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Antioxidants Reveal Protection against the Biochemical Changes in Liver, Kidney and Blood Profiles after Clindamycin / Ibuprofen Administration in Dental Patients

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***Abstract*—**The adverse effects of Clindamycin (Clind.) / Ibuprofen (Ibu.) combination on liver, kidney, blood elements and the significances of antioxidants (N-acetylcysteine and Zinc) against these effects were evaluated. The study includes: Group I; control n=30, Group II; patients on Clind.300mg/Ibu.400mg twice daily for a week n=30, Group III; patients on Clind.300mg/Ibu.400mg+N-acetylcysteine 200mg twice daily for a week n=15 and Group IV; patients on Clind.300mg/Ibu.400mg+Zinc50mg twice daily for a week n=15. Serum malondialdehyde (MDA), alanine transferase (ALT), aspartate transferase (AST), γ glutamyl transferase (GGT), creatinine, blood urea nitrogen (BUN) were measured. Applying one way ANOVA followed by Tuckey Kramer post test, Group II showed significant increase in ALT, AST, GGT, BUN and decrease in Hb, RBCs, platelets than Group I. Group III showed significant decrease in ALT, AST, GGT, BUN than Group II. Moreover, Group IV showed significant decrease in ALT, AST, GGT and increase in Hb, RBCs, and platelets than Group II. Conclusively, Adding Zinc or N-acetylcysteine buffer the oxidative stress and improve the therapeutic outcome of Clindamycin/Ibuprofen combination.

***Keywords*—**Clindamycin, Ibuprofen, Adverse effects, Antioxidant, Zinc, N-acetylcysteine.

I. Introduction

CLINDAMYCIN is a suitable antibiotic for treating dental patients because of its high degree of activity against gram positive cocci and most of the anaerobes that cause these dental infections [1]; Meanwhile, Ibuprofen, with its analgesic, anti-inflammatory, and antipyretic properties, has been shown to control dental pain [2]. Therefore, Clindamycin / Ibuprofen combination can be used to control infection and inflammation [2]. Clindamycin therapy has been rarely associated with blood changes, jaundice, raised liver enzyme levels and/or hepatotoxicity [1]; While, Ibuprofen as one of the non-selective NSAIDs may produce GI toxicity, including bleeding, ulceration, and perforation. In addition, renal side effects, including renal failure and interstitial nephritis may be aroused [3].

These adverse effects of the widely prescribed Clindamycin / Ibuprofen for dental patients may be due to oxidative stress. Oxidative stress stimulates inflammatory response by activating particularly the redox sensitive nuclear factor (NF)-κB that leads to generation of various inflammatory cyto- and chemokines [4]. Reactive oxygen species (ROS) such as O_2–, H_2O_2 and OH– are highly toxic to cells. ROS-mediated lipid peroxidation, oxidation of proteins, and DNA damage are well-known outcomes of oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death [5]. Moreover, the intracellular glutathione (GSH) pool is reduced in chronic inflammation [6]. Cellular antioxidant enzymes and the free-radical scavengers normally protect a cell from toxic effects of the ROS that lead to various pathological conditions [7]. It has been suggested that the impairment of oxidant-antioxidant balance in saliva causes oral diseases [8]. Several antioxidants are used successfully to treat certain diseases and act against tissue injury either directly or by induction of endogenous antioxidants such as vitamins (C & E), N-acetyl cysteine and Zinc [9].

The clinical importance of N-acetylcysteine (NAC) is an antioxidant, which is a thiol-containing compound that interacts and detoxifies free radicals by non-enzymatic reactions either by conjugation or by reduction [9]. In many tissues, NAC is deacetylated to form cysteine, which supports glutathione biosynthesis. In high concentrations, NAC can protect cells against oxidative damage by two mechanisms; by reacting directly with H_2O_2 extracellularly as a direct antioxidant, and by increasing the cytoplasmic reserve of glutathione [10].

Many drugs and poisons are detoxified through conjugation with GSH (e.g. paracetamol, among others) and additional GSH, provided by administering NAC, has been shown to be effective in intoxication by such drugs and poisons [10]. Moreover, NAC might be of benefit in the treatment of hepato-renal syndrome [11].

Zinc may act as an antioxidant and anti-inflammatory agent. The ability of zinc to function as an antioxidant and stabilize membrane suggests that it has a role in the prevention of free radical induced injury during inflammatory process [12]. Zinc has the ability to modulate the oxidative stress by producing

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metallothionein, which is very rich in cysteine and is an excellent scavenger of ROS [13]. Moreover, the increased amount of cytokines is associated with decreased zinc status in cutaneous leishmaniasis patients and increased lipid peroxidation products are associated with decreased zinc status in children with chronic giardiasis [13].

