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Introduction of Antibiotics in Cancer Treatment

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Abstract

As one of the most fatal disease for human being, cancer treatment is always a significant topic in the field clinical medicine. In this article, the author explores two antibiotic medicines that have strong anti-cancer effects: Mitomycin C and Bleomycin. Generally speaking, antibiotics are used exclusively to treat bacterial infections. Therefore, it's intriguing that how antibiotic works to destroy cancer cells. The article introduces multiple aspects of mitomycin C and bleomycin, including mode of action, biosynthesis, chemical synthesis, self-resistance. Concepts such as DNA alkylation, DNA cleavage, PKS /NRPS system and etc. The author concisely summarized the significant research conclusions in the past few decades and organized different resources into a comprehensive and intact description of mitomycin C and Bleomycin. To the best of today's knowledge, both mitomycin C and Bleomycin damage DNA molecules of cancer cells. Mitomycin C alkylates the DNA molecules and causes cross linkage of DNA. On the other way, bleomycin directly cleaves the DNA molecules of cancer cells. Although many chemical synthesis pathways have been proposed for mitomycin and bleomycin, fermentation is still the mainstream of industrial manufacture due to the low yield of chemical synthesis. The current literatures regarding the anti-cancer antibiotics are extremely focused on a specific aspect. The article aims to provide a review and summary of literatures and gives audience a general idea of mitomycin c and bleomycin in cancer treatment as well as the progress of scientific research on these anti-cancer antibiotics.

Keywords

Antineoplastic antibiotics, Cancer Treatment, Mitomycin C, Bleomycin, DNA Damage, Biosynthesis, Antibiotic Synthesis, Gene Resistance.

1. Introduction

Cancer, one of the leading death-causing diseases in the world, claims approximately 10 million's people life every year. Human's fight against cancer has a long history. In United States, the chance for a person to develop cancer during their lifetime is 50%. The rate is higher for male than female. The mortality rate of cancer is extremely high. The exact mortality rate of cancer depends on the stage of malignant cancer. Overall, the 5-year survival rate for cancer is approximately 66% in US. Nowadays, we have discovered many drugs for cancer treatment. Even so, the complete cure of cancer is still challengeable.

Antibiotics are generally used to cure bacterial infection. However, modern medicine studies have discovered few antibiotics which possess strong anti-cancer effects. We have learned there are at least three antibiotics that can be used or have potential to treat cancer in combination with other drugs: Mitomycin C, Bleomycin and epothilone. In these three medicines, epothilone is still at its clinical trial stage and its features and effects are not fully understood yet, while Mitomycin C and Bleomycin

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has been approved for cancer treatment for many years and their functions and effects are already well-understood.

1.1 Mitomycin C

Mitomycin C is mainly used as a chemotherapeutic drug for adenocarcinoma of stomach or pancreas. It is classified as an antitumor antibiotic [1]. Structurally, the major functional group of Mitomycin C is aziridine, carbamate and 1,4-benzoquinone, which actively involve with the reductive activation and DNA alkylation of mitomycin C. Nowadays, fermentation of mitomycin c is still the mainstream of industrial manufacture [2].

