2-Methoxyestradiol (2ME) as an Anti-Angiogenic and Anti-Proliferative Agent on *in vivo* Sarcoma 180 Tumour

Comment [B1]: Please, rephrase as "**InvivoAnti-Angiogenic Activity of 2-Methoxyestradiolon in Sarcoma 180 Tumour cell line".**

InvivoAnti-Angiogenic Activity of 2-Methoxyestradiolon in Sarcoma 180 Tumour cell line

Abstract

Cancer is a complex genetic disease. Since the last few decades, the incidence of cancer has increased in different parts of the world, and now the situation is very alarming. Cancer or tumour cells are disorganized, mitotically uncontrolled, and autonomous race of cell populations, which consistently violate the basic rules of cell division. Most primary tumours arising in humans are benign, ~~i.e.~~ harmless, with few exceptions. Some tumour cells may cause clinical problems and harm that migrate to distant sites of the body, where they form secondary tumours. This process is known as metastasis, which is dependent on angiogenesis. The process of angiogenesis (~~i.e.~~ the formation of new blood vessels) plays a critical role in tumour growth and metastasis. ~~So,~~ most cancer treatments aim to target against metastasis. Presently, surgery, radiation therapy, chemotherapy, combination chemotherapy, immunotherapy, hormone replacement therapy, and anti-angiogenic therapy, ~~etc~~ are standard methods of cancer treatment. However, low-dose chemotherapy combined with an anti-angiogenic drug therapy seems to be a promising approach for the treatment of cancer. Therefore, the biology of cancer cells is different than normal cells, and cancer biology research involves the realization of the biology of cancer to develop more avenues to diagnose, prevent, and treat cancer. ~~We will try to~~This study provide an overview of some aspects of cancer biology ~~in this book chapter~~.

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Introduction

Sarcoma 180 ascitic tumour

The Sarcoma 180 ascitic tumour cell line is a stable murine cell line (1-5) maintained in the inbred Swiss albino mouse. These ascitic tumour cells are injected intraperitoneally for the development of an ascitic form of tumour, and the tumour cell line is maintained generation after generation in different laboratories. Tumour development in mice depends on various factors such as age and weight of the mouse, health of the mouse, specific breed of mouse, environment and well-maintained, ventilated animal house of the laboratory. The whole process of tumour-bearing mouse maintenance, tumour volume or size measurement and therapeutic study can be carried out carefully and aseptically. All animal experiments are performed according to the Institutional Animal Ethics Committee (IAEC) guidelines.

Growth and maintenance of Ascitic form of tumour

Sarcoma-180 ascitic cells are a transplantable tumour (6). The ascitic form of Sarcoma 180 tumour cell line is maintained in the laboratory and propagated into transplantable tumours in the peritoneal cavity of Swiss albino mice. Tumour cells are injected intraperitoneally into normal mice for induction of ascitic tumour (1,7).

Growth and maintenance of Solid tumour

Cells from the ascitic form of sarcoma 180 cell line are injected subcutaneously ventrally into the leg to induce a solid tumour. A solid tumour's average appearance time is generally 7 – 8 days (2, 7). A pathologist or an expert animal house specialist can handle the animals properly and measure the solid tumour size before or after the treatment. Small solid tumour size may also be measured under a microscope to study the therapeutic potential of any drug for prognosis purposes.

Cancer is a complex genetic disease. This disease is a significant public health burden in developed and developing countries. Information was obtained from the different reports that the incidence of cancer has increased in different parts of the world since 2002, and the situation is very alarming nowadays. Moreover, cancer mortality rates are rapidly growing worldwide (8,9).

Greek physician Hippocrates coined the term cancer as 'karkinoma'. The term cancer comes from the word 'crab.' Generally, cancer or tumour cells are disorganized, mitotically uncontrolled, and autonomous race of cell populations, which consistently violate the basic homeostatic principle of the body. Tumours are of two types – a) Benign tumour – which is

usually not life-threatening, and its surgical removal generally results in complete cure; b) Malignant tumour – which is very harmful, capable of invading normal tissue and spreading to the distant organs of the body. In most cases, malignant tumours are dangerous and fatal because malignant tumours constitute important characteristics such as immortalization with uncontrolled cell proliferation, angiogenesis or neovascularisation, metastasis, etc.

