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The comparison of sodium sulfacetamide and erythromycin in treating trachoma

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Abstract. Trachoma is a common eye infection caused by Chlamydia trachomatis and there are many medicines for it already. Erythromycin is a type of antibiotic and sodium sulfacetamide is an antibacterial drug, they are both commonly used for treating trachoma. This passage contrasts the use of sodium sulfacetamide and erythromycin in the concepts of synthesis process, targets, and the resistance built up. Sodium sulfacetamide is a synthesized substance, generally contains 2 steps: prepare sulfacetamide and then let it hydrolysis to form the salt. It works by impede the synthesis of folic acids. If the Chlamydia tachomatis start to get folic acids from host cell, then sodium sulfacetamide can't work any more. Erythromycin is produced by fermentation, it works by bind to ribosomes of the trachoma to impede synthesis of protein. The Chlamydiae can built up resistance by transporting erythromycin out, changing structure of their ribosomes, or destructing the erythromycin molecules. They don't share many things in common, but the importance of them is significant, and they represents the main treatments of trachoma.

Keywords: trachoma, sodium sulfacetamide, erythromycin.

1. Introduction

Trachoma is a type of chronic infection. It appears as keratoconjunctivitis and is caused by Chlamydia trachomatis infection. About 5% of the trachoma sufferers got blind, and these people made up 25% to 30% of the total blindness in the middle of the 20th century. The WHO simplified trachoma grading system gives 5 levels to the severity of trachoma gives 5 levels: trachomatous inflammation: follicular (TF), trachomatous inflammation: intense (TI), trachomatous scaring (TS), trachoma trichiasis (TT), and corneal opacities (CO). These levels tell us the symptoms of trachoma: the follicular hyperplasia and corneal pannus take place together at first, then the scaring appears, and the TT and CO are sequelae or complication of trachoma. The TS is an important judgement basis for if it is trachoma and both TT and CO can cause visual impairment or even blindness. [1,2,3] Fig. 1. shows the appearance of trachoma in each level.

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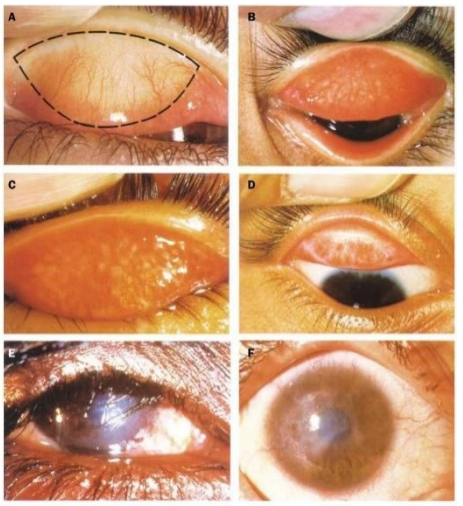

Figure 1. Staging card of trachoma by WHO [1,4]
A: normal everted upper conjunctiva, which is pink, smooth, thin and transparent; B: trachomatous inflammation: follicular (TF); C: TF and trachomatous inflammation: intense (TI); D: trachomatous scaring (TS); E: trachoma trichiasis (TT); F: corneal opacities (CO).

The trachoma has been together with people for too many years: the remains of human bones in Australia 10,000 years ago showed the characteristics of trachoma sequelas. There were records of trachoma symptoms and treatments for it at 1500 BCE in Egypt. In the 1950s in China, about 90% of people in villages were infected with trachoma. It is easy to be infected by trachoma if contact with the eye and nasal secretions of the infected, so the trachoma can spread easily under poor sanitary conditions. With the poor water treatments and the lack of facial cleanliness, the trachoma was a threat for people in 55 countries. But with the improvement of sanitation and the discovery of Chlamydia trachomatis in 1956, the number of the infected has been reduced from 360 million in 1985 to 80 million now.