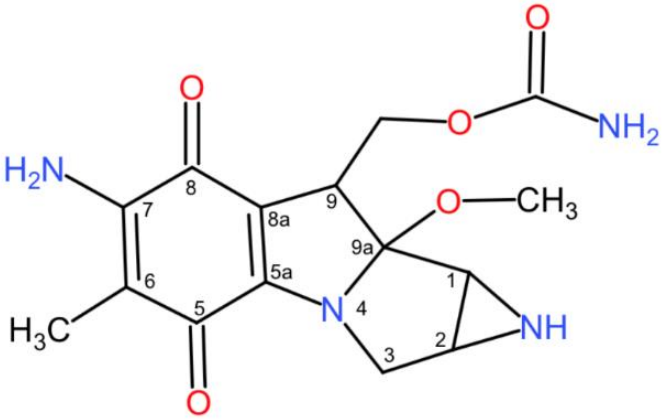

Figure 1. The Structure of Mitomycin C

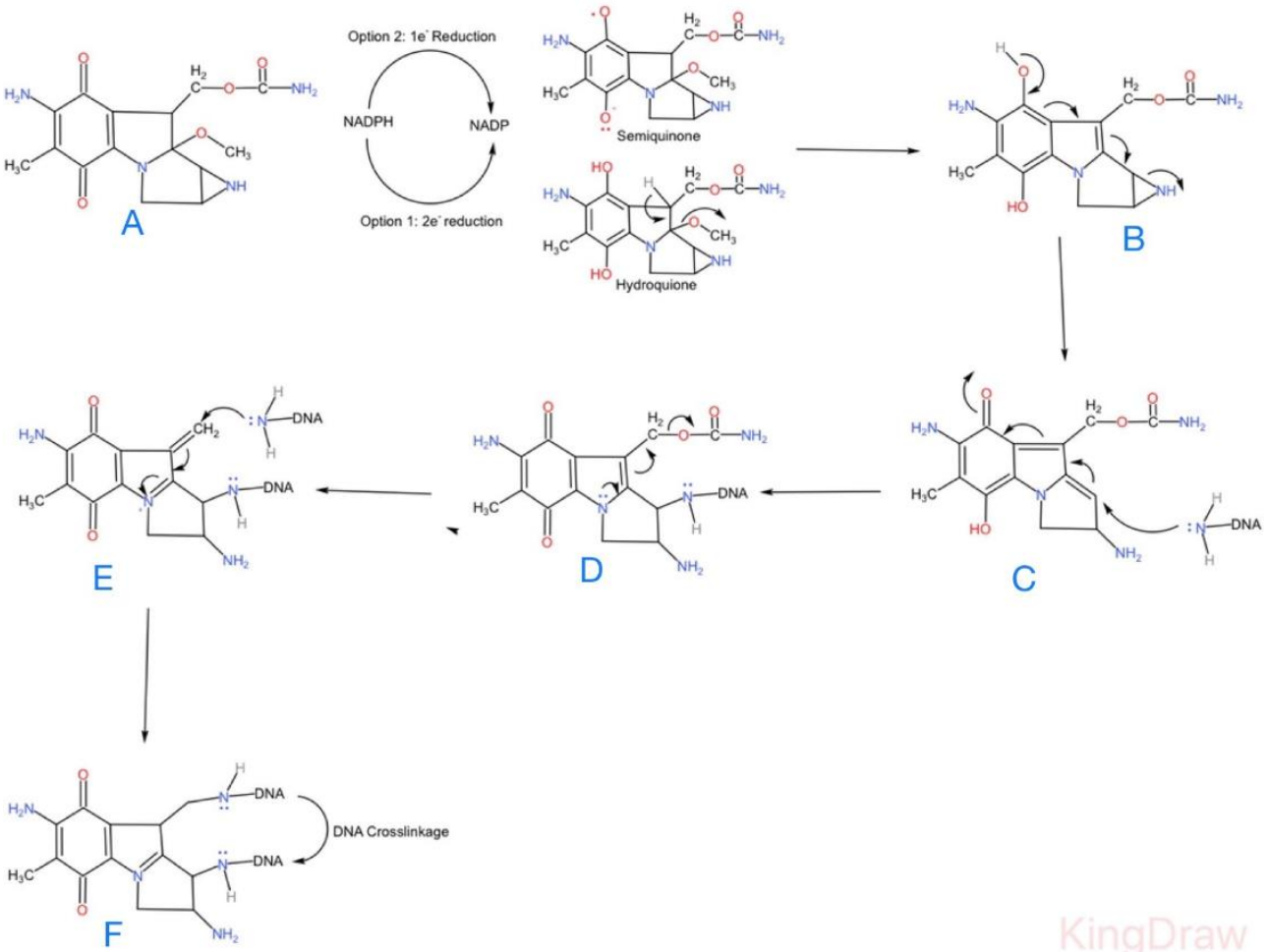

Figure 2. Reductive alkylation of Mitomycin C and Alkylation of DNA

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Essentially, Mitomycin C's anti-cancer effect is achieved through alkylation of DNA, generating interstand and intrastrand DNA cross linkage. DNA cross linkage blocks the separation of DNA strands and the formation of replication fork. This directly results in the termination of RNA transcription and DNA replication. Therefore, DNA cross linkage is extremely detrimental to the process of cell division.

