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MYELOTOXIC EFFECTS OF CHOLARAMPHENICOL AND ITS METABOLITES

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Abstract: Chloramphenicol has a broad spectrum of activity against both gram negative and gram positive bacteria, richetsia, and psittacozis-lymphogranuloma group. The most important unwanted effect of chloramphenicol is depression of the bone-marrow resulting in pancytopenia- an effect which may occur even with very low doses in some indivudials.Within the Turkey the drug is approved for use only in non-food producing animals; it is illegal to use the drug in food animals.Prohibition of chloramphenicol for use in food animals is based in the tendency of chloramphenicol or its metabolites to produce aplastic anemia in certain susceptible people. In this review, the myelotoxic effects of chloramphenicol and its metabolites are summarized.

Key Words: Chloramphenicol, Chloramphenicol metabolites, myelotoxic effect.

KLORAMFENİKOL ve METABOLİTLERİNİN MYELOTOKSİK ETKİLERİ

Özet: Kloramfenikol; gram negatif ve gram pozitif bakterileri, riketsiyalara ve psittakozis-lenfogranuloma grubu mikroorganizmalara karşı etkili, geniş spektrumlu bir antibiyotiktir. En önemli istenmeyen özelliği pansitopeni ile sonuçlanan kemik iliği depresyonudur. Bu durum bazı duyarlı kişilerde çok küçük dozlardaki kullanımlarda bile meydana gelebilir. Türkiye'de ilacın sadece gıda olarak tüketilmeyen hayvanlarda kullanımına müsaade edilmiştir. Ancak illegal kloramfenikol kullanımları mevcuttur. Kloramfenikol veya metabolitlerinin duyarlı kişilerde aplastik anemi yapma eğilimi gözönünde bulundurularak, gıda olarak tüketilen hayvanlarda kullanımı yasaklanmıştır. Bu makalede, kloramfenikol ve metabolitlerinin myelotoksik özellikleri incelenmiştir

Anahtar Kelimeler: Kloramfenikol, kloramfenikol metabolitleri, myelotoksik etkiler.

Chloramphenicol (CAP) was obtained from *Sreptomyces venezuelae* found in a soil sample from a mulched field in Venezuela in 1947, but it is now synthesised commercially for clinical use (Rang, 1991). CAP interferes with bacterial protein synthesis by ribosomes. Its primarily bacteriostatic, but also may be bactericidal against *H. influenza, N. meningitis, and spp* (Feder, 1989; Huber, 1982).

CAP must not be used for any purpose that would result in presence of residues in food for consumption by humans. Within the Turkey the drug is approved for use only in non-food producing animals; it is illegal to use this antibiotic in food animals (Ministry of Agricultar, May 1993). No residues in edible tissues are permitted by FDA (Huber, 1982). Prohibition of CAP for use in food animals is based in the tendency of CAP or its metabolites to produce aplastic anemia in certain susceptible people (Rang, 1991; Huber, 1982).

CAP has three well established toxicities-aplastic anemia, the gray syndrome, and bone marrow suppression have been better defined, the risk factors for aplastic anemia remain controversial (Huber, 1982; Lubran 1989).

It is generally accepted that CAP causes two types of bone marrow toxicity: Common dose-dependent reversilb marrow supression, affecting primarily the erythroid elements; and a rare complicating characterized by bone marrow aplasia, pancytopenia, and, in most cases, a fatal outcome. Ample evidence indicates that CAP-induced reversibl bone marrow supression result from inhibition by the drugs of mitochondrial protein synthesis. In addition to its in vitro effect on mitochondrial protein synthesis, CAP, when given the patients in large doses, produces a mitochondrial ultrastructural lesion, the extent of which correlates well with the blood levels. The mitochondrial lesions becomes undetectable following discontinuation of CAP theraphy, which suggests that, like the erythropoietic lesions, it is reversibls. It should be added that recent experiments indicated that the CAP effect on mitochondrial protein synthesis in vitro also, to a

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large extent, reversibl upon removal of the drug (Yunis, 1976; Yunis, 1980a; Yunis, 1980b; Yunis, 1981a).

Thiamphenicol, the analogue of CAP in which the NO_2 group in the para position of the benzen ring is replaced by a SO_2CH_3 group, also produces the dose-related reversilb, and therefore predictible, inhibition of the erythropoiesis, but has not been associated incidence of aplastic anemia (Yunis, 1976; Yunis, 1981b).