The chlamydiae are obligate intracellular organisms. They cannot replicate outside their eukaryotic host cells and have a special life-cycle. They can be observed as two forms: the elementary body (EB) and the reticulate body (RB). The elementary body has 2 layers of membranes: an inner cytoplasmic membrane and an outer membrane (made by lipopolysaccharide), and there is no peptidoglycan layer. Instead of peptidoglycan, there are cysteine-rich outer membrane proteins on the membrane. A kind of lipoprotein of size of 12-15kD has its lipid part buried in the plasma membrane and its protein part outside can form S-S bonds with another protein buried in the middle of outer membrane of size of 60kD, this structure can help to stabilize the osmotic pressure and hold the contents inside. The diameter of an EB is about 0.3 micrometers. Only the elementary bodies can live outside of the host cells and infect other cells: they can attach to the cells (with help of some lipopolysaccharide on their membrane) and get into the host cells by endocytosis. Then the EBs would differentiate to reticulum bodies, which takes about 8 hours. During this procedure, new proteins are synthesized, the number of S-S bonds on the membrane is reduced so the membrane proteins described before are unlinked and the ATPase are activated. The reticulum bodies are metabolic-active, they can synthesize giant molecules like nucleic acids and proteins and phospholipids. As the reticulum bodies of C trachomatis, they can synthesize glycogen with glucose and ATP and also synthesis folate. But they do not have respiratory enzymes and flavoprotein, which is why they have to rely on host cells. The reticulum bodies reproduce by binary fission and they change into EBs and dissolve the cells membrane to release them again. A whole cycle takes about 48-72 hours. [2,4,5] Fig. 2. shows the procedure of a life cycle of C trachomatis.

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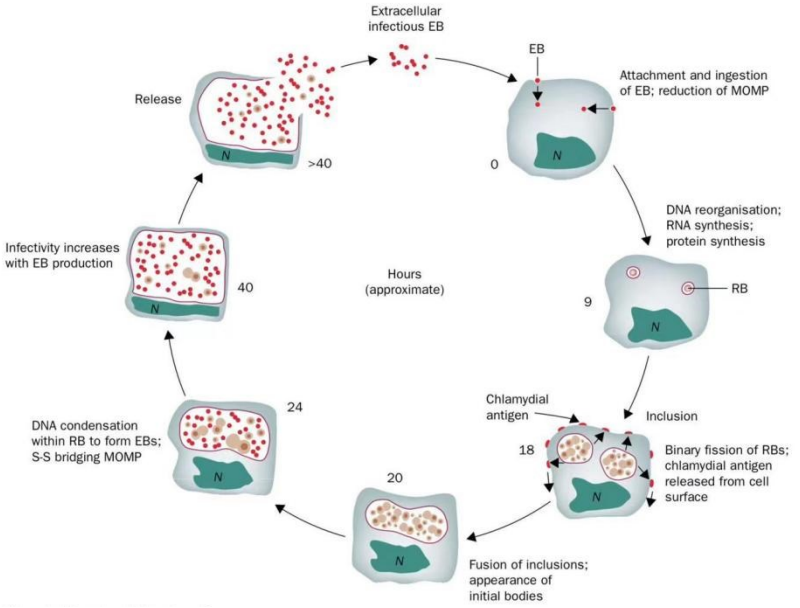

Figure 2. Life cycle of C trachomatis [4]
EB=elementary body; MOMP= major outer membrane protein; RB= reticulate body

There is a few researches about the toxin made by C trachomatis, but nearly no research has been done in the past 50 years. The evidence suggests they do produce a kind of toxin which is unstable under temperature changes. It can be inactivated within 30 minutes under 56 Celsius degrees and within 2 hours under 37 Celsius degrees. Only under 4 to -40 Celsius degrees is the toxin rather stable and can be stored for more than one week. In murine model, the mice die in 6 hours after being injected with 3 MLD of the toxin, and generally die between 10-24 hours with 1 MLD of toxin. The activity of the toxin could be separated from the chlamydiae: adding 10000U/ml penicillin to the C trachoma solution under 4 Celsius degrees for 4 hours can make the chlamydiae lose their infectivity without having influence on the activity of toxin. [1]

The C trachomatis also cause the symptoms by delayed type hypersensitivity (DTH). The heat shock protein 60 of chlamydiae share most of the sequence with that of humans, they can cause autopathological immune response as allergen. The T cells can be activated by human hsp60 without making responses. After activation, the T cells can produce interleukin-10, which promotes the increase of T cells. The receptors of T cells are occupied by human hsp60 at this time. But the nonreactive state of T cells can be eliminated by interleukin-2, then the T cells are induced by hsp60 of C trachomatis and reinforced by the human hsp60 and cause immunopathological response. The change of cells' state and the increase of lymphocytes acting finally lead to inflammatory damage. [1]

The reproduction of C trachomatis can be inhibited by interferon gamma, however, the production of hsp60 would be increased. The time a life cycle of C trachomatis takes also increases, so the trachoma lasts longer. The infection would be continuous and inapparent.

