

An Updated Review on Pharmacology and Toxicities Related to Chloramphenicol

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ABSTRACT

Chloramphenicol is an antimicrobial drug primarily its bacteriostatic, though at high concentration it shows bactericidal actions on some bacteria e.g. H. Influenzae. Initially chloramphenicol is obtained from *Streptomyces venezuelae* in 1947, now it is synthesized chemically and all the commercial product is synthetic. Chloramphenicol displays a broad-spectrum bacteriostatic activity by specifically inhibiting the bacterial protein synthesis. In certain but important cases, it also exhibits bactericidal activity, namely against the three most common causes of meningitis, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*. Resistance to Chloramphenicol has been frequently reported and ascribed to a variety of mechanisms. However, the most important concerns that limit its clinical utility relate to side effects such as neurotoxicity and hematologic disorders. In this review, we present previous and current research on Chloramphenicol and its derivatives with improved pharmacological properties.

Key words: -Chloramphenicol, Aplastic anemia, H. Influenzae, Gray Baby Syndromes

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1. INTRODUCTION: -

Chloramphenicol chemically is D-(-)-threo-1-p-nitrophenyl-2-dichloroacetamido 1, 3-propanediol. It is a broad-spectrum antibiotic affecting gram positive and gram negative organisms, aerobic and anaerobic bacteria and many intracellular organisms. Chloramphenicol, a useful antibiotic is extensively used for life threatening infection because it is cheap and used against different pathogens. It is effective despite its known haemotoxicity and linkage to fatal aplastic anemia¹. The antibiotic is still widely employed in ear and eye drop formulations & it is also used for the treatment of bacterial conjunctivitis and in typhoid fever. In humans chloramphenicol is haemotoxic and induces two forms of toxicity. First, a commonly occurring,

dose-related, reversible bone marrow depression, which develops during treatment. Second, a rarer aplastic anemia (AA), developing after treatment, irreversible and often fatal. CAP is well known to have serious health effects like Gray Baby Syndromes in infants' different hypersensitive reactions and also increases the risk of cancer.² Harmful effects that occur at the sites where the substance comes into contact with the body are referred to as local effects and if the substance is absorbed from the sites of contact, they or products of their bioconversion may produce toxic effects in the cells, tissues or organs, these responses are referred to as systemic toxicity.

1.2 CHEMISTRY:

Chloramphenicol has the following structural formula

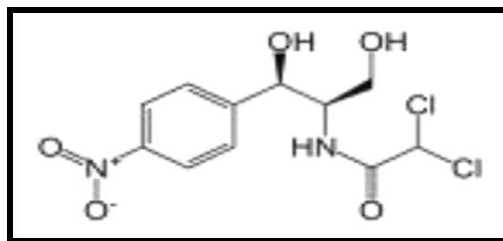
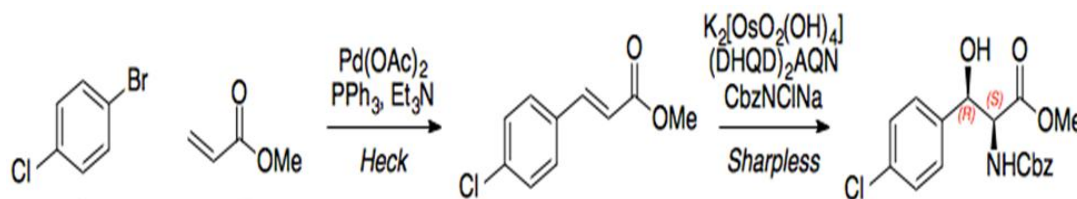


Figure 1: Chemical structure of Chloramphenicol.