Reductive activation of Mitomycin C is a necessary step that precedes the alkylation of DNA. From A to C, the proto-Mitomycin C undergoes one- or two-electron reduction on the 1,4-benzoquinone, followed by the loss of methoxide. The resulting highly reactive intermediate C is subsequently rearranged for DNA nucleophilic substitution. The first nucleophilic substitution enables the DNA to bind the five-membered ring as shown in E. Meanwhile, the nucleophilic site of DNA can also attack the electrophilic carbon of carbamate group, and add onto the activated Mitomycin C as shown in F. The entire process of A to F generates a di-alkylated product and causes the cross linkage of DNA.

Multiple enzyme systems can be selected to trigger the reductive activation of Mitomycin C: DT-diaphorase, NADPH-cytochrome P-450 reductase, NADPH-cytochrome C reductase and etc. The relevant studies on the binding sites of DNA nucleotide base and activated Mitomycin C have shown the possible position on DNA is N2, N7 of guanine residues and N6 position of adenine residues due to their relatively high nucleophilic strengths [3].

Mitomycin C is mostly used as anti-tumor drug and barely prescribed for bacterial infection unless it poses a severe threat to patient's life. The mechanism of action for Mitomycin C manifests its significant cytotoxicity. The reason that Mitomycin C can successfully kill tumor cell is due to its cytotoxic selectivity for hypoxic cells which characterize the malignant tumor cells. Therefore, Mitomycin C works most dominantly in those rapid division cells such as cancerous cells as well as Hair follicle stem cells, which also explains its side effects of hair loss.

1.2 Biosynthesis of Mitomycin C

The biosynthesis of Mitomycin C is achieved through the condensation of 3-amino-5-hydroxybenzoic acid (AHBA) and D-glucosamine [4] as well as the subsequent addition & elimination reaction between D-glucosamine and carbamoyl phosphate. The biosynthesis of Mitomycin C can be initiated in two ways: formation of the C-C bond by aromatic alkylation or acylation or formation of Schiff base at D-glucosamine aldehyde with subsequent ring closure reaction [7]. Meanwhile, both reactions jointly contribute to the formation of the five membered rings. The completion of Mitomycin C biosynthesis also requires several necessary tailoring modifications to the premature product, mitosane core. The tailoring of the mitosane core involves functionalization at different sites: Methylation of carbonyl, Reduction of carbonyl, oxidation of hydroxyl and formation of aziridine ring. Based on the retrosynthetic analysis, AHBA is recognized as the precursor of the Mitomycin C.

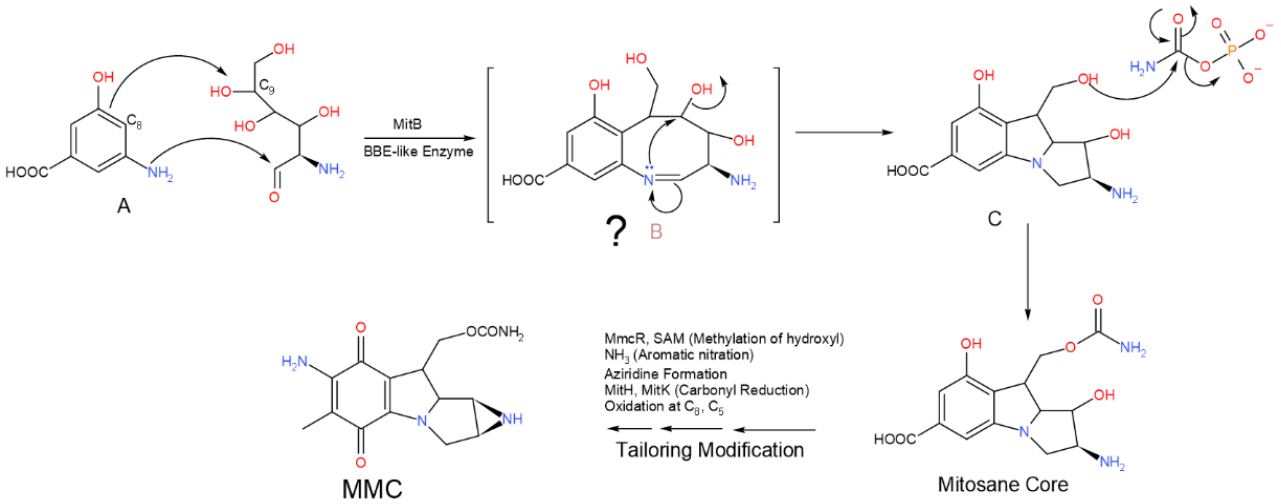

Figure 3. Proposed Mechanism for biosynthesis of Mitomycin C

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1.3 Chemical synthesis of Mitomycin C