The Chlamydia trachomatis is sensitive to tetracycline, macrolide antibiotics and fluoroquinol drugs. 0.1% rifampicin eye drops, 15% sodium sulfacetamide eye drops, tetracycline ointment and erythromycin ointment are commonly used in treating trachoma.

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2. Sodium Sulfacetamide

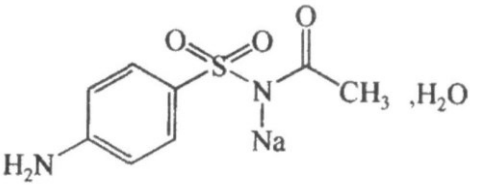

Figure. 3. Sodium trachomatis

The sulfonamides were first introduced in 1908, and the sodium sulfacetamide was soon made. The sulfonamides are the first synthetic antibacterial drugs.

The sodium sulfacetamide is a short-acting drug. It is not supposed to be used for a long time as the resistance may be built up. It should be used 3-4 times per day and 1-2 drops per time during treatment. A small number of people may be hypersensitive to sodium sulfacetamide and appear as erythra, dyspnea, blisters and so on.

The formula of sodium sulfacetamide crystal is $C_8H_9N_2NaO_3S \cdot H_2O$. In the sodium sulfacetamide eye drops, the concentration of sodium sulfacetamide is about 15% and the pH value is at 8.0 to 9.8. The solution is colorless or pale yellow.

2.1. Synthesis

The most common method is using a series of reflux reactions: the acetic anhydride is added to sulfanilamide(4-aminobenzenesulfanomide) to form N1, N4-diacetyl-sulfanilamide-d4 and sodium; and acetyl-(4-aminobenzene) sulfonylazanide is hydrolyzed to form N-{(4-aminophenyl) sulfonyl} acetamide(sulfacetamide) with presence of NaOH. The N- {(4-aminophenyl) sulfonyl} acetamide is then refluxed under sodium hydroxide to form the target molecule, sodium sulfacetamide. However, these reflux reactions have a slow rate, so the reactions are always done under hot conditions to speed them up.

Here are some possible routes of synthesis:

Route 1 is reflux of 4-aminobenzenesulfanomide with acetic anhydride;

Route 2 is reflux of 4-aminobenzenesulfonyl chloride with acetamide;

Route 3 is ultrasonic irradiation of 4-aminobenzenesulfanomide with acetic anhydride;

Route 4 is ultrasonic irradiation of 4-aminobenzenesulfanomide with acetamide. [6]

But no matter which route is used, the basic synthesis of sodium sulfacetamide just contains 2 main steps: to prepare a sulfacetamide and then let it hydrolysis to form the salt we want.

2.2. How it works

Sulfonamides are structural analogues and competitive antagonists of para-aminobenzoic acid(pABA). They compete with 4-Aminobenzoic acid for the dihydrofolic acid synthetase, which catalysis the incorporation of pABA into dihydrofolic acid, the immediate precursor of folic acid. [7]

The folic acid is important in the methyl metabolism and production of DNA and RNA. It is important for the production of S-adenosynthetase, the primary methyl donor for DNA methylation. Folate deficiency may deplete cellular S-adenosynthetase levels, causing DNA hypomethylation and inappropriate activation of oncogenes for animals. [8]

The folic acid also takes part in a series of reactions. The conversion of deoxyuridine monophosphate(dUMP) to thymidine monophosphate(dTMP) requires folic acid in the form 5,10, methylenetetrahydrofolate as methyl donor. Imbalances in deoxyribonucleotide pools caused by folate deficiency can negatively affect cell replication and DNA repair. With folate depletion, methylation of dUMP to TMP would be blocked and leads to an increase in the cellular levels of deoxyuridine triphosphate and uracil misincorporation into the DNA molecule in place of thymine. Also, the normal DNA repair includes removing the uracil, but if there is lack of folate, the conversion of dUMP to

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thymidine would be limited and the uracil may be misincorporated and induce double strand breakage. [8] The picture on the right is about how the folate metabolism cycle works with the major reactions.