1.3 Forward synthesis

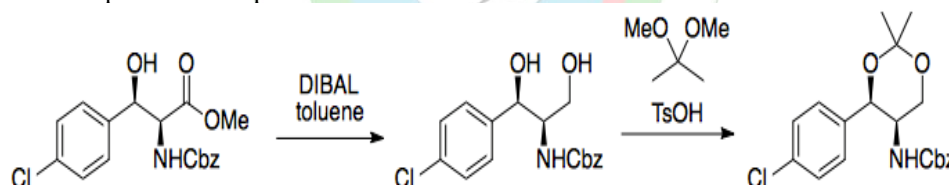
Chloramphenicol is a unique compound among the natural compound and in its chemical structure it contains a nitrobenzene moiety and it is a derivative of dichloro acetic acid and biologically active form is levorotatory. Its nitrobenzene substitution is probably responsible for its antibacterial activity.

Methyl 4-chlorocinnamate is commercially available, but the restriction on starting materials to 8 carbons or fewer means that we need to make it. The most common way seems to be a Heck reaction, but olefin metathesis and the Horner–Wadsworth–Emmons have also been used. This cinnamate can then undergo the sharplessaminohydroxylation as described previously, which creates the key C–O and C–N bonds with the desired stereochemistry.



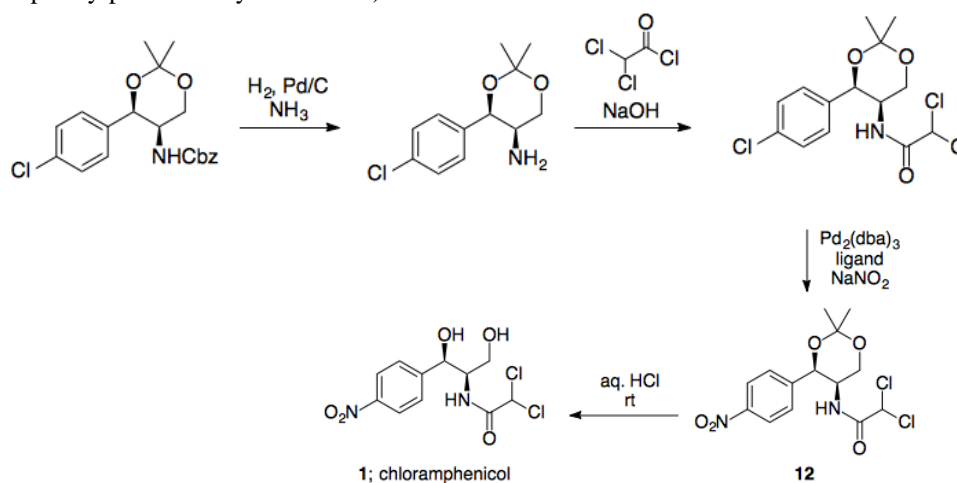
The next step is DIBAL reduction of the ester to the alcohol, followed by protection of the 1,3-alcohol as the acetonide. I inserted this protection step as I'm afraid that

the free hydroxyl/amino groups adjacent to each other would poison the Pd catalyst in the nitration step.



The synthesis is completed by hydrogenolysis of the Cbz group (the use of ammonia as a solvent inhibits benzyl ether cleavage, eliminating any possible risk of destroying the acetonide) and acylation on N (the use of the acetonide also nicely sidesteps any potential acylation on O). I chose

to do this before the nitration, just in case the free amine interferes with the Pd. The Pd-catalysed nitration, which seems to have quite a broad substrate scope, and removal of the acetal with aqueous acid, preserving the amide ³.



1.4. PROPERTIES:

- Yellowish white crystalline solid
- Stable in aqueous solution
- Resist high temperature
- Bitter taste
- Light sensitive

1.5 TRADE NAME:

- Amphicol
- Chloromycetin (U>S, intravenous preparation)
- Chlorsig (U.S, Australia eye drops)
- Renicol (eye drop)
- OftanChlora (eye ointment)
- Synthomycine (skin ointment)

2.0 MECHANISM OF ACTION:

Chloramphenicol inhibits the growth of bacteria via inhibiting the protein synthesis and to a lesser extent it also affects the eukaryotic cells. Drug readily penetrates the bacterial cells and probably binds to the 50 s ribosomal subunit reversibly. Although binding of t RNA at the recognition site on the 30 s ribosomal subunit is thus undisturbed, the drug appears to prevent the binding of the amino acid containing end of the aminoacyl t RNA to the acceptor site on the 50 s ribosomal subunit. Due to the presence of the Chloramphenicol the interaction between the peptidyltransferase and its amino acid substrate cannot occur and thus the peptide bond formation is inhibited and the functional⁴.

