BACTERIAL THERAPY: A NOVEL APPROACH FOR CANCER TREATMENT

Priyanka A. Mohite*, Dhanashree H. Surve, Manisha Karpe, Dr. Vilasrao Kadam

Department of Pharmaceutics, Bharati Vidyapeeth’s College of Pharmacy, CBD Belapur, Navi Mumbai-400614.

ABSTRACT

In the wake of growing global burden of cancer, newer cancer prevention and control modalities are being explored. One such novel experimental strategy is the implication of natural and genetically modified non-pathogenic bacterial species as potential antitumor agents. The first scientifically observed treatment dates back to the Nineteenth century, when bacteria were not even known to cause disease. The use of live, attenuated or genetically modified, non-pathogenic bacteria has begun to emerge as potential antitumor agents, either to provide direct tumoricidal effects or to deliver tumoricidal molecules. Bacteria also use as vector to delivering either tumoricidal agents or prodrug converting enzyme. Bacterial toxins are also showing anticancer activity, bacterial toxins along with antibody gives immunotoxins. Bacterial spores are also use in the treatment. Although bacteria showing promising results but there are some limitation using bacteria as anticancer agent.

KEYWORDS: Bacteria, prodrug, tumoricidal agents, immunotoxins, cancer.

INTRODUCTION

Cancer has become the second ranking cause of death in the industrialized world. It is a complex disease involving numerous tempo spatial changes in cell physiology, which ultimately lead to malignant tumors. Abnormal cell growth (neoplasia) is the biological endpoint of the disease. Tumor cell invasion of surrounding tissues and distant organs is the primary cause of morbidity and mortality for most cancer patients. The biological process by
which normal cells are transformed into malignant cancer cells.\textsuperscript{1} Conventional therapies for cancer such as chemotherapy and radiotherapy are characterized by poor survival rates due to multiple factors including tumor development of drug-resistance and their lack of tumor specificity, resulting in undesirable side effects on healthy cells and therefore limitations on therapeutic dose.\textsuperscript{2}

The idea to manage tumors with the aid of bacteria is not something new. The first scientifically observed treatment dates back to the Nineteenth century, when bacteria were not even known to cause disease. At that time, W. Busch (1868) tried to cure a female patient by first cauterizing her neck tumor and subsequently placing the woman into a bed, which has previously been occupied by a patient with “erysipelas”, a \textit{Streptococcus pyogenes} infection. As intended, the woman became infected and rapid tumor shrinkage occurred. In the following decades, several clinicians tried independently of each other to advance this therapy using different bacteria and bacterial components. “Coley’s toxin”, a mixture of inactivated Streptococci and \textit{Serratia narcescens} is still well known to oncologists. The use of live, attenuated or genetically modified, non-pathogenic bacteria has begun to emerge as potent antitumor agents, either to provide direct tumoricidal effects or to deliver tumoricidal molecules.\textsuperscript{1}

\textbf{Overview of Bacteria}

Bacteria constitute a large domain or kingdom of prokaryotic microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from shapes to rod to spirals. Most of the terminology used to define the host-microbe interaction has been in use for nearly a century. Early in this period, microbes were thought to be primary aggressors that governed the host-pathogen interaction, resulting in disease.

Later, new information about the attributes of microbes and their hosts resulted in the understanding that the host pathogen interaction does not always result in disease. This recognition, in turn, led to the introduction of terms to explain states in which microbes only cause disease in certain hosts. The understanding of the most intimate molecular mechanisms of the pathogen-host interaction might enable us to identify microbial functions required for pathogenesis or disease transmission that can be specifically target by research in the field of microbial pathogenesis.
Important concepts in microbial pathogenesis

Abundance/Diversity/Distribution of bacteria is present in nature. More recent views of microbial pathogenesis emanating primarily from studies of bacterial virulence have continued to focus on the ability of a pathogen to cause disease. Bacterial pathogenesis based on the identification of the genes responsible for causing disease. Pathogenesis/virulence factors/experimental approaches are important to study bacterial pathogenesis. Bacterial secretion are having role in virulence.