2-Methoxyestradiol (2-ME)

2-Methoxyestradiol (2-ME) is an endogenous estrogen metabolite of 17 β-estradiol (E2); this metabolite has recently emerged as an up-and-coming agent for cancer treatment (10). 2-ME is synthesized by sequential hydroxylation of the parent compounds followed by methylation in the liver (11-14). 2-ME is a potent inhibitor of estrogen-induced tumour angiogenesis and the inhibitory activity is strongly associated with the downregulation of vascular endothelial growth factor-A (VEGF-A) expression (15 - 18).

Interestingly, VEGF-A – the selective endothelial cell growth factor, is synthesized and secreted by various tumour cells and by several transplantable animal tumours (19 - 22). After secretion from tumour cells, this growth factor may play critical role in the initiation of angiogenic process to synthesize tumour vessels for the metastatic pathway.

This metabolite also acts as a potent inhibitor of tumour angiogenesis, targets proliferating cells with relatively high specificity, and does not kill non-dividing cells (17, 23,24). The effect of 2ME has also been investigated in different animal models by some investigators (25 - 28). In these models, 2ME inhibited local inflammation, angiogenesis, leukocyte infiltration, etc. Banerjee et al., 2002; 2003;2008 (16, 17, 24) studied the effect of different concentrations of 2ME at various time points on some tumour cell lines (viz. MCF-7 and GH3) and showed that 2-ME is antiangiogenic. 2ME induces G2/M arrest and apoptosis in many actively dividing cells (29). According to Mallick et al. (2), low-dose 2ME treatment can induce a robust antiangiogenic effect on solid-bearing mice and can protect the mouse from bone marrow toxicity as it inhibits different types of chromosomal aberrations. According to Peta et al. (30) 2 ME promotes early-stage palpable mammary tumour development.

2ME has been used for the treatment of different types of cancer at the preclinical and clinical levels (14,17, 31). Mallick et al. (3 -5) advocated that combination therapy 2ME + CP is less

toxic and safe for tumour-bearing mice. It also increases the survival time of the mice by decreasing the ascitic fluid volume and arresting the tumour growth.

Angiogenesis

Angiogenesis (i.e. the formation of new blood vessels) plays a critical role in the growth of cancer, particularly in the case of solid tumours that need a blood supply, which is the essential component for metastasis. This process is critical during tumour growth and metastasis (32,33). The objective of antiangiogenic therapy is quite different from other therapeutic approaches, as antiangiogenic drugs stop new blood vessels from forming around the tumour and break up the existing network of abnormal capillaries that deliver nutrients and oxygen to rapidly growing tumour cells. (33 - 35). The angiogenic process involves endothelial cells' migration, growth, and differentiation.

One of cancer's main characteristics is metastasis, i.e., the migration of cancer cells through the circulatory systems to the different organs of the body within a very short time. Therefore, most current cancer treatments aim to target against uncontrolled growth and metastasis.

Cancer treatment

Cancer treatment may be more successful when it is detected early, before it has spread. The metastatic process and tumour growth are angiogenesis-dependent.

Presently, surgery, radiation therapy, chemotherapy, immunotherapy, hormone replacement therapy, etc, are standard methods of cancer treatment.

1. **Surgery**: This is the earliest therapy established for cancer treatment and still the most widely used approach. Surgery is usually the first treatment option for cancer. According to Hellman and Vokes (36), surgical excision of a tumour is quick and effective and accounts for the most significant number of cures. Surgery is limited because the tumour cannot be treated or operated if the cancer cells are metastasized elsewhere in the body (37).
2. **Radiation Therapy** often eliminates or destroys cancer cells (38). During therapy, powerful X-rays or gamma rays are used to irradiate the region of the patient's tumour. Radiation treatments act either by inflicting genetic damage sufficient to kill cells directly or by inducing apoptosis. Cancer of the cervix, larynx, uterus, early stages of both prostate cancer as well as Hodgkin's disease are treated with radiation therapy (36). However, the standard cancer treatment consists of surgery plus radiotherapy, sometimes followed by chemotherapy (39,40).

