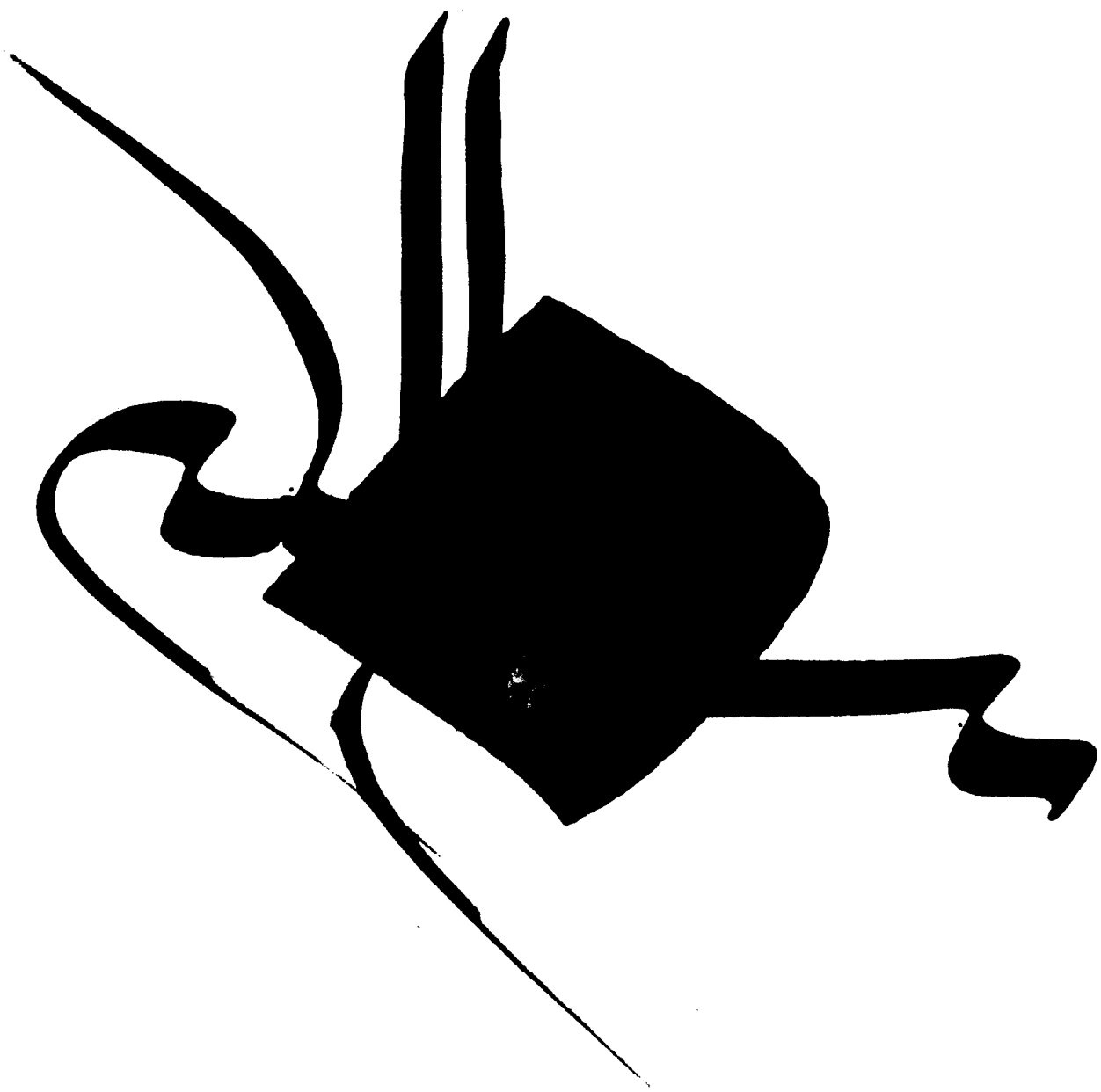

In the name of God

Islamic Republic of Iran

Kerman University of Medical Sciences

Faculty of Dentistry

For the Certificate of General Dentistry

Title:

Investigation of the cytotoxic effects Cyclooxygenase enzyme inhibitors drugs (COX1, COX2 inhibitors) on KB cell, SAOS-2, glioma (1321N,REYF-1), neuroblastoma(U-87MG, DAOY) in vitro

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By:

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To my parents

Who brighten for me the way of life

with their real love

AND

To my brothers

My protectors for ever

To Dr.Hashemi pour

For her worthy and remarkable encouragement ,positive suggestion and support trough all steps of this study.