History

The observation that bacteria could be used as anticancer agent dates back 150 years. The German physician W. Busch and F. Fehleisen separately observed that certain types of cancer regressed following accidental Erysipelas an Streptococcus pyogenes infection that occurred whilst patient was hospitalized. Later the American William coley noticed that one of his patients suffering from neck cancer began to recover following an infection with Erysipelas. He developed a safe vaccine in late 1800’s composed two killed bacterial species S. pyogenes /Serratia marcescens the vaccine was known as “Coley’s toxins.” The vaccine has been used to treat carcinomas, lymphomas, melanomas, myelomas able to induce recoveries in final stages of disease. In 1931 Modern Operative Surgery states “after amputation, prophylactic injections of Coley’s fluid should be given in doses sufficient to cause a sharp febrile response.” In 1936 Coley’s vaccine endorsed in the New and Nonofficial Remedies of the American Medical Association “its use as a prophylactic in conjunction with conservative or radical surgery” and “inoperable cases may be quite justified” Decline in use of Coley’s toxins because of in 20th century the sterile cancer surgical procedures initiated. After Coley’s death in 1936, introduction of radiotherapy cancer treatments & gaining of acceptance to chemotherapy (both treatments more easily standardized) but counter to immunotherapy both highly immunosuppressive. During World War II increased in use of antibiotic further decreased post-surgical infections / severity & duration of infections. Antipyretics came into routine use to eliminate discomforting symptoms of immune response. In comparison of 10 year survival rates of past successes using Coley’s regimen to modern conventional cancer treatment patients receiving modern conventional therapies did not fare better than patients receiving treatment initiated by Coley over 100 years ago.
Mechanism of bacteria as antitumor agent

After Coley's initial observations, scientists discovered that certain species of anaerobic bacteria, such as those belonging to the genus *Clostridium*, thrive and consume oxygen-poor cancerous tissue whereas die when they come in contact with the tumor's oxygenated sides, meaning they would be harmless to the rest of the body. These findings provided the rationale for using the bacteria as anticancer or antitumor agents. However, bacteria don't consume all parts of the malignant tissue thus underlying the need of combining the therapy with chemotherapeutic treatments. Thus bacteria can be implied as sensitizing agents for chemotherapy. Bacterial products like endotoxins (Lipopolysaccharides) have to some extent already been tested for cancer treatment. Bacterial toxins can be used for tumor destruction and cancer vaccines can be based on immunotoxins of bacterial origin. Bacteria can be exploited as delivery agents for anticancer drugs, as vectors for gene therapy. Spores of anaerobic bacteria can be used for the aforementioned strategies because only spores that reach an oxygen starved area of a tumour will germinate, multiply and become active. The use of genetically modified bacteria for selective destruction of tumors and bacterial gene-directed enzyme prodrug therapy have shown promising potential.[3]

Applications of bacteria in cancer therapy:
1. Whole live, attenuated or genetically-modified
2. Bacteria as vector
3. As immunotherapeutic agent
4. Bacterial toxin
5. Bacterial spors