The overall structure of Mitomycin C is compact and densely, causing the compound's functional groups to be highly interactive. The reaction-active sites of Mitomycin C pose lots of challenges for its chemical synthesis. The hemiaminal structure is likely to lose the methanol at C_{9a}, and the aziridine ring is also prone to ring opening reaction under various conditions.

However, Dr. Yoshito Kishi still overcame the difficulties and firstly synthesized mitomycin in 1977. The synthesis method devised by Kishi is briefly illustrated in Fig.4. Kishi's synthesis starts with the phenol A and the following Claisen Rearrangement with Allyl Bromide. A series oxidations and reductions are carried out with incorporation of benzyl protecting group gives the compound C. In Kishi's synthesis, benzyl protection is crucial for stabilizing the aziridine group and other electrophilic sites of the intermediates. The introduction of aziridine ring is achieved by transferring nitrogen to the alkene. The transferring of nitrogen to the alkene requires the use of strong oxidizing agent, osmium tetroxide, which takes a week of reaction time to produce the diastereomeric mixture of diol. And the diol D is selectively obtained through separation techniques such as chromatography. The diol D is then treated with sodium hydride to form an epoxide E. Subsequently the epoxide is opened by lithium azide to introduce the nitrogen into the system. The azide can be easily reduced to amine, and subsequently a simple Sn2 reaction will result in the aziridine ring G. In consideration of the high reactivity caused by the ring string, the aziridine is protected by a phosphate protecting group which can be later deprotected by lithium aluminum hydride. The addition of H_2/Pd-C removes the benzyl protecting groups, and the O_2/MeOH oxidize the para-catechol to quinone. The eight membered ring formation results from the Michael addition of primary amine. The key step of Kishi synthesis is the trans-annulation in the presence of tetrafluoroboric acid as the catalyst, leading to two pyrrole-indole-like five membered rings. At last, the removal of aziridine protecting group and carbamylation lead to the formation the mitomycin A, and mitomycin A can be simply converted to mitomycin C with ammonia.