I also wish to thank the staff of cell culturing laboratory in kerman neuroscience center.

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1. Introduction

1.1. Cancer

Cancer (malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. Today, the Greek term carcinoma is the medical term for a malignant tumor derived from epithelial cells. It is Celsus who translated carcinos into the Latin cancer, also meaning crab. Galen used "oncos" to describe all tumors, the root for the modern word oncology(1).

There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Also, malignant tumors (cancers) are usually named using carcinoma, sarcoma or blastoma as a suffix, with the Latin or Greek word for the organ of origin as the root (2).

Cancer affects people at all ages with the risk for most types increasing with age. Cancer caused about 13% of all human deaths in 2007 (7.6 million)(2).

Cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome(4).

Definitive diagnosis requires the histologic examination of a biopsy specimen,

although the initial indication of malignancy can be symptomatic or radiographic imaging abnormalities. Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. There is no single treatment for cancer, and patients often receive a combination of therapies and palliative care. Treatments usually fall into one of the following categories: surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or gene therapy. Experimental cancer treatments are also under development (5).

Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness. Surgery often required the removal of a wide surgical margin or a free margin. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue (4,6).

As research develops, treatments are becoming more specific for different varieties of cancer. There has been significant progress in the development of targeted therapy drugs that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. The prognosis of cancer patients is most influenced by the type of cancer, as well as the stage, or extent of the disease. In addition, histologic grading and the presence of specific molecular markers can also be useful in establishing prognosis, as well as in determining individual treatments(6).

Cancer has a reputation as a deadly disease. While this certainly applies to certain particular types, the truths behind the historical connotations of cancer are

increasingly overturned by advances in medical care. Some types of cancer have a prognosis that is substantially better than nonmalignant diseases such as heart failure and stroke(2). In the developed world, one in three people will develop cancer during their lifetimes. If all cancer patients survived and cancer occurred randomly, the lifetime odds of developing an second primary cancer would be one in nine. However, cancer survivors have an increased risk of developing a second primary cancer, and the odds are about two in nine (3).

1.1.1. Oral cancer

Oral cancer or oral cavity cancer, a subtype of head and neck cancer, is any cancerous tissue growth located in the oral cavity (7). It may arise as a primary lesion originating in any of the oral tissues, by metastasis from a distant site of origin, or by extension from a neighboring anatomic structure, such as the nasal cavity or the maxillary sinus. Oral cancers may originate in any of the tissues of the mouth, and may be of varied histologic types: teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment producing cells of the oral mucosa. There are several types of oral cancers, but around 90% are squamous cell carcinomas(8), originating in the tissues that line the mouth and lips. Oral or mouth cancer most commonly involves the tongue. It may also occur on the floor of the mouth, cheek lining, gingiva (gums), lips, or palate (roof of the mouth). Most oral cancers look very similar under the microscope and are called squamous cell carcinoma(9). These are malignant and tend to spread rapidly.

Cancer of oral cavity comprises approximately 30% of malignant tumors of head and neck. This malignancy accounts for 3% of all cancers in the United States. Oral

cancer remains a serious & constant problem and is one of the most prevalent cancers and is one of the 10 most common causes of death. It is the fifth most common cancer of all sites and accounts for approximately 4% of cancers world wide(10).

In 2008, in the US alone, about 34,000 individuals were diagnosed with oral cancer. 66% of the time these will be found as late stage three and four disease.