1. Whole live, attenuated or genetically modified

Some bacterial species can also preferentially replicate and accumulate within tumors. In contrast to viruses, the bacteria reside primarily in the extracellular tumor micro environment and possess certain features that may be advantageous in the treatment of cancer. Thus, bacteria are motile, which facilitates their spread throughout the tumor and can help target systemic disease.[4] Our body is comprised of numerous cells which undergoes cell division. All these activities are controlled by molecules in our body. Normal cells regenerate in proper manner and they will be shattered after the proposed cell life. But in a cancerous site, the cells regenerate again and again inconsistently and pass through uncontrolled multiple cell divisions. This abnormal activity leads to the formation of cancer lumps and mass at different
parts of body, which in turn leads to solid tumors. Cancerous cells multiply continuously contrary to normal cell growth. Nutrients are one of the essential elements required for the body. Our body has its own mechanism in procurement of nutrients. Normally, blood vessels act as carriers of nutrients all over the body. In a cancerous state, nutrient won’t be reaching the cancerous affected areas properly. In this state, alternative blood vessels will be formed by the cells for meeting the adequate nutrients requirements. Even though alternative blood vessels exist, they won’t be able to meet the normal functionality of a blood vessel. Moreover enormous cancer masses will be present here. This area will be oxygen deficient and are commonly termed as oxygen deprived areas. Since the cancerous areas consist of anaerobic portion too, by using of anaerobic bacteria for the treatment of solid tumors play a new lead against solid tumors as shown in Fig.1. Several scientist and genetically expertise people discussed this and conclude anaerobic Bactria as a promising assisting agent for solid tumors or cancers. Anaerobic bacteria become active in oxygen deprived environment, the anaerobic sites of cancerous area were considered as perfect sites for breeding of these species. These bacteria multiply and destroy the cancerous cells present in the solid tumors. This was considered as an efficient treatment procedures against cancer. The primary bacteria experimented for cancer therapy was bacteria belonging to clostridium class. Even though it showed better results the animals were experimented on died due to acute toxicity. This resulted to focus of a non-pathogenic strand of Clostridium such as M55, which showed promising results.\[5\]

Fig.1: Mechanism of anaerobic bacteria.
DISADVANTAGES
1. Bacteria cannot be directly incubated inside the body.
2. Several risk factors like occurrence of bacterial infections, disturbing the immune system of the body etc.
3. Bacterial don’t consume all parts of malignant tissue thus underlying the need of combining the therapy with chemotherapeutic treatments.

Examples of attuned bacteria
Bacillus Calmette-Guerin (BCG) is an attenuated, live culture of the bacillus Calmette and Guerin strain of *Mycobacterium bovis*, which induced a granulomastus reaction at the site of administration. By nuclear mechanisms, this preparation is active against tumors and is indicated for treatment and prophylaxis of carcinoma in most successful agent so far is used specifically for the treatment of superficial bladder cancer. Clostridium and Bifidobacterium, will be evaluated in human clinical trials in further.[6] New strains of bacteria begin investigated as anticancer agents which are *Salmonella choleraesuis*, *Vibrio cholerae*, *Listeria monocytogenes* and even *Escherichia coli*.[3]

Bacteria as vector
The major problem with using bacteria as anti-cancer agents is their toxicity at the dose required for therapeutic efficacy and reducing the dose results in diminished efficacy. The basic obstacle in cancer gene therapy is the specific targeting of therapy directly to a solid tumor. One approach to overcome these limitations has been the use of bacteria, genetically engineered to express a specific therapeutic gene. By producing the protein of interest specifically in the tumor micro-environment, these bacterial vectors can provide a powerful adjuvant therapy to various cancer treatments as shown in Fig.2. Thus bacteria serve as vectors or vehicles for preferentially delivering anticancer agents, cytotoxic peptides, therapeutic proteins or prodrug converting enzymes to solid tumours.[7][8] Various preclinical trials have shown the ability of different bacterial strains to transport and amplify genes encoding factors such as prodrug converting enzymes, toxins, angiogenesis inhibitors and cytokines specifically within tumors. Indeed, systemic delivery can be achieved not only through IV administration but for some species, also through oral administration of commensal non-pathogenic or pathogenic strains.[2]
Bacteria as vector for gene therapy divide into two types.