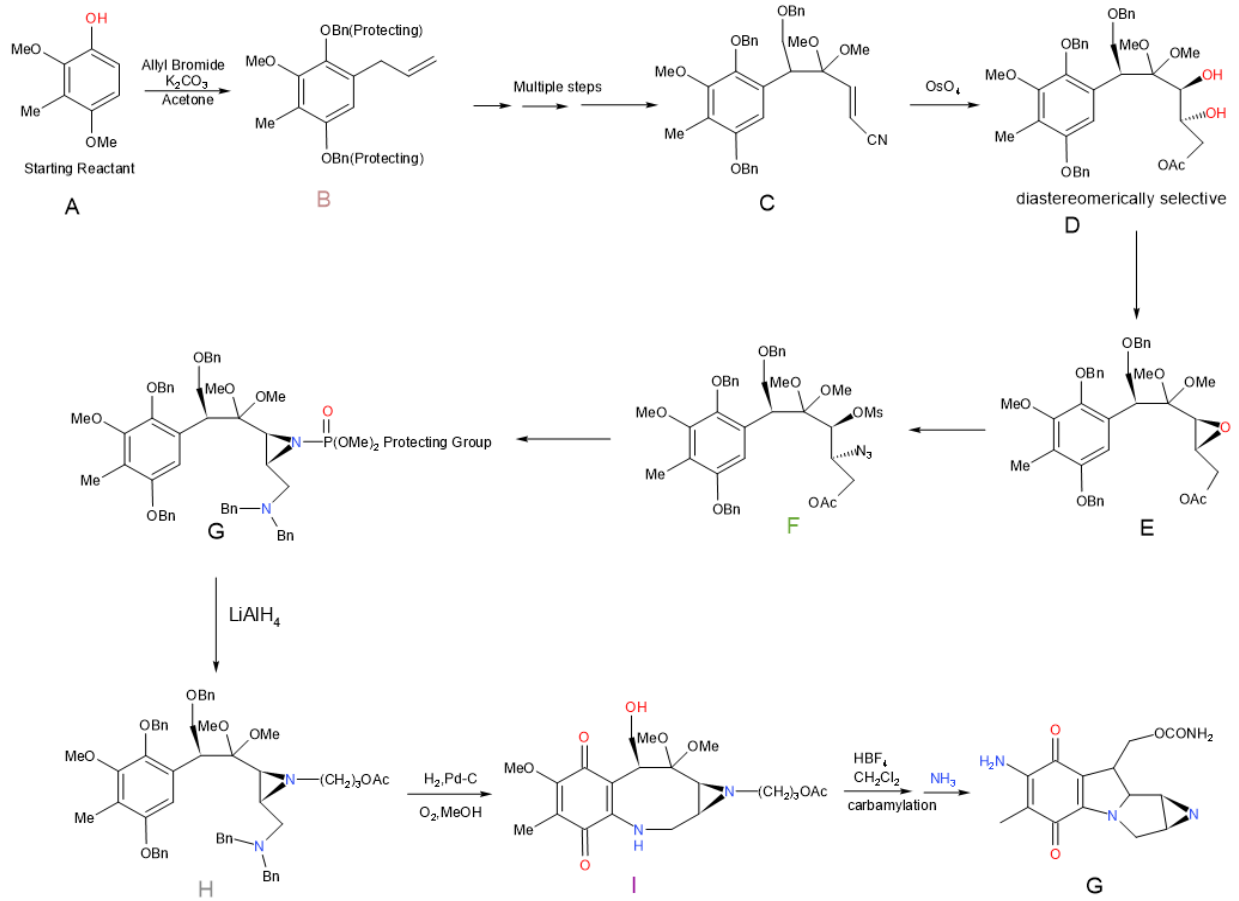

Figure 4. Brief Illustration of Kishi's Synthesis of Mitomycin

ISSN: 2414-1895 DOI: 10.6919/ICJE.202101_7(1).0043

The total Kishi synthesis involves more than 40 steps. Consequently, the global yield of Kishi synthesis is just 0.16% [5]. Despite the low yield, Kishi synthesis still set a landmark as the first successful synthesis of mitomycin.

1.4 Antibiotic Gene Resistance of Mitomycin

The high-level resistance gene is determined to be mcrA [4]. McrA encodes the mitomycin oxidases which catalyze the oxidation reaction of semiquinone or hydroquinone. The re-oxidation of semiquinone and hydroquinone convert them back to the prototypical mitomycin C and stops the activation of mitomycin C. This process deprives the mitomycin c of its ability to alkylate DNA and avoids the DNA cross linkage.