In southwest Asia and particularly in India, cancer of the oral cavity is the most common cancer, compromising 35% of all cancer men and 18% of all cancers in woman. The overall incidence and mortality rates for oral cancer combined are 10.4 per 100,000 population and 2.9 per 100,000 population, respectively(11).

All cancers are diseases in the cancer cells. Oncogenes are activated as a result of mutation of the DNA. The exact cause is often unknown. Regardless of the cause, treatment is the same surgery, radiation with or without chemotherapy. Risk factors that predispose a person to oral cancer have been identified in epidemiological studies(8).

Some oral cancers begin as leukoplakia a white patch (lesion), red patches, (erythroplakia) or non healing sores that have existed for more than 14 days. In the US oral cancer accounts for about 8 percent of all malignant growths. Men are affected twice as often as women, particularly men older than 40/60(9).

Smoking and other tobacco use are associated with about 75 percent of oral cancer cases, caused by irritation of the mucous membranes of the mouth from smoke and heat of cigarettes, cigars, and pipes(9).

Alcohol use is another high-risk activity associated with oral cancer. There is known to be a strong synergistic effect on oral cancer risk when a person is both a heavy smoker and drinker. Their risk is greatly increased compared to a heavy smoker, or a

heavy drinker alone. Recent studies in Australia, Brazil and Germany point to alcohol-containing mouthwashes as also being etiologic agents in the oral cancer risk family(7).

Some studies suggest that not eating enough fruits and vegetables may increase the chance of getting oral cancer. Scientists also are studying whether infections with certain viruses (such as the human papillomavirus) are linked to oral cancer.

Chemotherapy is useful in oral cancers when used in combination with other treatment modalities such as radiation therapy. It is not used alone as a monotherapy. When cure is unlikely it can also be used to extend life and can be considered palliative but not curative care. Treatment of oral cancer will usually be by a multidisciplinary team, with treatment professionals from the realms of radiation, surgery, chemotherapy, nutrition, dental professionals, and even psychology all possibly involved with diagnosis, treatment, rehabilitation, and patient care(10).

1.1.2. Osteosarcoma

A sarcoma (from the Greek 'sarx' meaning "flesh") is a cancer that arises from transformed connective tissue cells (12). These cells originate from embryonic mesoderm, or middle layer(13) which forms the bone, cartilage, and fat tissues. Sarcomas affect people of all ages.

Osteosarcoma is an aggressive cancerous neoplasm arising from primitive transformed cells of mesenchymal origin that exhibit osteoblastic differentiation and produce malignant osteoid. It is the most common histological form of primary bone cancer(12).

Osteosarcoma is the eighth most common form of childhood cancer, comprising 2.4% of all malignancies in pediatric patients, and approximately 20% of all bone

cancers(14). Incidence rates for osteosarcoma in U.S. patients under 20 years of age are estimated at 5.0 per million per year in the general population, with a slight variation between individuals of black, Hispanic, and white ethnicities (6.8, 6.5, and 4.6 per million per year, respectively. It is slightly more common in males (5.4 per million per year) than in females (4.0 per million per year(15).

There is a preference for origination in the metaphyseal region of tubular long bones, with 42% occurring in the femur, 19% in the tibia, and 10% in the humerus. About 8% of all cases occur in the skull and jaw, and another 8% in the pelvis(12).

Complete radical surgical en bloc resection is the treatment of choice in osteosarcoma. Although about 90% of patients are able to have limb-salvage surgery, complications, such as infection, prosthetic loosening and non-union, or local tumor recurrence may cause the need for further surgery or amputation.

Deaths due to malignant neoplasms of the bones and joints account for unknown amount of childhood cancer deaths. Mortality rates due to osteosarcoma have recently been declining at approximately 1.3% per year. Current long-term survival probabilities for osteosarcoma have improved dramatically in recent decades and now approximate 68%(16).

1.1.3. Brain tumors

Brain tumors do not discriminate. Primary brain tumors - those that begin in the brain and tend to stay in the brain - occur in people of all ages, but they are statistically more frequent in children and older adults. Metastatic brain tumors – those that begin as a cancer elsewhere in the body and spread to the brain - are more common in adults than in children(17).