It has been reported that zinc is effective against intoxication by drug and poisons by increasing the synthesis of GSH [10].

II. Materials and Methods

A. Subjects

Ninety subjects were divided over the studied groups. All samples were obtained from outpatients' Dental Clinic at Modern Sciences and Arts University (MSA Uni.). All subjects are males, more than 18 years old, with normal body weight and non-smokers. They were not suffering any chronic diseases and not taking any other medications. Selected patients were classified into three groups; Group I (Control group): included 30 patients and received no drugs, Group II: included 30 patients received Clindamycin (300mg) + Ibuprofen (400mg) twice daily for one week, Group III: in which 15 patients received Clindamycin (300mg) + Ibuprofen (400mg) + N-acetyl cysteine (200mg) twice daily for one week and Group IV: in which 15 patients received Clindamycin (300mg) + Ibuprofen (400mg) + Zinc (50mg) twice daily for one week.

B. Blood Sampling, Collection and Storage

Blood samples were collected from all 90 subjects (10mls). Five mls of the blood was collected into vacutainer tubes with K3_Edeta as anticoagulant in a concentration of 50 micron for the analysis of hemoglobin, RBCs and platelets; While, the other 5mls of blood was collected into plain vacutainer tubes, centrifuged at 15,000 xg then, serum was separated for the assessments of liver (sALT, sAST and sGGT), kidney (sCreatinine and sBUN) and blood (Hb, RBCs and platelets) profiles and lipid peroxide test (MDA).

The whole blood was stored at 4-8°C until assay, usually within a week. Serum was assayed same day, otherwise, stored at -80°C until time of assay.

C. Laboratory Assessment

The whole blood obtained for the measurement of Hb, RBCs & platelets while, serum obtained was aliquoted for the measurements of liver profile: ALT, AST and GGT, kidney profile: creatinine and BUN, blood profile: Hb, RBCs, platelets, and lipid peroxide test (MDA).

The liver profile and creatinine were assessed and measured by using kinetics method described in the kit of QM lab prietest ECO and biochemistry analyser, Germany; While BUN and MDA were assessed and measured by using colorimetric method in QM lab prietest ECO+ biochemistry analyser, Germany. The blood profile was assessed and measured by using Automated ERMA cell counter, model PCA 210 N, Japan.

D. Statistical Assay

Results were expressed as mean ± standard error of mean (M ± S.E.M). Different groups were compared by analysis of variance (One Way ANOVA) at $P<0.05$ and followed by Tukey Krakmer posttest using Graph Pad Prism 4 software.

III. Results

The clinical parameters of liver, kidney and blood profiles of the studied groups are shown on Tables I-III.

As shown in Table I, administration of Clindamycin / Ibuprofen combination (Group II) by dental patients significantly increased serum activities of liver profile; ALT, AST and GGT if compared to the control group (Group I).

Co-administration of NAC / Zinc along with Clindamycin / Ibuprofen (Group III and Group IV) respectively, the activities of serum ALT, AST and GGT were markedly reduced than in patients received Clindamycin / Ibuprofen only (Group II) although they were still at higher levels in comparison with the control group (Group I). Meanwhile, the co-administration of Zinc along with Clindamycin / Ibuprofen (Group VI) showed a marked decrease in sAST and sGGT levels compared to, the co-administration of NAC along with Clindamycin / Ibuprofen (Group III).

Table II shows the administration of Clindamycin / Ibuprofen combination by dental patients (Group II) produced no significant effect on serum creatinine level. Moreover, the co-administration of NAC / Zinc along with this combination (Group III) or (Group IV) respectively did not alter serum creatinine level. However, BUN level was markedly increased in (Group II) compared with the control group (Group I), while the co-administration of either NAC or Zinc with the combination (Group III or Group IV) respectively, resulted in normalization of BUN level.

Table III explains that Clindamycin / Ibuprofen administration (Group II) resulted in marked reduction in hemoglobin level, RBCs count and platelet count when compared to the corresponding control values (Group I).

Administration of NAC or Zinc along with Clindamycin / Ibuprofen led to normalization of hemoglobin level. Moreover, (Group III) shows a significant increase of both RBCs and platelet counts along with reduction of MDA level than (Group II), while (Group IV) nearly normalizes both RBCs and platelet counts and shows a significant reduction in MDA level than (Group II).