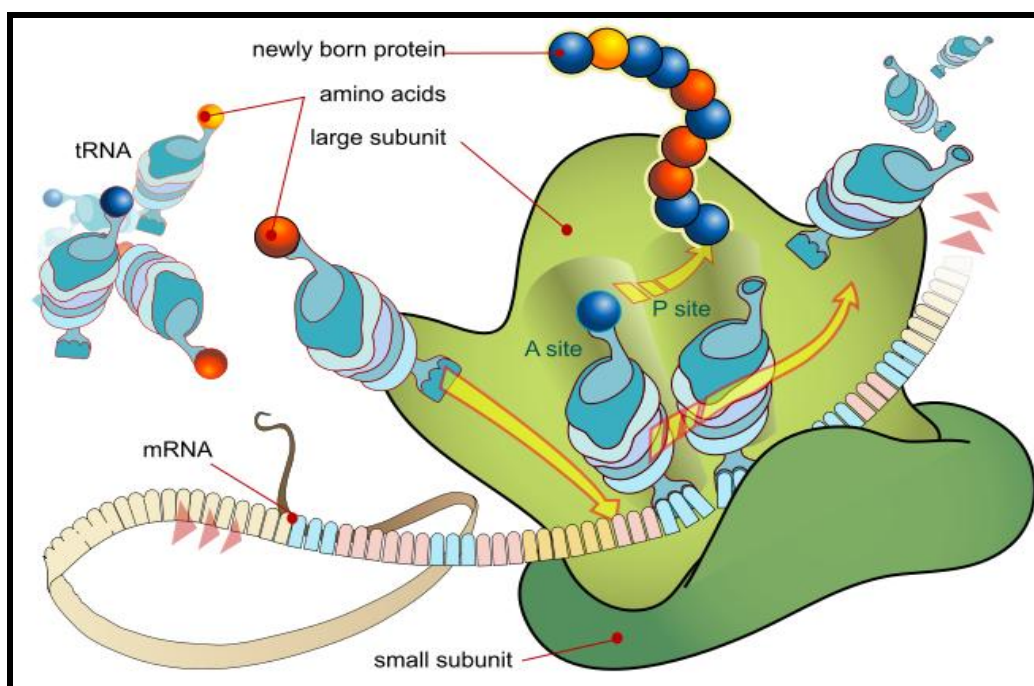


Figure: 2 Mode of action of Chloramphenicol

Chloramphenicol also can inhibit the mitochondrial protein synthesis in mammalian cells, perhaps because mitochondrial ribosomes are similar to the bacterial ribosomes (70 s) therefore it cause many adverse effects. Erythropoietic cells are much more sensitive to the drug.

2.1 ANTIMICROBIAL ACTIONS:

It inhibits bacterial protein synthesis therefore it shows broad spectrum of activity. Chloramphenicol exhibits a broad spectrum of antimicrobial activity. Chloramphenicol is primarily bacteriostatic it can be bactericidal for certain species such as *H. influenza*, *Streptococcus pneumoniae* and *Neisseria meningitidis*. Most of the strains of Gram negative bacteria are inhibited *in vitro* by 8.0µg/ml or less of chloramphenicol – *H.influenzae*, *N.meningitis*, *N.gonorrhoeae*, *Brucella spp.* and *Bordetellapertusis*. Likewise, most anaerobic bacteria, including Gram positive bacteria like cocci and *Clostridium spp.* And Gram negative rods including *Bacillus fragilis* are inhibited by this

concentration of the drug. Most strains of *E.coli* and *Klebsiella pneumonia* are susceptible⁵.