An estimated 52,236 new cases of primary brain tumors are expected to

diagnosed in 2008. This is based on an overall incidence rate of 16.5 per 100,000 persons. Sources that quote the incident number of brain tumors at about 22,000 people (12,000 males and 10,000 females) diagnosed per year do so based on data counting only malignant brain tumors. In the United States, approximately 3,750 children younger than age 20 were expected to be diagnosed in 2007 with primary brain tumors, of which 2,820 were under age 15(17).

Brain tumors are the most common of the solid tumors in children, and the leading cause of death from solid tumors. Brain tumors are the second most frequent malignancy of childhood(18).

The incidence of malignant brain tumors appears to increase steadily with age. The lowest incidence rate is among children less than 20 years (4.5 per 100,000 persons). The rate increases steadily until age 75—84, when it peaks at 57 per 100,000 persons. After age 85, the incidence rate drops to 56(17).

Meningiomas represent 32% of all primary brain tumors, making them the most common primary brain tumor. Gliomas, a broad term which includes all tumors arising from the gluey or supportive tissue of the brain, represent 39% of all brain tumors and 81% of all malignant tumors. Glioblastomas represent 19% of all primary brain tumors, and 51% of all gliomas(17).

In 2008, the American Cancer Society reported a significant decrease in the number of brain and central nervous system cancer deaths over the past 13 years. Deaths due to malignant brain tumors decreased 14.36% between 1991 and 2004(18).

In 1973-2001, five year survival rates for those with malignant brain tumors showed improvement over a three decade period: 21% in the 1970's, 27% in the 1980's, and 31% in the 1990's(19).

1.2. Non-steroidal anti-inflammatory drugs or NSAIDs

The willow tree has been a source of remedies against fever and inflammation since ancient times. Just over a century ago, Hoffman isolated and modified an active compound from the willow tree and offered it for sale as aspirin. Subsequently, a number of similar compounds have been derived, and these have been classified as non-steroidal anti-inflammatory drugs or NSAIDs. These drugs inhibit the enzyme cyclo-oxygenase (COX), which catalyses the conversion of arachidonic acid to prostaglandins (PGs). PGs are important mediators of signal transduction pathways, and are involved in cellular adhesion, growth and differentiation. Aspirin and other NSAIDs are extensively used in cancer patients, primarily for analgesia. However, since the late 1970s, researchers have been interested in whether regular ingestion of aspirin and other NSAIDs can decrease cancer risk. The most persuasive evidence to date relates to colorectal cancer(20).

1.2.1. COX enzymes

Two isoforms of COX exist, with distinct tissue distributions and physiological functions. COX-1 is constitutively expressed in many tissues and cell types, whereas the inducible isoenzyme COX-2 is pro-inflammatory in nature, and expressed only in response to certain stimuli such as mitogens, cytokines and growth factors. NSAIDs may achieve different degrees of inhibition of COX-1 and COX-2. Specific COX-2 inhibitors such as celecoxib and rofecoxib have been developed, and these largely avoid the gastrointestinal side-effects associated with NSAID use, which are thought to be due mainly to COX-1 inhibition.

A number of studies have demonstrated overexpression of COX-2 in solid malignancies including colon, prostate, and breast(21,20,22), as well as pancreas,

non-small-cell lung, bladder, endometrium and skin basal and squamous cell(21). A significant relation between over-expression of COX-2and survival of patients with various cancers has been reported in retrospective studies. In general, COX2 expression is higher in well-to-moderately differentiated tumors and in metastases. Evidence of changes in COX-1 expression in cancer cells is more limited. Oncogenes, growth factors, cytokines, and tumor promoters stimulate COX-2 transcription via protein kinase C and RAS-mediated signaling(23).

