A Direct Comparison of Anti-ulcer Effects of Coenzyme Q10 and Vitamin C on Indomethacin-induced Gastric Ulcer in Rat: A Controlled Experimental Study

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ABSTRACT

Introduction: Indomethacin increases generation of mitochondrial reactive oxygen species (ROS) which have a crucial role in the indomethacin-induced gastric ulcer. Coenzyme Q10 has an antioxidant activity on mitochondria and cell membranes and protects lipids from oxidation and is essential for stabilizing biological membranes. Superoxide dismutase (SOD) acts as one of the defense mechanisms against free radicals. When the generation of ROS overwhelms, the antioxidant defense, lipid peroxiation of cell membrane occurs and cause cell damage.

Materials and Methods: Male adult Wistar rats were divided into A and B groups. The rats in group A were then further divided into three subgroups of 6 animals each and received one of the following treatments: Animals in the first subgroup received saline. Animals in the second subgroup received saline and indomethacin. Animals in the third subgroup received vitamin C and indomethacin. The rats in group B were also further divided into 3 subgroups of 6 rats each and treated with one of the following treatments: Animals in first subgroup received 1% Tween 80 as vehicle. Animals In second subgroup received 1% Tween 80 and indomethacin. Animals in third subgroup received CoQ10 and indomethacin. Four hours after the last treatment, animals were killed by an overdose of ether and 2 ml blood was drawn from left ventricle into syringe containing EDTA (1mg/ml) and the stomachs removed were cut and gastric mucosal lesions were examined). Ulcer indexes were determined and SOD activity measured in plasma.

Results: Pre-treatment with both vitamin C and coenzyme Q10 was associated with attenuation of ulcer index and increased SOD activity compared with animals treated with indomethacin alone (P<0.001).

Conclusion: This effect of CoQ10 may be due to its electron donating property that inhibits the decrease in SOD activity in gastric tissue (replenishment of endogenous SOD) and inhibiting lipid peroxidation.

Introduction:

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to induce gastric mucosal damage in patients chronically taking these drugs and also used to produce experimental ulcer in animals (1,2,3). Although it has been proposed that inhibition of prostaglandin synthesis by NSAID is involved in this side effect, it has recently been proposed that microvascular injuries may be important events that lead to mucosal injury induced by these drugs and lipid peroxidation mediated by free radicals plays a crucial role in the indomethacin-induced gastric mucosal injury (4). NSAIDs damage mitochondria and cause Mitochondrial dysfunction which is associated with increased generation of intra- mitochondrial reactive oxygen species (ROS) leading mitochondrial oxidative stress (5).

Scavenging the ROS prevents indomethacin-induced mitochondrial changes. It has been suggested that the ROS and lipid peroxidation play an important part in the pathogenesis of gastric mucosal injury induced by indomethacin and the decreased gluthathion peroxidase (GSH-PX) activity aggravates the injury due to accelerated accumulation of ROS in gastric mucosal cells (6). Coenzyme Q10 (CoQ10) is a producing center of the cell known as the mitochondria and has an effect on electron transport and energy production (ATP). CoQ10 has also an antioxidant effect on mitochondria and cell membranes and protects lipids from oxidation and thereby stabilizes biological membranes, so it is essential for the health of all human tissue and organs (7,8). Antioxidants are substances that scavenge oxidants (free radicals), compounds that alter cell membrane, tamper with DNA and even cause cell death. Oxidative stress is an imbalance between the oxidant and antioxidant systems of the body, in favour of the oxidants and has been linked to the development of

diseases such as cardiovascular diseases, cancer, neurodegenerative diseases and diabetes. CoQ10 is a potent free radical scavenger. This effect related to its electron donating property that neutralizes free radicals and its ability to replenish endogenous antioxidants such as GSH-PX and superoxide dismutase (SOD) (9). Protective effect of CoQ10 against cardiovascular and neurodegenerative diseases is well established (9). It has also been shown that CoQ10 acts against gastric ulceration and healing of chronic acetic acid gastric ulcer (10) and acts against indomethacin induced acute gastric ulcer in rats (11). The oxidized and reduced forms of CoQ10 called ubiquinone and ubiquinole respectively. Ubiquinole is biologically active form of CoQ10 and has received more interested due to its higher bioavailability and its rapid and better effects (12). Acid ascorbic (vitamin C) is a known antioxidant agent and can reduce and thereby neutralize ROS. It has a biological role as a reducing agent in a number of hydroxylation reactions and protects essential substances in the body such as proteins, lipids, carbohydrates and DNA from damage by free radicals (13). It has been suggested that vitamin C reduced development of indomethacin induced gastric damage in rats and reverse the indomethacin induced decrease in the level of antioxidant enzymes (13). It has been suggested that gastroprotective properties of vitamin C could be related to its positive effects on the antioxidant enzymes activity in indomethacin induced gastric ulcers in rats (14). In this study we investigated the effect of CoQ10 and vitamin C on indomethacin induced gastric ulcer. Vitamin C has been used as a known antioxidant for comparing the antioxidant effects of CoQ10.

