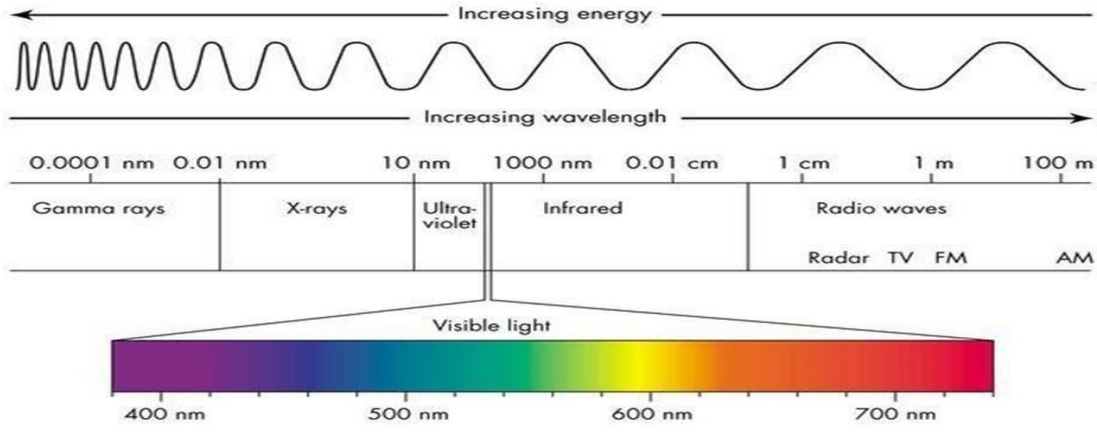

Figure 2.4 Electromagnetic spectrum

Source: Chestikov (2022)

2.3.1 Ionizing radiation

Radiation with sufficiently high energy may ionize atoms, which means it can knock electrons out of atoms and form ions. This happens when one electron is removed (or "knocked out") from the atom's electron shell, resulting in a net positive charge. Because this ionization may harm live cells and, more critically, the DNA inside those cells, it increases the risk of cancer. Thus, "ionizing radiation" is intentionally segregated from particle radiation and electromagnetic radiation, owing to its high potential for biological harm. While a single cell has trillions of atoms, only a small fraction of them will be ionized at low radiation levels the probability of ionizing radiation causing cancer is proportional to the absorbed dose and is determined by the harmful nature of the radiation (equivalent dose) and the sensitivity of the irradiated organism or tissues (effective dose). Photons and particles with energy greater than around 10 electron volts (eV) ionize.

Alpha particles, beta particles, cosmic rays, gamma rays, and x-ray radiation are all energetic enough to ionize atoms.

Furthermore, unbound neutrons are ionizing because their interactions with matter are almost always more energetic than this threshold. Radioactive materials, x-ray tubes, and particle accelerators all emit ionizing radiation, which is naturally present in the environment. It is invisible and cannot be detected directly by human senses; thus, devices such as Geiger counters are often used to detect its existence. In certain situations, such as Cherenkov radiation and radio-luminescence, it may cause secondary discharge of visible light when it interacts with materials. Ionizing radiation has several practical applications in medicine, research, and construction, but it poses a health risk if handled incorrectly. Radiation exposure damages living tissue. High doses induce skin burns, radiation sickness, and death, while low but persistent doses produce cancer tumors and genetic damage (Ng, 2003). However,