TABLE I
The Effect of Antioxidants on Liver Profile Parameters in Clindamycin / Ibuprofen-Treated Patients

Liver profile parameters	Group(I) Control Mean ± SEM	Group(II) Clind+Ibu Mean ± SEM	Group(III) Clind+Ibu+NAC Mean ± SEM	Group(IV) Clind+Ibu+Zn Mean ± SEM
a. ALT (U/L)	15.20 ± 0.85	33.90 ± 1.07a	19.40 ± 0.75a,b	22.00 ± 0.92a,b
b. AST (U/L)	13.10 ± 0.40	31.80 ± 1.05a	21.00 ± 0.79a,b	14.50 ± 0.87a,b
c. GGT (U/L)	24.10 ± 0.43	39.30 ± 10.96a	30.40 ± 0.85a,b	25.10 ± 0.78b

a: Significantly different from control group (Group I)
b: Significantly different from Clind. + Ibu. Group (Group II)

TABLE II
THE EFFECT OF ANTIOXIDANTS ON KIDNEY PROFILE IN CLINDAMYCIN / IBUPROFEN-TREATED PATIENTS

Kidney profile parameters	Group(I) Control Mean ± SEM	Group(II) Clind+Ibu Mean ± SEM	Group(III) Clind+Ibu+NAC Mean ± SEM	Group(IV) Clind+Ibu+Zn Mean ± SEM
a. creatinine (mg/dl)	1.10 ± 0.05	1.00 ± 0.02	0.95 ± 0.04	0.89 ± 0.03a
b. BUN (mg/dl)	25.00 ± 1.20	36.50 ± 0.97 a	24.30 ± 0.79	22.10 ± 0.86a,b

a: Significantly different from control group (Group I)
b: Significantly different from Clind. + Ibu. Group (Group II)

TABLE III
THE EFFECT OF ANTIOXIDANTS ON BLOOD PROFILE PARAMETERS IN CLINDAMYCIN / IBUPROFEN-TREATED PATIENTS

Blood profile parameters	Group(I) Control Mean ± SEM	Group(II) Clind+Ibu Mean ± SEM	Group(III) Clind+Ibu+NAC Mean ± SEM	Group(IV) Clind+Ibu+Zn Mean ± SEM
a. Hb (g/dl)	14.50 ± 0.56	11.60 ± 0.28a,b	13.20 ± 0.46	15.80 ± 0.36
b. RBCs (million/m3)	5.20 ± 0.36	2.80 ± 0.19a	3.90 ± 0.21a,b	4.80 ± 0.31
c. Platelets (million/m3)	0.36 ± 0.02	0.10 ± 0.006a	0.21 ± 0.003a,b	0.39 ± 0.002
d. MDA	6.95 ± 0.31	31.60 ± 0.89a	15.60 ± 0.49a,b	19.20 ± 0.68a,b

a: Significantly different from control group (Group I)
b: Significantly different from Clind. + Ibu. Group (Group II)

IV. DISCUSSION

Clindamycin / Ibuprofen combination shows along with the gastrointestinal tract side effects, recent evidences indicated their adverse effects on liver, kidney and blood [14], [15]. Meanwhile, the Clindamycin / Ibuprofen combination is widely prescribed for dental patients; the present study evaluates its undesirable effects on liver, kidney and blood as well as the underlying mechanism of these adverse effects. Because oxidative stress is implicated in the majority of drug-induced adverse effects, it will be valuable to explore the possible protection by administration of NAC or Zinc.

The present data revealed that administration of Clindamycin / Ibuprofen resulted in abnormal elevation of liver function enzymes (ALT, AST and GGT) and increased BUN in dental subjects. These findings are in harmony with previous studies indicated that administration of Clindamycin lead to elevation of liver enzymes and induce jaundice and hepatotoxicity [1]. The elevated level of BUN after administration of Clindamycin / Ibuprofen combination is in accordance with the previous findings that acute kidney failure with the raise of blood urea, creatinine and potassium was shown few days after starting treatment with NSAIDs [15]. Because there were no previous reports for adverse effects of Clindamycin alone on renal functions and for Ibuprofen alone on liver functions it could be suggested that the produced effects in the present work is a combined effect of Clindamycin (on the liver) and Ibuprofen (on the kidney). Moreover, oxidative stress might be implicated in these adverse effects.

Our findings explained that Clindamycin / Ibuprofen combination significantly reduced blood Hb, RBCs and platelets. This effect can be explained by the fact that the non-selective inhibition of cyclooxygenase-induced by NSAIDs-induces a reduction in thromboxane A2 synthesis, which in turn leads to the inhibition of platelet aggregation which is an important step in hemostasia [16]. The incapacity of the platelets to produce cyclooxygenase explains why acetylation of this enzyme induces an irreversible toxic effect that persists for the full life of the platelet (7-10 days) in the case of aspirin, and is reversible in the case of the rest of NSAIDs including ibuprofen [16].