3. **Chemotherapy and combination Chemotherapy**: Chemotherapy is a systemic treatment that inhibits DNA synthesis and mitotic cell division of proliferating tumour cells (41). It is a relatively new type of treatment for cancer in comparison to surgery and radiotherapy. Based on the stage and type of cancer, chemotherapy may be applied before or after surgery (38). In most cases, the drug is needed to reach every organ in the body for effective treatment. Although a large number of chemotherapeutic agents have been introduced to fight against cancer, only a few have found acceptance for clinical use. The use of 2 ME has been shown to enhance the effects in case of breast cancer treatment.

   Interestingly, 2-ME in combination with paclitaxel has shown promising results in preclinical models of breast cancer (42). Some common antineoplastic chemotherapeutic agents, including cyclophosphamide (CP), mitomycin-C, and cisplatin, have antitumour effects in different animal tumour models (1,4,5,7,43, 44). Therefore, various chemotherapy schemes are used, most of which consist of the association of separate drugs. The tumour's nature, distribution, and cell type determine the treatment mode. The patient's overall health condition is essential in chemotherapy and combination chemotherapy.
4. **Antiangiogenic therapy :** Antiangiogenic therapy seems to be a promising approach for treating cancer because antiangiogenic agents can break up new blood vessels of the tumour and also inhibit the process of angiogenesis. In 1989, a clinical trial of an antiangiogenic agent – interferon alpha- was initiated to treat hemangioma (32). Anti-angiogenesis is a novel approach to the treatment of gliomas (45,46). The combination effect of low-dose chemotherapy and antiangiogenic treatment showed promising results in some experimental tumours, i.e. lung carcinomas, retinoblastomas, lymphoma, and leukaemia (40,47,48). In the last few decades, scientists have identified numerous antiangiogenic agents that can trigger blood vessel growth (23, 24, 31,35).
5. **Immunotherapy**: Immunotherapy is a type of treatment which boosts the body's immune system to fight against cancer. It also stimulates the immune system to kill cancer cells by using antibodies. Cancer immunotherapy is a promising, innovative treatment for many forms of cancer, particularly melanoma (49). Monoclonal antibodies, Oncolytic virus therapy and T-cell therapy are some of the many types of immunotherapies (50, 51). These forms of immunotherapy are designed to target a specific antigen on the tumour or to enhance the host's immune system. Though

immunotherapy represents a new avenue in cancer treatment, specific immunotherapies can affect the intestine, kidney, heart and other organs. Moreover, immunotherapies affect the immune system, which may cause autoimmune disorders and other health problems.

6. **Hormone therapy:** The role of hormones in cancer treatment has been developed in the last few decades. Huggins and Hodges (52) used oestrogens to treat prostate cancer. Breast carcinoma is responsive to endocrine therapy. However, it has some toxic side effects like thrombosis, osteoporosis, etc. Exogenous oestrogen treatments may induce nausea, fluid retention, and cardiovascular problems (37). According to Fairchild et al. (53), exogenous hormones were administered to control the endocrine system by interfering with hormone production or the activity of hormone receptors.
7. **Targeted therapy:** An alternative strategy for cancer treatment is the development of drugs targeted specifically against the oncogenes that induce tumour growth (54). Target therapy is a type of cancer treatment that targets proteins that control cancer cells' growth and metastasis. This therapy uses new molecular drugs or other substances to identify and attack the cancer cell precisely. Targeted therapies can be designed so that they can interfere with the activity of cancer-causing genes. The pioneering drug in this category is Herceptin, a monoclonal antibody designed to combat the ErbB—2 oncogene protein, which is known to be overexpressed in breast cancer (54, 55). Other components, such as, bevacizumab is used for lung and colon cancer, and sorafenib for liver and kidney cancer (55).
8. **Stem cell Therapy:** Stem cells, with their unique and diverse biological actions such as self-renewal, differentiation, and modulatory effects on other cells, present a fascinating area of study in the field of cancer treatment (56). Stem cells have been identified in a wide variety of adult tissues such as the hematopoietic system, skin, intestine, heart etc. For example, epidermal stem cells can be used for skin grafts. Stem cells are also used during the treatment of cancer. Moreover, stem cell transplantation can restore blood stem cells in patients who have received high doses of chemotherapy or radiation therapy for cancer treatment. But in leukaemia, the stem cell transplant may work against cancer directly.