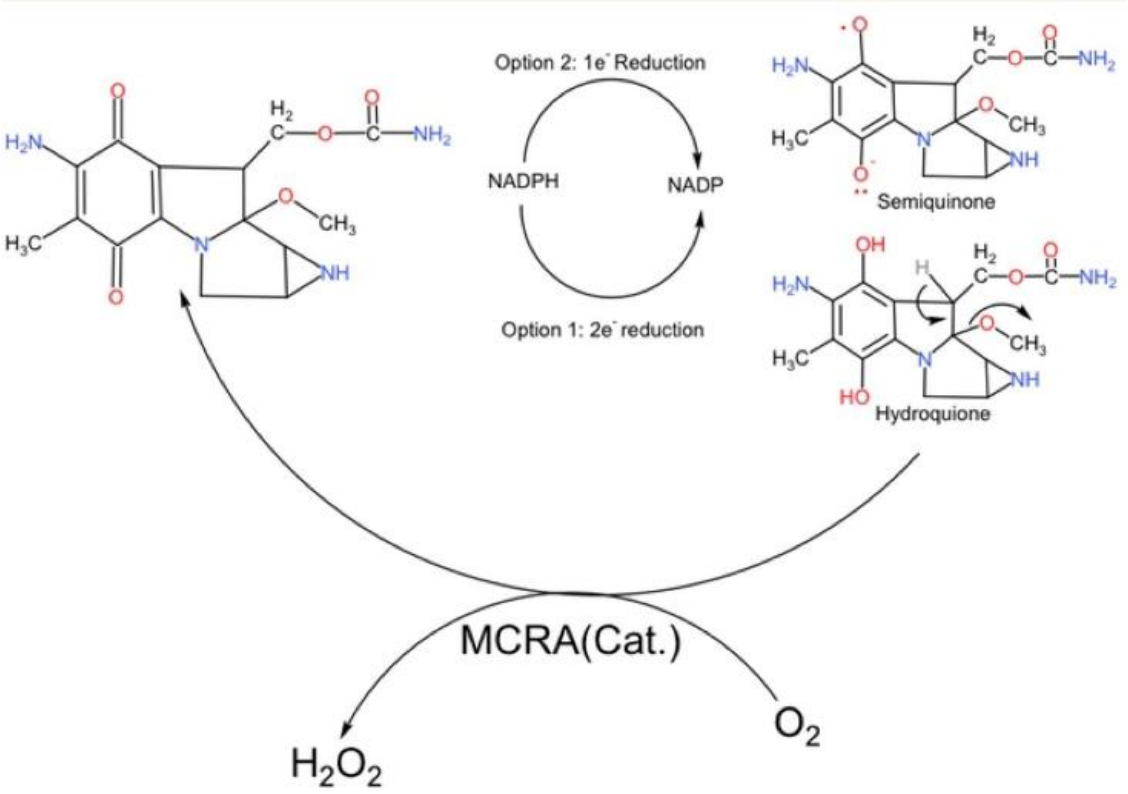

Figure 5. Antibiotic Resistance Mechanism of Mitomycin C

2. Main body

2.1 Bleomycin

Bleomycin is a glycopeptide antibiotic which generally used as anti-cancer chemotherapy drug [6]. Industrial manufacture of bleomycin is mainly achieved through fermentation. In terms of functionality, the structure of prototype bleomycin can be divided into four domains. The characteristics of metal binding domain and DNA binding domain are known through experimental studies, while the functions of disaccharide moiety are still not well-understood. It's clear that the metal binding domain is likely to share its nitrogen to constitute the metal chelate, and the tripeptide and bithiazole of DNA binding domain are related to the binding of bleomycin to DNA.

The Bleomycin-induced DNA degradation requires activated bleomycin chelate.[7] The activation mechanism of bleomycin is analogous to the activation of cytochrome P450 Mechanism. Activated Bleomycin can cleave the intramolecular bonds of DNA molecules, especially at the phosphodiester bonds. Experimental studies have identified more than 130 cleavage sites at DNA and RNA. Presently, a relatively common mechanism is already figured out, which might associate with both single strand and double strand break. It's worth noting that the DNA cleavage of bleomycin is highly sequence selective. The most preferred recognition sites for bleomycin is G-Py (G-C or GT) [8]. Different modes of DNA cleavage correspond to different preference sites.

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The activated bleomycin is expected to abstract C-H bond at C_4 of deoxyribose and converts it to a carbon-based radical. After that, the modified deoxyribose radical will be facing two pathways. Path A is triggered by the radical hydroxylation reaction and ends up with the cleavage of nucleotide base. Path B proceeds in the presence of O_2 and disassemble the nucleotide into three parts including the base propenal which is not observed in path A. In path B, there might be a branch which undergoes a series of reaction and transformation and eventually generate the exact same products as the other Path B1 [9].