In addition, the metabolic pathway for Clindamycin may explain these effects, as it is well known that Clindamycin induces cytochrome P450 3A enzyme, which is an oxidizing enzyme able to produce oxidative stress with subsequent destruction of blood elements, which is translated in the form of reduced number in RBCs as well as platelets. In this regard, the increased BUN level may be, at least in part, due to destruction of nitrogenous blood compounds such as heme protein. By this way, it may be proposed that the adverse effects of this combination on blood elements are an additive effect of both Clindamycin and Ibuprofen.

Administration of Clindamycin / Ibuprofen in the present work markedly increased the level of MDA, which indicates induction of lipid peroxidation. This effect can be due oxidative metabolism of Ibuprofen and Clindamycin in the liver [16]. Induction of oxidant enzymes lead to production of free radicals that attack vital cellular components. This may explain the increased level of MDA in the present study. In addition, induction of oxidative stress by Clindamycin and/or Ibuprofen can add further augmentation to our data where the disrupted renal and liver functions as well as abnormal blood pattern may be attributed to drugs-induced oxidative stress.

It has been reported that the clinical importance of NAC is due to its role in defense against oxidative stress insults [9]. In the present study, NAC administration markedly improved the therapeutic outcome of Clindamycin / Ibuprofen combination in dental patients by reduction of liver function enzymes activity, reduction of BUN and corrections of blood elements abnormality. These effects are accompanied with a significant decrease in MDA level. The produced data are in close agreements with previous sties in which NAC was successfully improved haemodynamics and oxygen transport in acute liver failure of different origins [17].

In addition, one study suggests that the "standard" high dose of NAC induces a net protein catabolism in the liver during transplantation [18]. Another Italian study revealed that combination therapy would be of benefit [19]. Moreover, it was found that administration of NAC resulted in improved liver functions in patients with peritonitis [19]

It has been concluded that the use of NAC for the prevention of contrast nephropathy was recommended [20]. NAC might be of benefit in the treatment of hepatorenal syndrome [11], buffers oxidative stress and inhibits cell death induced by ischaemia-reperfusion injury in the kidney [21]. Further augmentation to our data is that NAC is used as an antidote for acetaminophen-induced liver poisoning [22].

The decreased MDA level by NAC administration may explain its beneficial effects. In this respect, it is satisfactory to suggest that NAC could act against Clindamycin /

Ibuprofen-induced oxidative stress and protect against liver, kidney and blood deterioration. This suggestion is supported by the previous findings indicate the requirement of NAC for tissue GSH synthesis, the availability of cysteine is generally the limiting factor, and one of the effective precursors of cysteine is its synthetic derivative, NAC [9]. It has been also reported that NAC is effective against intoxication by drugs and poisons by increasing synthesis of GSH [10].

Moreover, N-acetylcysteine (NAC) is a thiol-containing compound that interacts and detoxifies free radicals by non-enzymatic reactions either by conjugation or by reduction [9]. Furthermore, high concentrations NAC can protect cells against oxidative damage by two mechanisms; by reacting directly with H2O2 extracellularly as a direct antioxidant, and by increasing the cytoplasmic reserve of glutathione [10].

Meanwhile in the present study, the co-administration of zinc with the combination emphasiasis the ability of Zinc to retard the oxidation process mechanism. This goes along with the study that shows the capability of Zinc to reduce post-ischemic injury to a variety of tissues and organs through the mechanism of antagonizing copper reactivity [23].

In the present study, Zinc administration significantly improved the therapeutic outcome of Clindamycin / Ibuprofen combination in dental patients by reducing the liver function enzymes activity, BUN and corrections of blood element deformity. These effects are accompanied with a significant decrease in MDA level. The current outcomes were supported by previous studies that pointed to the requirement of Zinc for tissue GSH synthesis in which zinc provides SH-groups and scavenge ROS. Moreover, Zinc has important role in inhibiting neutrophil activation, decreasing microbial attachment and vasodilatation [24]. Also, it has been reported that Zinc is effective against intoxication by drugs and poisons by increasing the synthesis of GSH [10].

Recently, in a large study organized by the National Eye Institute - National Institutes of Health - it was reported that Zinc and (Vitamin C, Vitamin E and H-carotene) significantly reduced the odds of developing advanced age-related macular degeneration (AMD) and prevented blindness in a high-risk group of elderly subjects. Although the mechanism of Zinc effect was not defined, one may hypothesize that Zinc reduced the oxidative stress and was thus beneficial in AMD [25].

From above, it could be suggested that Clindamycin / Ibuprofen impaired liver and kidney functions and induced blood abnormalities at least in part by induction of oxidative stress, which is expressed by increased level of MDA. On the other hand, NAC and Zinc ameliorated these effects by buffering oxidative damage. This may provide a new strategy by using Zinc and NAC as adjuvant therapies for dental patients to improve therapeutic outcome of Clindamycin / Ibuprofen combination.

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