Based on this observation, Yunis (1984) has put forth the following hypothesis: The *p-nitro* group is the structural feature underlying the aplastic anemia that follows CAP treatment. The susceptible host provides the milieu in which the *p-nitro* group undergoes reduction, resulting in the formation of higly reactive intermediates, such as *nitroso* compound and *hydroxylamine,* which in turn result in stem cell damage. He answered this question by examinig fresh liver tissue from ten kidney donors with brain death. All ten livers were capable of reducing the *p-nitro* group of CAP to the amino group.

Chloramphenicol and Its Nitroso Derivative: Possible Role in Chloramphenicol-Induced Bone Marrow Injury

Several investigators (Yunis, 1976; Abou-Khalil, 1987) reported that CAP inhibits colony growth *(CFU-C)* in a concentration-dependent manner, with 50 % inhibition occuring at concentration within therapeutic levels ($1.6x10^{-4}$ M or 50 mcg/ml). However, inhibition of colony formation was much more pronounced, with nitroso-CAP (NO-CAP) becoming complete (zero growth) at $5x10^{-5}$ M or 15 mcg/ml (Yunis, 1980a). On the other and, incubation of human bone marrow cell with $5x10^{-5}$ M nitroso-CAP resulted in 50 % cell death in 48 hr. At a concentration of $3x10^{-4}$ M 85 % of cells were dead in 24 hr., and over 90 % were dead in 48 hr. In addition, incubation of a rapidly growing human lymphoid cell line (*Raji cell*) for 12 hr. with the NO-CAP concentration of $3x10^{-5}$ M (approximately 9 mcg/ml) resulted in accumulation of cell in the G_2M phase and significant cell death. In another study was performed by Yunis at all. (1976; 1980a; 1981a; 1981b) observed that CAP does not inhibit DNA synthesis until concentrations above $3x10^{-4}$ are reached. Thus, 50 % inhibit was obtained at $1x10^{-3}$ M. In sharp contrast significant inhibition of DNA synthesis was observed at $1x10^{-4}$ M NO-CAP reaching 80-90 % at $4x10^{-4}$ M. Furthermore, whereas the inhibition by CAP was reversed by removing the drug, that from NO-CAP was largely irreversibl.

In subsequent studies they (Yunis, 1984; Muray, 1982) have in vitro demonstrated that NO-CAP has the ability to cause breaks in double-stranded DNA . Levels of NO-CAP of >= 5 mM readily produce demonstrable levels of acid soluble fragments from radiolabelled DNA in 30 min., with complete hydrolysis occuring at a concentration of 100 mM. Furthermore, same authers have indicated that DNA damage in the form of a single strand breaks could be readily demonstrated in cultured Raji cells and phytohemagglutinin-stimulated normal human lymphocytes by small concentrations of NO-CAP (0.05-0.1 mM). A small but reproducible effetc was observed from large concentrations of CAP (2 mM) (Yunis, 1987) .

DNA Damage and Genetoxicity in Intact Cells Induced by Intestinal Bacterial Metabolites of Chloramphenicol

In recent studies, it clearly indicated that NO-CAP more toxic than main compound (Yunis, 1980a; Yunis, 1981b; Yunis, 1987). However, in order to shown toxic effects of NO-CAP, has to be accumulated in target tissues, such as the bone marrow. Abou-Khalil at all. (1988) observed that incubation of CAP and analogues with the blood or liver at 37 °C for up to 30 min. showed the following: CAP was stable in both tissues with full recovery; DH-CAP was stable for 5 min., than gradually decreased reaching 50 or 70 % of the initial amount after 30 min. of incubation with blood and liver, respectively; NPAP decreased at a faster rate than DH-CAP, and NO-CAP completely disappeared. The data suggeest that if and when formed in the body , DH-CAP and NPAP may stay in the circulation long enough to reach the marrow and interact with its cellular components.

The metabolites of CAP known (Isildar, 1988; Jimenez,1987) to be produced by intestinal bacteria are of three types (Fig. 1).

1- Aminochloramphenicol (H_2N-CAP) is a final nitroreduction product of CAP.

2- p-nitrobenzaldehyde (PNBA) and p-nitrophenyl-2-amino-3-hydroxy-propanone. HCl (NPAP) are products of CAP via oxidation at C-1-hydroxyl and hydrolysis.

3- 2-dichloroacetomido-3-hydroxypropio-p-nitro propa none (DH-CAP) possesses a structure very similar to that of CAP, except the C-1 hydroxyl group of CAP is oxidized to a keton moiety.