Bacteria delivering tumoricidal agents

It is the most direct gene therapy strategy to treat tumors involves introducing a vector and gene to a malignant cell that directly induces death of that cell. There are a number of mechanisms by which this can be achieved, including the delivery of genes cytotoxic to the cell (pro-apoptotic genes or so-called ‘suicide genes’) or through oncolysis induced by the bacterial vector itself (as is observed with Clostridium and Salmonella) Oncolytic vectors. The oncolytic approach uses replication competent bacteria that are capable of spreading through the tumor tissue to infect neighboring cells with cancer cells killed as a result of infection. Therapeutic trials employing clostridial species mainly rely on the natural oncolytic activity of the vector to achieve tumor therapeutic responses.\(^2\) *Salmonella typhimurium* has now been fully developed for use in cancer treatment. Deletion of two of its genes msbB and purI resulted in its complete attenuation by preventing toxic shock in animal hosts and dependence on external sources of purine for survival. This dependence renders the organism incapable of replicating in normal tissue such as the liver or spleen, but still capable of growing in tumours where purine is available. This bacterium showed long-lasting efficacy against a broad range of experimental tumors and was even able to target metastatic lesions. One attenuated *S. Typhimurium* strain, VP20009, appears to have a favorable safety profile in humans, having been administered systemically to colon cancer and melanoma patients in phase I trials with minimal side effects. This strain bears attenuating mutations that reduce the toxicity of its lipopolysaccharide and create a requirement for an external source of adenine.\(^2\)[9] Various therapeutic proteins, including TNF-α and platelet factor 4 fragment, have been cloned and expressed in VNP20009. hIL-12, hGM-CSF, mIL-12 and mGM-CSF have been cloned under the control of a cytomegalovirus (CMV) promoter into SL3261, an auxotrophic *S. typhimurium*. It was found that oral administration of Salmonella expressing mGM-CSF or mGM-CSF plus mIL12 caused tumor regression in mice bearing Lewis lung carcinomas. Functional TNF-α has been cloned and expressed in *C. acetobutyllicum*. *Bifidobacterium adolescentis* has recently been used as a delivery system for the antiangiogenic protein endostatin.\(^7\)

a) Bacteria mediated prodrug therapy

*Clostridium acetobutylicum* and *Clostridium sporogenes* expressing cytosine deaminase (CD) and nitroreductase (NTR) significantly delayed tumor progression. CD promotes tumor site-specific conversion of 5-fluorocytosine (5-FC) prodrug into its active form (5-fluorouracil, 5-
FU) whereas NTR catalyzes the reduction of the prodrug metrodinazole (Mtz) thereby producing a toxic compound.\[^{10}\]

![Fig. 2: Bacteria-Mediated Prodrug Therapy.](image)

3. **Immunotherapeutic agent**

Immunotoxins are proteins that contain a toxin along with an antibody or growth factor that binds specifically to target cells. Immunotoxins are created by chemically conjugating an antibody to a whole protein toxin, devoid of its natural binding domain. Immunologic proteins that are smaller than monoclonal antibodies (MoAbs), like growth factors and cytokines, have also been chemically conjugated and genetically fused to protein toxins. Radiation therapy has been considered as more reliable one for cancer therapy. However, radiation therapy for cancer severely lowers the quality of life for the treated patients, resulting in nausea, hair loss and a severe drop in energy. Drug therapy, or chemotherapy, most often is also accompanied by these side effects. The problem is that chemotherapeutics and radiation therapy are poorly selective in which cells they attack. By tethering therapeutic agents to antibodies, "magic bullets" could be produced that specifically bind and deliver the therapeutic agent to sick or kill cells, having little or no effect on healthy cells in the body. This works because antibodies are proteins that have exquisite binding selectivity which can be produced to bind to only one targeted protein, while ignoring a multitude of other proteins they might come in contact with. Immunotoxins are produced in *Escherichia coli* transformed with a plasmid encoding the recombinant toxin.\[^{11}\]
4. Bacterial toxin

Bacterial products like endotoxin (lipopolysaccharides) have to some extent already been tested for cancer treatment. Bacterial toxins can be used for tumor destruction and cancer vaccines can be based on immunotoxins of bacterial origins. Bacterial toxins can kill cells or at reduced levels alter cellular processes that control proliferation, apoptosis and differentiation. These alterations are associated with carcinogenesis and may either stimulate cellular aberrations or inhibit normal cell controls. Cell-cycle inhibitors, such as cytolethal distending toxins (CDTs) and the cycle inhibiting factor (Cif) block mitosis and is thought to compromise the immune system by inhibiting clonal expansion of lymphocytes. In contrast, cell-cycle stimulators such as the cytotoxic necrotizing factor (CNF) promote cellular proliferation and interfere with cell differentiation. Bacterial toxins act by two ways.