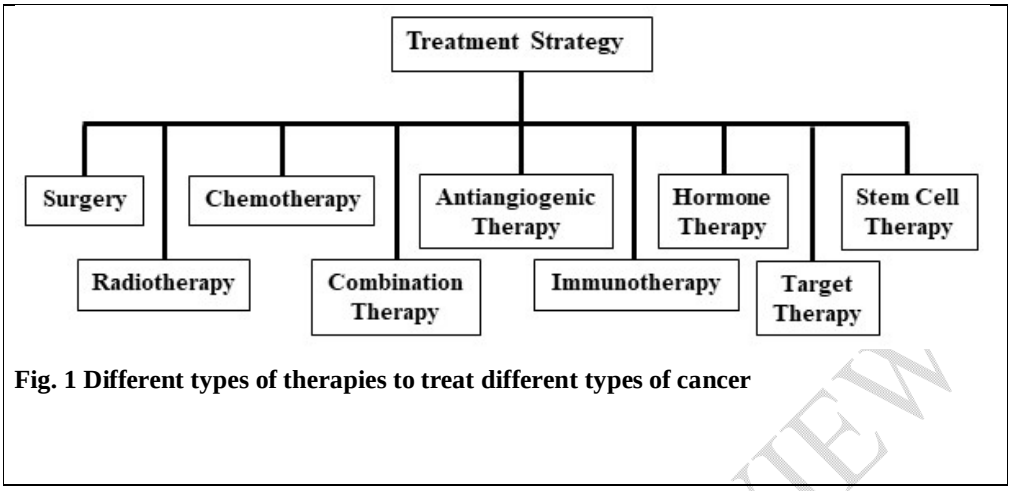

Fig. 1 Different types of therapies to treat different types of cancer

Natural Compounds and its effect on

Chemotherapeutic drugs often kill normal cells of the body during treatment. Natural compounds extracted from plants are presently used as medicines for cancer treatment and replace toxic chemotherapeutic drugs. Many herbs and spices also possess antioxidant and medicinal properties that exhibit anti-mutagenic and anti-carcinogenic potentialities. Thousands of herbal compounds are screened to investigate their antineoplastic properties (57 - 59). Scientists are trying to develop anticancer compounds from plants that may exhibit beneficial effects for cancer treatment with minimum toxic effects.

~~**Discussion**~~

Combination therapy, i.e. the use of two or three antineoplastic drugs, is more effective and less toxic than monotherapy. Bello et al. (40) demonstrated that low-dose chemotherapy combined with an antiangiogenic drug reduces tumour growth. Mallick et al. (3 - 5) advocated that combination therapy with a low dose of 2ME and CP significantly enhanced the therapeutic efficacy of monotherapy 2ME or CP. In addition, the combination effect of 2ME and Cyclophosphamide (CP) on the S-180 tumour cell line is anti-proliferative and less toxic. Moreover, the study also showed that treating 2ME and CP with lower concentrations antagonistically increased the life span of tumour-bearing mice and synergistically inhibited the viable cell population. Therefore, an effective combination dose induces a profound, significant result in cancer treatment.

~~**Conclusion**~~

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~~Our study describes the potential of combination therapy as a novel and reassuring approach to cancer treatment. It has the unique ability to inhibit haematological and cytological toxicities, thereby safeguarding the patient's body from the harmful effects of these drugs. In this book chapter,~~ we discuss the combination effect of 2ME and Cyclophosphamide (CP) on the sarcoma–180 tumour cell line. When used in combination, 2ME, an antiangiogenic drug, and cyclophosphamide (CP), ~~a conventional and cost effective chemotherapeutic drug,~~ do not only protect the host system but also inhibit tumour growth through the induction of apoptosis, thereby increasing the life span of tumour-bearing mice. This emphasis on reducing toxicities through combination therapy should instil confidence in its safety and efficacy.

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