2.2 THERAPEUTIC USES:

Therapy with chloramphenicol must be limited to infections for which the benefits of the drug outweigh the risks of the potential toxicities. If different antimicrobial drugs are available which are much effective and less toxic they should be used. Chloramphenicol was first indicated in the treatment of typhoid, but now the strains have become resistant and the treatment is given when organism is known to be sensitive.

- (a) **TYPHOID FEVER:** It is an important drug for the treatment of typhoid fever and it is used for other types of systemic *Salmonella* infections, but other safer drugs are available⁴.

DOSE: Adult dose of chloramphenicol for typhoid fever is 1g every 6 hrs for 4 weeks. Both oral and

intravenous administration is used but the response is more rapid with oral administration.

- (b) **BACTERIAL MENINGITIS:** Treatment with chloramphenicol produces excellent results in *H.influenzae*, meningitis equal to or better than those achieved with ampicillin. Chloramphenicol is referred as bacteriostatic but it is bactericidal for many meningeal pathogens such as *H.influenzae*.
- (c) **ANAEROBIC INFECTIONS:** Chloramphenicol is quite effective against most anaerobic bacteria, including *Bacterioids spp.* It is effective for treatment of serious intradominal infections or brain abscesses (caused by anaerobes). It is used for the treatment of Rickettsial disease and *Brucellosis*⁵.

2.3 ADMINISTRATION AND DOSAGE:

- 1st approved by FDA – August, 25 1982.
- Chloramphenicol Sodium succinate is intended for intravenous use only.

IN ADULTS: Adults should receive 50mg/kg/day in divided doses at 6-hour intervals. In some exceptional cases patients with infections due to moderately resistant organisms may require increased dosage upto 100mg/kg/day to achieve blood levels inhibiting the pathogen, but these high doses cause hypersensitive reactions and should be decreased as soon as possible.

IN CHILDREN: Dosage of 50mg/kg/day divided into 4 doses at 6-hour intervals yield blood levels in the range effective against most susceptible organisms.

STORAGE/STABILITY: stored between 15 °C & 25°C.

2.4. PHARMACOKINETICS:

Absorption:

Chloramphenicol has been available for oral administration in two forms.

- The active drug
- The inactive prodrug-chloramphenicol palmitate

Chloramphenicol is readily absorbed from the gastrointestinal tract. The preparation of chloramphenicol for parenteral use is the water soluble, inactive prodrug sodium succinate preparation. Similar concentration of chloramphenicol succinate in plasma is achieved after intravenous and intramuscular administration. Still it is not known where the hydrolysis of chloramphenicol succinate occur in vivo, but it is assumed that esterase of the kidney, liver and lungs all may be involved.

Distribution:

Chloramphenicol is well distributed in body fluids and readily reaches therapeutic concentrations in CSF, where values are approximately 60% of those in plasma in the presence or absence of meningitis. The drug may accumulate in the brain tissues. Chloramphenicol is present in bile, is secreted into milk and readily traverses the placental barrier. It also penetrates into aqueous humor after subconjunctival injection.

Excretion:

The major route of elimination of chloramphenicol is hepatic metabolism to the inactive glucuronide. This metabolite, as well as chloramphenicol itself is excreted in the urine by filtration and secretion. Over a 24-hour period, 75% to 90% of an orally administered dose is so excreted; about 5% to 10% is in the biologically active form. Chloramphenicol succinate is rapidly cleared from plasma by the kidneys. Poor renal functions in the neonate and other states of renal insufficiency result in increased plasma concentrations of chloramphenicol. Decreased esterase activity has been observed in the plasma of neonates and infants. Patients with hepatic cirrhosis or otherwise impaired hepatic functions have decreased metabolic clearance and dosage should be adjusted in these individuals.