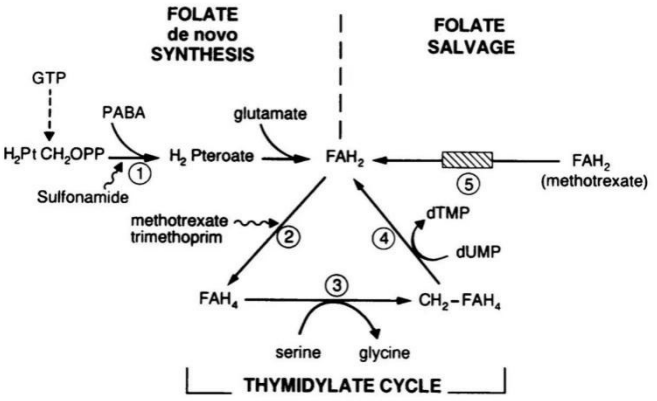

Figure 4. Steps of folate metabolism cycle [7]

The squiggly arrows represent steps inhibited by certain compounds. Some important enzymes are numbered: 1 is dihydropteroate synthase; 2 is dihydrofolate reductase; 3 is serine hydroxy methyltransferase; 4 is thymidylate synthase; 5 is a membrane transport system for folates. FAH_2, dihydrofolate; FAH_4, tetrahydrofolate; CH_2-FAH_4, 5,10-methylene tetrahydrofolate.

As the C trachomatis, it does encode a thymidylate synthase for synthesis of dUMP to dTMP and is capable of de novo folate synthesis. It does not require folates from the host, so if it can't make the folic acids itself, it could hardly survive.

Conclusively, the folate metabolism is impeded by sodium sulfacetamide and the chlamydiae then cannot synthesize the required purine and nucleic acid itself, thus inhibiting bacterial growth and reproduction.

However, the sodium sulfacetamide could not do harm to human cells because we obtain folates from dietary sources. while the chlamydia produce folate by themselves. The sulfonamides can only influence the microorganisms which synthesize their own folates.

2.3. Resistance

Some chlamydia take up special transport mechanisms: they synthesize transport protein to get folate from the hosts.

2.4. Other components common in sodium sulfacetamide eye drops

Although they are not inhibiting the C trachomatis, they are common components of sodium sulfacetamide eye drops. Here is a list of them with their roles.

Sodium bisulfite: Intermediate for sodium sulfacetamide.

Sorbic acid: C6H8O2, is a common preservative and bacteriostat. It can inhibit the growth and reproduction of most fungus as it can inhibit the dehydrogenase system of the organisms. Also, the sorbic acid is stable and not reactive. [9]

Sodium hydroxide: The sulfonamides are amphoteric due to the primary aromatic amino group and the sulfonamide group. They are more soluble in alkaline or acidic solution (NaOH or HCl solution). The sodium salts of them may form precipitates when meet with CO2 and it is better not to use acidic solutions as the solvent. So the NaOH is used to dissolve sodium sulfacetamide.

EDTA-2Na: The copper ions or iron ions can promote oxidation of sodium sulfacetamide easily. As a chelating agent, EDTA-2Na can catch these ions and prevent the oxidation of sodium sulfacetamide. It is a good stabilizer for sodium sulfacetamide.

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3. Erythromycin

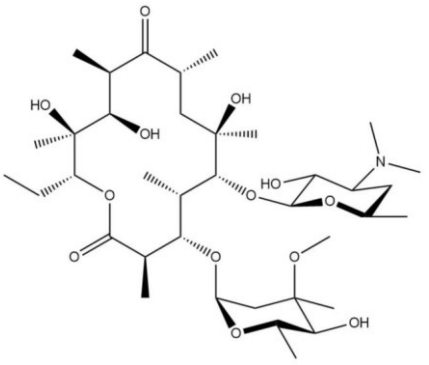

Figure 5. Erythromycin

The erythromycin was discovered in 1949. It is an antibiotic and was found in the soil.

At most use continuously for 5 days in treatment. About 0.5% to 1% of people may be allergic to erythromycin. The possible side effects also include the eye itching and aching.