a) Bacterial toxins binding to tumor surface antigens

Diphtheria toxin (DT) binds to the surface of cells expressing the heparin-binding epidermal growth factor like growth factor (HB-EGF) precursor. DT-HB-EGF complex is internalized after endocytosis via clathrin-vesicles. Subsequently DT undergoes several post translational modifications resulting in a catalytically active toxin, called DT fragment A. This catalytically ribosylates elongation factor-2 (EF-2) leading to inhibition of protein synthesis with subsequent cell lysis and/or induction of apoptosis. Like DT, Pseudomonas exotoxin A is also known to catalytically ribosylate EF-2 and thus leading to inhibition of protein synthesis shown in below figure.

![Fig.3: Mechanism of Diphtheria toxin (DT)](image-url)
b) **Bacterial toxins conjugated to ligands**

Protein toxins such as Pseudomonas exotoxin, diphtheria toxin, and ricin may be useful in cancer therapy because they are among the most potent cell-killing agents.

Although they are very lethal yet for therapeutic efficacy these toxins need to be targeted to specific sites on the surface of cancer cells. This process is accomplished by eliminating binding to toxin receptors by conjugating the toxins to cell-binding proteins such as monoclonal antibodies or growth factors. These conjugates bind and kill cancer cells selectively thus sparing normal cells, which don't bind the conjugates. A wide variety of DT ligands such as IL-3, IL-4, granulocyte colony stimulating factor (G-CSF), transferrin (Tf), EGF and vascular endothelial growth factor (VEGF) have been studied for targeted tumors. The transferrin-DT conjugate (Tf-CRM 107) and DT-EGF have reached the stage of clinical trials inpatients of brain tumor and metastatic carcinomas respectively. Similarly a large variety of antibodies and ligands to surface antigens overexpressed in different tumors have been conjugated to PE. Important ones tested in clinical trials are IL-4, IL-13, monoclonal antibody-recognizing a carbohydrate antigen Lewis Y, reacting with metastatic adenocarcinoma cells (Mab B3) and transforming growth factor (TGF-α).[^7]

5. **Bacterial spores**

The majority of all the anaerobic bacteria can form highly resistant spores which allow them to survive even in oxygen-rich conditions, although they cannot grow or multiply there. But once they meet favorable conditions, such as the dead areas inside tumors, the spores can germinate and the bacteria thrive, making them ideal to target cancers. *C. novyi-NT* spores were administered in combination with conventional chemotherapeutic agents like dolastatin-10, mitomycin C, vinorelbine and docetaxel. This strategy known as combination bacteriolytic therapy (COBALT) resulted in significant anti-tumour properties but still was not devoid of animal deaths.[^7]
**Limitation of Bacterial therapy**

i) Whole bacteria can be used in their live, attenuated, or genetically modified forms to stimulate immune responses, but this may potentially result in side effects.\(^{12}\)

ii) Systemic infection of bacteria is rather inconvenient and carries higher risk of obvious toxicity.

iii) Bacteria don't consume all parts of the malignant tissue thus necessitating the combination of therapy with chemotherapeutic treatments.

iv) Another major concern regarding bacterial therapy is the potential for DNA mutations i.e. any loss of functionality due to mutations may lead to wide variety of problems like failure of therapy or exaggerated infection.\(^{7}\)

**CONCLUSION**

Bacteria in the treatment of cancer showing promising result. Current tumor therapies have limited efficacy because they are extremely toxic, weakly infiltrate tumor tissues, and incompletely target tumors. By combination of two methods problem has been solved, COBALT is one of the method in which bacterial therapy combine with cytotoxic agents. Bacterial spores are having high affinity and low toxicity over live bacteria. Bacterial toxin with cell protein act sit specific so targeting is possible. The studies are going I and II clinical trials. To minimize side effect related to bacteria further studies are required to improve efficiency and efficacy of this type of system.
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