Materials and Methods:

Male adult Wistar rats (n=36) weighing

200 to 250 g were used in this study. Animals were housed 3 per cage at 22 ± 2°C with a controlled 12-hour light-dark cycle. Food was withdrawn 24 hours before the experiment but the animals were allowed free access to water. The animals were handled in accordance with the criteria and recommendations of ethics committee on animal experiments of the School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Animals were divided into A and B groups of 18 animals each. The rats in group A were then further divided into three subgroups of 6 animals each and received one of the following treatments: animals in the first subgroup received saline (1ml/kg, SC) at 10 and 11 AM, animals in the second subgroup received saline (1ml/kg, SC) at 10 AM and indomethacin (30 mg/kg, IP) at 11 AM ,animals in the third subgroup received vitamin C (350 mg/kg, IP) at 10 AM and indomethacin (30 mg/kg, IP) at 11 AM. The rats in group B were also further divided into 3 subgroups of 6 rats each

and treated with one of the following treatments: Animals in first subgroup received 1% Tween 80 as vehicle (9) (1 ml/kg, IP) at 10 and 11 AM. Animals in second subgroup received 1% Tween 80 (1ml/kg, IP) at 10 AM and indomethacin (30 mg/kg, IP) at 11 AM . Animals in third subgroup received CoQ10 (200 mg/kg, IP) at 10 AM and indomethacin (30 mg/kg, IP) at 11 AM. Four hours after the last treatment, animals were killed by an overdose of ether and 2 ml blood was drawn from left ventricle into syringe containing EDTA (1mg/ml) and the removed stomachs were cut open along the great curvature and washed out with saline and fixed in 10% neutral buffered formalin and gastric mucosal lesions were examined under 5-fold binocular magnification to asses lesions (15,16). Ulcer indexes were determined and SOD activity measured in plasma. The different groups of study has schematically illustrated in figure 1:
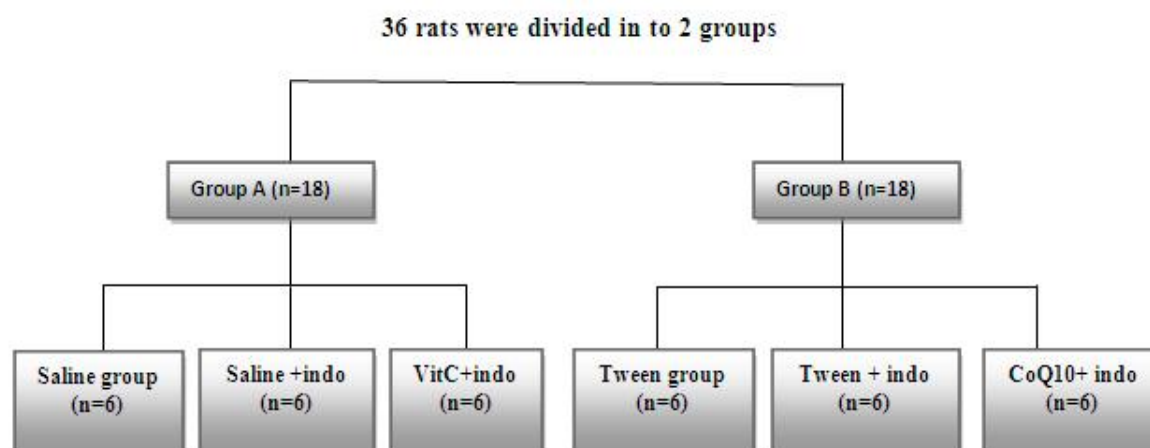

Figure. 1: Schematic illustration of experiment

The following chemicals were used in this study: Ubiquinol (Kaneka,Japan), vitamin C (Osvah, Pharmaceutical Co., Iran), EDTA (Merck, Germany), SOD kit (Randox laboratories, UK), indomethacin (Sigma Chemical Co., UK), Tween 80 (Sigma Chemical Co., UK). Removed stomach cut open and washed out with salin. Samples from each group fixed in

10% neutral buffered formalin. Ulcers were examined by 5-fold binocular magnification to assess lesions. The number of ulcers, ulcer surface area, ulcer score and ulcer index were determined. Ulcers were scored intensify using a 4 point scale (0 = no lesion, 1- superficial mucosal erosion, 2- deep ulcer or transmural ulcer, 3- perforated or