Several metabolites of chloramphenicol were identified in urine samples obtained from male Wistar rats and from a human volunteer given tritiated chloramphenicol at a dose of 10mg/kg bw by oral route. In rats two most abundant metabolites detected in the 1st 24 hours by High Performance Liquid Chromatography (HPLC) AND Gas Chromatography-Mass Spectrophotometry (GC-MS) was chloramphenicol-base and chloramphenicol acetylarlyamine. The remaining metabolites were unchanged chloramphenicol, chloramphenicol-oxamic acid, chloramphenicol-alcohol, chloramphenicol-glucuronide, and chloramphenicol-oxamylethanolamine. The formation of chloramphenicol – oxamylethanolamine as an end product of the metabolism of chloramphenicol by the liver was proven by the release of chloramphenicol-oxamylethanolamine after incubation of tritiated chloramphenicol with hepatocyte microsomes from rats treated with phenobarbital⁶.

3.0 ADVERSE REACTIONS:

(a) Untoward effects:-

Chloramphenicol inhibits the synthesis of proteins of the inner mitochondrial membrane, probably by inhibiting the ribosomal peptidyltransferase. These include subunits of Cytochrome C. Oxidase, Ubiquinone, Cytochrome C reductase and the proton translocating ATPase critical for aerobic metabolism.

(b) Hypersensitive reactions:-

Although relatively uncommon, macular or vesicular skin rashes results from hypersensitivity to chloramphenicol. Fever may appear simultaneously or be the sole manifestation. Angiodema is a rare complication. Jarisch – Herxheimer reactions may occur after administration of chloramphenicol therapy for syphilis, brucellosis and typhoid fever.

(c) Hematological toxicity:-

The most important adverse effect of chloramphenicol is on the bone marrow. Chloramphenicol affects the hematopoietic system in two ways:

- A dose related toxicity that presents as anemia, leucopenia or thrombocytopenia
- An idiosyncratic response manifested by aplastic anemia, leading in many cases to fatal pancytopenia.

Chloramphenicol is associated with sideroblastic bone marrow changes. Chloramphenicol principally suppresses erythropoiesis in a consistent and dose dependent manner. The effect is distinct from and unrelated to the rare complication of aplastic anemia. Sideroblastic anemias are characterized by the presence of a variable number of hypochromic cells in the peripheral blood and an excess of iron in the bone marrow and it causes development of erythroblasts containing iron granules arranged in a ring around the nucleus and these changes lead to ineffective erythropoiesis. Chloramphenicol causes alterations of iron kinetics and along with a reticulocytopenia, the serum iron rises and plasma iron clearance is prolonged. These can be accomplished by the development of ring sideroblasts. The major toxic effect of chloramphenicol on the function of the differentiated marrow cells is inhibition of mitochondrial protein synthesis, specifically of certain cytochromes and cytochrome oxidase. Chloramphenicol inhibit both bacterial and mitochondrial protein synthesis as it reversibly binds to the 50s subunit of 70s ribosome, inhibiting peptidyltransferase in both prokaryotic organisms and mitochondria⁷.

3.1 Chloramphenicol Induced Aplastic Anemia:

Aplastic anemia is defined as a syndrome of unexpected pancytopenia (anemia, leucopenia and thrombocytopenia) with marrow hypoplasia, in which normal haemopoietic marrow is replaced by fat cells. Aplastic anemia is a serious condition where by bone marrow is unable to produce sufficient levels of red blood cells, WBC and platelets. The term “**aplastic**” refers to the bone marrow malfunction responsible for the insufficient blood cell production, while the term “**anemia**” refers to insufficient blood cell production itself. Chloramphenicol is a nitrobenzene compound with a dichloroacetamide side chain. The mechanism of chloramphenicol induced aplastic anemia is unknown. Aplastic anemia is a rare case, but can develop after months or weeks of exposure; mortality is about 50%. It has been observed and many evidences are given that aplastic anemia is related to leucopenia and chloramphenicol is carcinogenic. (IARC, 1990). Due to reduction of p-nitro group of nitroso-chloramphenicol is produced which causes DNA damage and is related to leukemia⁹.

Effiongetal; 2010 detected through the experiment that chloramphenicol cause hepatotoxicity and it affects the liver enzymes and it increases the enzymes in the blood levels. The dose range of chloramphenicol was 50mg/kg B.wt and 100mg/kg B.wt and in one group chloramphenicol was dissolved in coconut water. The results suggest that coconut water can be exploited in the amelioration of chloramphenicol toxicity in a dose dependent therapy¹⁰.