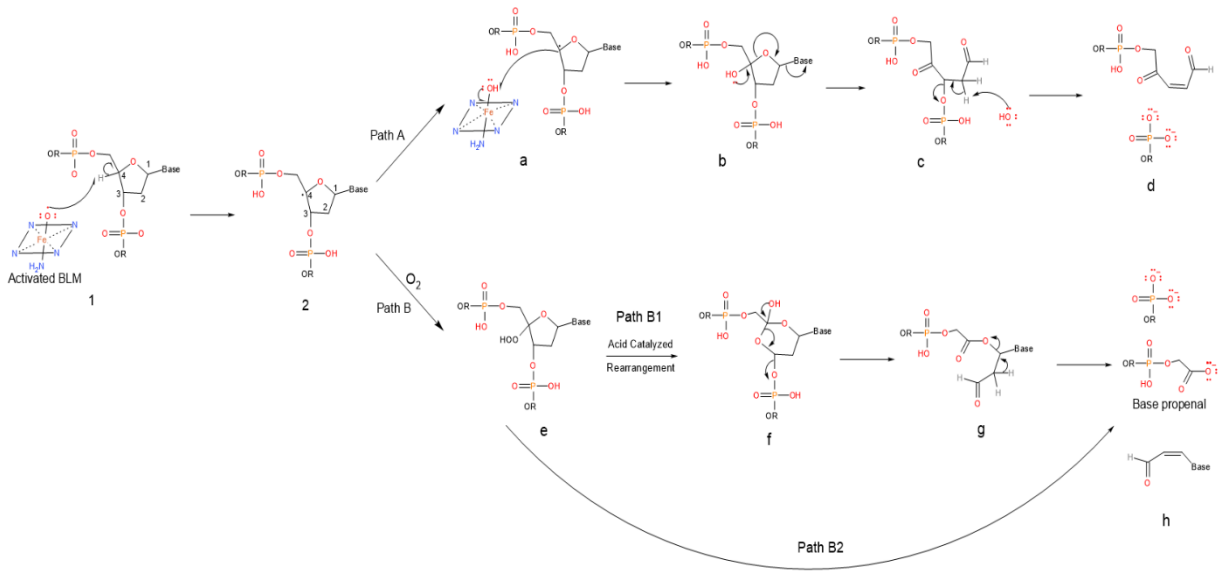

Figure 6. The Proposed mechanism for Bleomycin-induced DNA degradation

2.2 Biosynthesis of Bleomycin

Bleomycin is isolated from the metabolites of Streptomyces verticillus. Structurally, bleomycin consists of both peptide chains and polyketide segments. The proposed biosynthesis of bleomycin was inspired by the nonribosomal peptide synthesis and polyketide synthesis.

The proposed biosynthesis begins with the condensation of five amino acids: L-serine, L-histidine, L-Alanine and two L-asparagine. After the polypeptide condensation, the decarboxylative polyketide condensation proceeds with Malonyl Coenzyme A and S-adenosylmethionine. The resulting intermediate is pushed to undergo a β-keto reduction. At this point, the polyketide segment is successfully inserted. In the next few steps, L-threonine, β-alanine and two L-cysteines are added on through non-ribosomal peptide synthesis, which completes the synthesis of aglycone [10].

2.3 Chemical Synthesis of Bleomycin

The chemical synthesis of bleomycin is built of 5 structural subunits: disaccharide moiety (A), (S)-erythro-β-hydroxyhistidine (B), 4-amino-3-hydroxy-2-methylvaleric acid (D), threonylbithiazole moiety (F) and pyrimidoblamic acid (H). For chemical synthesis of bleomycin, the choice of protecting group directly affects the outcome of the strategy. As shown in Fig. 7, the strategy is a revised version which successfully increased the overall yield to 23%.

The revised synthesis starts with a modified disaccharide moiety. One of the unprotected carbamoyl groups is replaced by a glucopyranosyl chloride. And this modified disaccharide moiety is catalyzed to react with the (S)-erythro-β-hydroxyhistidine protected by tert-Butyl carbamate. The interesting part of this strategy is the deprotection of either benzyl group or Boc must be conducted in each step so that the sequential condensation can proceed without obstacles. In the following steps, the 4-amino-3-hydroxy-2-methylvaleric acid (D) and threonylbithiazole moiety (F) are condensed onto the intermediate C, E in sequence. The entire synthesis ends with the intermediate G condenses onto the Boc-pyrimidoblamic acid (H) in the presence of Benzytriazol-1-yloxytris phosphonium (BOP Reagent) and gives the Bleomycin [11].