Isildar at all. (1988b) have investigated myelotoxic effect of CAP, DH-CAP, NPAP and NO-CAP, in a result they demonstrated that DH-CAP and NPAP much more toxic than CAP, and toxic effects of DH-CAP and NO-CAP were similar. In the same study, they demonstrated that at concentrations of $<=10^{-4}$ M/L, DH-CAP caused totaly irreversible inhibition of myeloid colony (*CFU-GM*) growth and 80 % inhibition of DNA synthesis in human bone marrow. In addition, they have determined that incubation of human bone marrow cells with 10^{-4} m/L NO-CAP or DH-CAP for 24 hours resulted in 75 % and 65 % cell death respectively, and DH-CAP was 10-20 fold more cytotoxic than CAP. Jimenez at all. (1990) have showed that DH-CAP inhibited CSF(G-CSF, GM-CSF) production without affecting cell viability. Colony stimulating factors (CSFs) play an essantial role in hematopoietic cell growth. In the same study they found that increasing concentrations of rhGM-CSF or rhG-CSF completely reversed the inhibitory effect of CAP on human CFU-GM growth, but inhibition of DH-CAP , NO-CAP and NPAP was not affected by either CSF. Same authors have also suggested that the daul toxic-inhibitory effect of some intestinal metabolites of CAP such as DH-CAP on hemapoietic cell growth on the one hand, and on CSF production on the other.

Isildar at all. (1988) clearly demonstrated that DH-

CAP was capable of inducing DNA single strand breaks in Raji cells, activeted human lymphocytes and human marrow cells at concentration of 10^{-4} M.

In recent studies (Yunis, 1980a; Yunis, 1980b; Yunis, 1981b; yunis, 1984) showed that NO-CAP has cytotoxic and genotoxic effect. Although nitroreduction of CAP can be carried out by the liver , presumably resulting in the production of the toxic nitroso-intermediate, it appears unlikely that nitroso-CAP can reach its target organ, the bone marrow, because of extreme instabilty. Thus, nitroso-CAP is eliminated from whole blood within seconds. The nitroreduction of DH-CAP by human and rabbit bone marrow cell homogenates, as Raji cells was observed under aerobic condition by Isildar at all. (1988a).

These results suggest that genetoxicity of DH-CAP may be related to its nitroreduction by the target tissue with in situ production of toxic intermediates. Together with previus studies (Yunis, 1984; Abou-Khalil, 1987; Muray, 1987; Yunis, 1987; Abou-Khalil, 1988; Isildar, 1986b; Jimenez, 1987), these observations lend support to the thesis that the p-nitro group may be the structural feature underlying aplastic anemia from CAP.

Efforts have been made to find a replacement for chloramphenicol. Thiamphenicol has been investigated in more than 12 million human patients in Europe without producing a single case of aplastic anemia. Unfortunatally, thiaphenicol does not have the same broad antibacterial spectrum of CAP. Another possibility in a analog of thiamphenicol, florphenicol, which replaces the 3' hydroxyl group with an atom of flourine. This compound has a good antibacterial spectrum of activity and may eventually serve as a chloramphenicol replecament (Huber,1982)

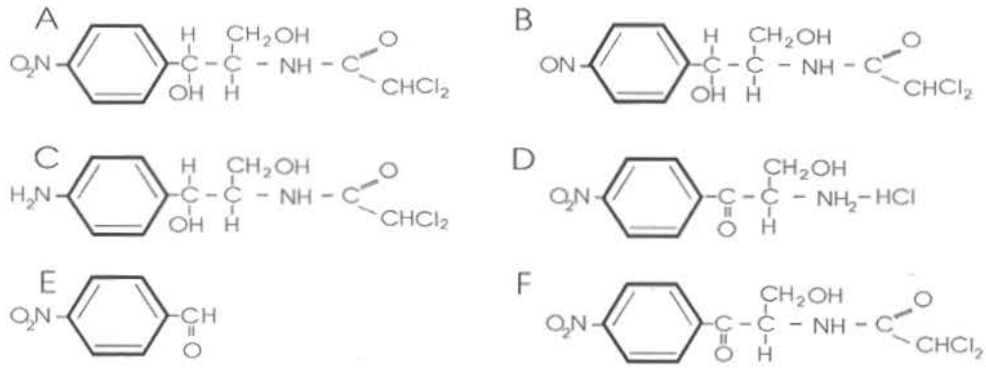

Fig. 1. Chloramphenicol and its metabolites (A) CAP (B) NO-CAP (C) H_2N-CAP (D) NPAP (E) PNBA (F) DH-CAP

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