penetrated ulcer) (13,16). The ulcer index was calculated by the following formula $U_I = U_N + U_S + \frac{U_A}{10}$ where UI is the ulcer index, UN is ulcer number, US is the ulcer score and UA is the ulcer surface area for each stomach. The ulcer surface area was estimated using transport tapes divided into cells each 1 mm^2 in area; the number of cells was counted and the ulcer area was thus measured for each stomach. For histopathologic assessment, the formalin-fixed specimens were embedded in paraffin, sectioned (5μm), and stained with hematoxylin and eosin, and then histochemical sections were evaluated by light microscopy. Blood samples transferred to plastic tubes, centrifuged for 10 minutes at 3000 rpm, and then aspirated of the plasma. The red blood cells (RBCs) were washed 4 times with 3 ml of normal salin and centrifuged for 10 minutes at 3000 rpm after each wash. Then, 2 ml of cold redistilled water was added to the washed, centrifuged RBCs, mixed and left to stand at 4°C for 15 minutes before being subjected to lysis. The lysate was diluted with 0.01 mol/L phosphate buffer (pH=7) and SOD activity was determined using an enzyme kit. This method employs xanthine and xanthine oxidase to produce superoxide radicals which react with INT (idophenyl-nitrophenol-enyltertrazolium) to form a redformazan dye. The SOD activity was measured photometrically by the degree of inhibition of this reaction at 505 nm and 37°C and expressed as U/mL (13). Data were analyzed statistically by Student t-test and expressed as mean ± SEM. P<0.05 was considered statistically significant. Calculations were performed using SPSS version16.

Results:

In animals treated with saline, ulcer index and SOD activity were zero and 78.50 ± 0.93 (U/ml) respectively. In animals treated with indomethacin alone, mean ulcer index was significantly increased

and SOD activity was significantly decreased compared with saline treated animals (p<0.001) (Table 1). In animals pre-treated with vitamin C, ulcer index was significantly decreased and SOD activity was significantly increased compared with animals treated with indomethacin alone (Table 1). In animals treated with 1% Tween 80 (vehicle), ulcer index and SOD activity were zero and 75.10 ± 0.62 (U/ml) respectively. In animals treated with indomethacin alone, mean ulcer index was significantly increased and SOD activity was significantly decreased compared Tween 80 treated animals (p<0.001) (Table 2).

Table 1: Ulcer index and activity of SOD in group A

Subgroups (n=6)	Ulcer index	SOD (U/ml)
Saline (1 ml/kg)	0	78.50 ± 0.93
Indomethacin (30 mg/kg)	8.33 ± 0.66	30.10 ± 0.68
Vitamin C (350 mg/kg) + Indomethacin (30 mg/kg)	2.5 ± 0.42*	62.66 ± 2.29
	(P<0.001)	

*P<0.001 versus indomethacin alone
SOD=Superoxide dismutase

In animals pretreated with CoQ10 ulcer index was significantly decreased and SOD activity was significantly increased compared with animals treated with indomethacin alone (Table 2).

Table 2: Ulcer index and activity of SOD in group B

Subgroups (n=6)	Ulcer index	SOD (U/ml)
Tween 80 (1 ml/kg)	0	75.10 ± 0.62
Indomethacin (30 mg/kg)	7.43 ± 0.26	28.18 ± 0.52
CoQ10 (200 mg/kg)+ Indomethacin (30 mg/kg)	2.66 ± 0.33*	4 9.96 ± 2.32*
	(P<0.001)	

P<0.001 versus indomethacin alone
SOD=Superoxide dismutase

Discussion:

An increase in aggressive factors like free radicals or decrease in defensive factors like antioxidant enzymes lead to loss of mucosal integrity resulting in ulceration (17). SOD is one of the main antioxidant enzymes that constitutes a part of the physiologic defence against oxidative stress and its activity is estimated as an index of the antioxidant status (18). When the generation of ROS overwhelms The antioxidant defense, lipid peroxidation of the cell membrane occurs, which cause disturbance in the cell integrity leading to cell damage (13). It seems that, in the presence of indomethacin, free radical production overwhelms antioxidant defence and this situation cause disturbances in the cell membranes. The effects of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction, but later, enzyme depletion take place, resulting in the oxidative cell damage (13). Blood concentration of vitamin C and other antioxidant decrease due to the accelerated consumption in the blood (19). CoQ10 (Ubiquinole) is one of the key substances in the myocardial energetic metabolism and also important for cell membrane stability, and with CoQ10 deficiency, myocytes could be prone to damage in the form of myopathy or myositis or even rhabdomyolysis (20) so it is essential for the health of all human tissue and organs (7,8). Protective effect of CoQ10 against cardiovascular and neurodegenerative diseases is well established (9). It has also been shown that CoQ10 acts against gastric ulceration and healing of chronic acetic acid gastric ulcer (10) and acts against indomethacin induced acute gastric ulcer in rats (11). In this study, vitamin C and CoQ10 were used as antioxidants, which produce attenuation of the ulcer index and enhanced SOD activity suggesting that CoQ10 has protective effect against indomethacin-induced gastric ulcer.

Histological analysis confirmed the biological results.

Conclusion:

In conclusion, this effect of CoQ10 may be due to its electron donating property that inhibits the decrease in SOD activity in gastric tissue (replenishment of endogenous SOD) and inhibiting lipid peroxidation. Limitations of the present study include the open-label design and the small number of rats used in each group. Large animal studies and controlled studies in humans are needed to confirm the results.

Conflict of Interests:

The authors have no conflict of interests.

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