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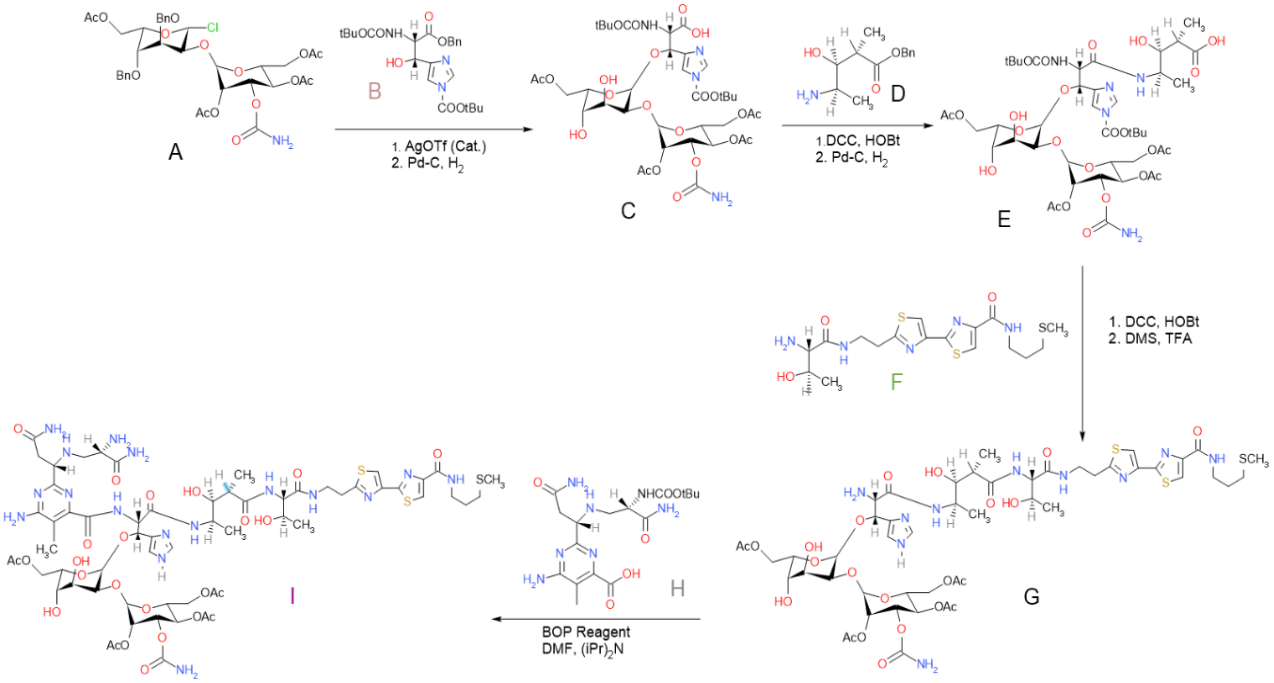

Figure 7. The Chemical Synthesis Mechanism of Bleomycin

2.4 Antibiotic Gene Resistance of Bleomycin

The primary resistance mechanism of Bleomycin is dictated by BlmA. BlmA can directly bind to the metal coordinated bleomycin and hinder the further oxidative activation. The alternative way is considered to involve with BlmB. The mechanism for BlmB's antibiotic resistance is slightly different from BlmA. BlmB dictates the acetylation of BLM's primary amine, which requires the metal-free form of bleomycin. Compared to the metal-free bleomycin, the structure of metal-coordinated bleomycin is so compact and densely that primary amine can hardly be exposed to the acetyl coenzyme A. In this case, acetylation of bleomycin can block the binding of O_2 and the activation of bleomycin, which precludes the DNA cleavage in a very similar way as BlmA [12]

3. Conclusion

The discovery of mitomycin and bleomycin in 1950-1960s starts the chapter of antibiotic treatment for cancer. In clinical practice, mitomycin c and bleomycin are commonly used as chemotherapeutic agents in combination with other anti-cancer medicines. In summary, this article discusses the DNA alkylation mechanism and DNA cleavage mechanism of mitomycin c and bleomycin respectively. Mechanistically, both antibiotics damage DNA molecules of cancer cells. Mitomycin C can cause DNA cross linkage, while bleomycin kills cancer cells by cleaving their DNA molecules. The author also covers the proposed mechanism pathways for biosynthesis, chemical synthesis as well as the antibiotic resistance. The mainstream industrial manufacture method for mitomycin C and bleomycin is still fermentation.

Even though both antibiotics have already been approved for clinical use, there are still lots of problems and controversies remaining unresolved. Even until today, little is known about the sophisticated action mechanism of mitomycin and bleomycin. Unfortunately, antibiotic treatment of cancer is not a popular topic. It is a field of study needed to be further explored. Composing this research article, the author tried to use simple and clear explanation to make this neglected topic better-known. Recently, a new anti-neoplastic antibiotic, epothilone, was identified and characterized. We are still expecting further study on epothilone's functions so that it can be safely used as an anti-cancer treatment soon.

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