Less is known about negative modulators. Wild-type (but not mutant) p53 markedly suppresses transcription of COX-2. Several recent studies of human tumors highlight the importance of p53 status as determinant of COX-2 levels. For example, COX-2 levels are higher in cancers of the oesophagus, stomach, lung and breast that express mutant (rather than wild-type) p53(24,25). It is likely that other factors, such as hypermethylation of the promoter12 and post-transcriptional mechanisms, also determine COX-2 levels in neoplastic tissues. Genetic studies support a relationship between COX-2 and tumorigenesis.

Increased expression of COX-2 is observed in polyps from the Min mouse, which is an animal model for human familial adenomatous polyps. Removal of the COX-2 gene by knockout mutation reduces the number and size of intestinal polyps in Apc_716 mice. COX-2 over-expression in experimental models promotes tumor development. Multiparous female transgenic mice that are engineered to over-express human COX-2 in mammary glands develop focal mammary gland hyperplasia, dysplasia and metastatic tumors. Also, transgenic mice that over-express COX-2 in skin develop epidermal hyperplasia and dysplasia(26).

1.2.2. Cox and cancer

1.2.2.1. Activation of carcinogens

The peroxidase part of COX can convert procarcinogens to carcinogens and thus initiate tumor formation. Substantial amounts of xenobiotics (natural non-human organic compounds) can be co-oxidized into mutagens by the peroxidase activity of COX. In the liver, these types of oxidative reactions are catalyzed principally by cytochrome P-450s, thus preventing the formation of mutagens. In contrast, the colon has low concentrations of P-450s and other monooxygenases, leading to co-oxidation of significant amounts of xenobiotics to mutagens by the peroxidase activity of COX (27).

This reaction could be relevant at organ sites that are exposed to tobacco carcinogens such as lung, oral cavity and bladder. Similarly, estrogens, oxidized to diethylstilbestrol demonstrate transforming and genotoxic activity (27).

1.2.2.2. Apoptosis

There is accumulating evidence that NSAID treatment can restore apoptosis in several experimental and clinical settings. Apoptosis can be increased in cultured HT-29 human colon cancer cells by salicylate, sulindac or sulindac sulphide, and other conventional NSAIDs. NSAIDs increase apoptosis in rats exposed to chemical carcinogens,and normal apoptosis can be restored in familial adenomatous polyposis patients by a 3-month treatment with sulindac (28).

1.2.2.3. Angiogenesis

Another cellular function by which NSAIDs may exert their anti-tumoral effects involves the suppression of angiogenesis and neovascularization.COX-2 induces pro-angiogenic factors such as vascular endothelial growth factor (VEGF), inducible

nitric oxide synthase, interleukin 6 and 8, and TIE2 and it produces prostaglandins that have both autocrine and paracrine effects on proliferation and migration of endothelial cells in vitro. COX-2 is over-expressed in tumor endothelial cells. A significant association of COX-2 with tumor vascularization, microvessel density and VEGF has been reported in human head and neck cancer, and microvessel density was related to COX-2 expression in gastric adenocarcinoma. In vivo models show that COX-2 derived prostaglandins stimulate angiogenesis, and that COX-2 inhibition slows neovascularization. For example, celecoxib has been shown to inhibit fibroblast growth factor (FGF)-induced corneal angiogenesis in rats. Celecoxib reduced both the number and length of sprouting capillaries in a dose-dependent fashion. Tsujii and co-workers, by co-culturing endothelial cells and colon carcinoma cells, demonstrated that COX-2 modulates the production of angiogenic factors by tumor cells, whereas COX-1 regulates angiogenesis of endothelial cells in normal tissues (29,30).

1.2.2.4. Invasion and metastasis

An important issue in the progression of solid tumors is the ability of cells to invade locally and to spread to distant sites. Matrix degradation and cell motility are essential in this process.

Matrix metalloproteinases (MMPs) are a family of matrix degradation enzymes. Their expression is associated with tumor cell invasion of the basement membrane and stroma, blood vessel penetration and metastasis. It has been demonstrated that COX-2 induces MMP expression in human colon cancer cells and therefore promotes metastasis (31). Further, Fernandez et al. have described that COX-2 inhibition in the human prostate tumor cell line DU-145 resulted in a decreased secretion of MMP-2 and MMP-9 (32).