3.2 CHLORAMPHENICOL AS A CARCINOGEN-

Administration of chloramphenicol in mouse induces abnormal cell differentiation and it does not allow apoptosis, which is the cause of development of leukemia like syndrome¹¹. Adverse effects caused by overuse and overdose of chloramphenicol include aplastic anemia, gray baby syndrome and leukemogenesis¹².

3.3 GREY BABY SYNDROME:

This phenomenon occurs in newborn infants because they do not have fully functional liver enzymes (i.e., UDP-glucuronyltransferase) and so chloramphenicol remains unmetabolized in the body. This causes several adverse effects, including hypotension and cyanosis.

Two mechanisms apparently are responsible for chloramphenicol toxicity in neonates:

1. A developmental deficiency of glucuronyltransferase, the hepatic enzymes that metabolizes chloramphenicol in the first 3 to 4 weeks of life.
2. Inadequate renal excretion of unconjugated drug. Chloramphenicol also induce hepatotoxicity which can be analyzed by liver marker enzymes such as ALT, ALP, LDH etc. if any type of tissue damage occurs, the enzyme normally present in sub cellular organelles will be released in the circulation. Increased plasma enzyme activities may indicate loss of hepatocyte integrity.

3.4 Other adverse reactions:

Pregnancy: animal reproduction studies have not been conducted with chloramphenicol. There are no adequate and well controlled studies have been done to establish safety of this drug in pregnancy. It is not known whether chloramphenicol can cause fatal harm when administered to a pregnant woman. Orally administered chloramphenicol has been shown to cross the placental barrier

Observations in humans

Aplastic anemia in humans is an idiosyncratic reaction to chloramphenicol, which has an immunological basis and which is related to the nitrobenzene structure. This hypothesis is supported by clinical evidence showing that 40-50% patients with aplastic anemia have a partial or complete response to a variety of immunosuppressive agents^{13,14}. (Young et al., 1994) reviewed the pathophysiology of aplastic anemia and reported that most cases can be characterized by a T-cell mediated destruction of bone-marrow haematopoietic cells. The potential for an adverse reaction induced by treatment with chloramphenicol is of critical importance in seriously ill or compromised patients^{15,16}. In patients with pre-existing haematologic abnormalities or hepatic failure, or in neonates, chloramphenicol is only used when no other effective antibiotics are available. Chloramphenicol has not been determined to be safe for use during pregnancy¹⁷. The drug may decrease protein synthesis in the fetus, particularly in the bone marrow. Chloramphenicol is found in human milk at 50% of serum concentrations in humans and therefore the drug should be given with extreme caution to nursing mothers^{18,19}.

4.0 CONCLUSION:

Currently, Chloramphenicol is indicated in developed countries only for the treatment of serious infections, in which alternative medication is ineffective or contraindicated. For these reasons, CAM has been modified using various synthetic approaches aiming to optimize its pharmaceutical profile. Despite important progress made to address problems related to side effects and resistance against CAM caused by CAM-modifying enzymes, the Achilles' heel of the new synthesized CAM derivatives seems to be their general inability to penetrate the bacterial cell envelope, coupled with their susceptibility to multi-drug efflux pumps. Its efficacious activity against a broad spectrum of pathogenic bacteria is hampered by adverse effects causing hematologic disorders, immunosuppression and cancer invasion. As CAM is an ancient microbial metabolite, genetic elements conferring resistance against this drug have been retained by and are frequently dispersed in microbial communities. In parallel, resistance was expanded by the misuse of CAM in the medical and veterinary practice. Engineering nanolayered particles for specific CAM delivery is a challenge for the future. It is clear from the past six decades of efforts that CAM derivatives, with ideally improved pharmaceutical properties are incredibly difficult to be found and that there is an urgent need to Antibiotics. Therefore, deeper understanding of the biology and the interactions with the drug of a variety of microorganisms is needed.

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