The formula of erythromycin molecule is $C_{37}H_{67}NO_{13}$. The concentration of erythromycin in the erythromycin ointment is 0.5%.

3.1. Synthesis

The production of erythromycin is still using fermentation traditionally. After fermentation, butyl acetate and water are used to filter and extract.

Erythromycin is basic and insoluble in water. It forms salts with butyl acetate, which is more soluble.

3.2. How it works

There are two ways erythromycin can go through:

Firstly, erythromycin can bind with the 50S subunit of ribosomes, so the tRNA can no longer bind to the P site of ribosomes and the bacteria can't synthesize proteins. By this way, the erythromycin inhibits the transcription from tRNA to polypeptides. Secondly, the erythromycin can also bind with the 50S subunit before it is made, so the 50S subunit can't be produced.

There are 5 five active sites related to polypeptide synthesis on the ribosome: a mRNA binding part, a part to accept or bind with AA-tRNA, the part to accept or bind with peptidyl-tRNA (formed when tRNA bind toto the ribosome the P site and a peptidyl transferase. In the first way, the erythromycin inhibits the function of the peptidyl transferase. The center of peptidyl transferase is located on center ring of 23S rRNA domain V. Several hairpin loops are formed around the center ring. Under the peptidyl transferase there is a nascent peptide release channel, which is made of RNA, L4 and L22 proteins. The two proteins are close to each other on the backside of channel like a gate and may regulate the release of peptide chain. The polypeptide chain goes out of the peptidyl transferase center, goes through channel and pasts domain V, domain II, domain IV, L4, L22, and then domain I and domain III in sequence. The erythromycin is bind to the 50S unit at lower place of peptidyl transferase and at the entrance of the nascent peptide release channel. The nucleotide groups form two units on the wall of channel: one is in the domain V and another one is between A nucleotide in hairpin loop in domain II and a U nucleotide on the back of channel. The erythromycin also binds with L4 and L22 proteins. Two effects help in the inhibition of transcription: the inhibition of extension of polypeptide chain and the promotion of drop of nascent peptide release channel. The unit the domain V can cut off the polypeptide chain when it is 3 to 5 amino acids long and the transfer of peptidyl-tRNA from A site to P site would be impeded and fall off. [10]

In the second way, the erythromycin binds with the 50S subunit when it is not already formed, and the components of 50S unit cannot be connected then.

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However, the erythromycin can't do harm to human cells because of the difference in the structure of ribosomes.

3.3. Resistance

There are 3 major ways to cope with inhibitory action: (1) altered antibiotic transport; (2) antibiotic modification; (3) target site alternation.

Many microorganisms can use the first method by increasing the number of efflux pumps (a kind of transport protein to transport the toxic compounds from the inside of cell to the outside). 1 erythromycin molecule can only bind with 1 50S subunit, if the concentration of the erythromycin is low enough, the microorganisms can then survive.

Some enzymes are also made to destruct the structure of erythromycin, for example, by hydrolysis of the lactone ring of erythromycin or by glycosylation of the OH group on the macrocyclic lactone.

Reports of resistance results from mutations of structure of 50S subunits is also common. There may be mutations to the nucleotide groups in domain V and domain II, so the erythromycin cannot bind to these sites and the affinity of it to ribosomes is lowered. Mutation of L4 and L22 proteins also can inhibit the combination of erythromycin and ribosomes. [10,11]

3.4. Other components in erythromycin ointment

There is Vaseline in the ointment to keep the moisture.

4. Conclusion

In summary, this paper briefly introduces the life form of chlamydia trachomatis and how it causes the symptoms. As the sodium sulfacetamide and erythromycin, the basic information about them, their targets, the resistance against them, the procedures of synthesizing them are also introduced basically. The sodium sulfacetamide and erythromycin are completely different drugs. They share nothing in the synthesis procedures, aiming targets and uses. They are contrasted just because they cure the same disease: trachoma, a disease which has been disturbing us for centuries but is losing its power now. With the development of society and technology, it is hopefully to get it under control in the next few years. However, we still can't get relaxed- the chlamydia trachomatis can infect people in other ways than on eyes. Also, some of the poorest regions cannot get rid of trachoma soon due to their poor sanitation, which is favorable for the spread of trachoma.

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