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Research article

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Astaxanthin and Coenzyme Q10 are not synergistic against oxidative damage in cerulein-induced acute pancreatitis

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Ethics Committee Approval
All experimental protocols were approved by Marmara University Animal Care and Use Committee (63.2018.mar) according to Turkish law on the use of animals in experiments and New York Academy of Sciences guidelines.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The anti-inflammatory effects of Astaxanthin (ATX) and Coenzyme Q10 (Ubiquinone) are studied in different inflammation models. The study aims to investigate the synergistic effect of astaxanthin and Coenzyme Q10 in a rat model of acute pancreatitis.
Methods: Twenty-four female Wistar rats were grouped as control (C), vehicle-treated control (AP), astaxanthin-treated (ATX; 40 mg/kg), astaxanthin+coenzyme Q10- treated (ATX+Q10; 40 mg/kg and 1 gr/kg) groups. Cerulein was administered (50 µg/kg) twice, one hour apart. Seven hours after the first cerulein injection, the rats were sacrificed. Pancreatic oxidative damage was evaluated with an increase in the serum activity of lipase and amylase, tissue levels of myeloperoxidase activity (MPO), malondialdehyde (MDA), luminol and lucigenin and decrease of glutathione.
Results: In all AP groups, MDA and MPO increased while GSH decreased ($P<0.001$). ATX and ATX+Q10 both decreased MDA and MPO ($P<0.001$) and increased GSH ($P<0.01$). Both therapies significantly increased luminol levels ($P<0.001$, and $P<0.01$, respectively). Lucigenin markedly decreased in the ATX+Q10 group ($P<0.01$).
Conclusion: Antioxidant combination therapy does not alleviate oxidative damage in pancreatic tissue better than astaxanthin alone.

Keywords: Astaxanthin, Coenzyme Q10, Oxidative damage, Acute pancreatitis

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Introduction

Acute pancreatitis (AP) is an inflammatory condition which develops with interstitial pancreatic edema [1]. Oxidative stress has a well-known role in the pathophysiology of acute pancreatitis. Many reports revealed that while lipid peroxidation products increase, glutathione decreases in accordance with the severity of the disease.[2]

Epidemiologic data show an increase in the incidence of AP worldwide with significant morbidity and mortality [3]. The disease ranges from mild to severe, and patients with severe disease have a 30 % mortality rate.

Evidence-based data show an association between acute pancreatitis and oxidative stress. Based on these findings and experimental studies, antioxidants are beneficial in the management of acute pancreatitis [5,6].

Astaxanthin (ATX) is a naturally reddish carotenoid pigment derived mostly from marine environment. *Haematococcus pluvialis* is used as a dietary supplement [7]. Astaxanthin is ten times more effective than the other carotenoids such as zeaxanthin, lutein, and canthaxanthin [8,9]. Experimental studies also support its powerful antioxidant effect, which is due to the chain breaking role in free radical reaction [10,11].

Coenzyme Q (CoQ) a naturally occurring vitamin-like essential compound predominant in humans, is biosynthesized from a quinone structure [12]. It has antioxidant activity and a beneficial role in experimental models are reported [13-15]. It is also preferred as a dietary supplement worldwide as an antiaging agent because its levels decrease by age [16].

To the best of our knowledge, there is limited data regarding the role of astaxanthin and CoenzymeQ10 in pancreatitis. Recent studies revealed that astaxanthin may have a beneficial effect in acute pancreatitis. In the present study, we investigated the therapeutic activity of naturally occurring antioxidants, astaxanthin and CoQ10, in a rat model of acute pancreatitis.

Materials and methods

Animals

Twenty-four female Wistar rats (4-6 months old) were obtained from Marmara University Experimental Animals Research and Implementation Centre (DEHAMER) (Istanbul, Turkey). Animals were kept under standard conditions of humidity (65-70 %), temperature (22 ± 2°C) and constant light/dark (12 h/12 h) cycles and fed pellets and water *ad libitum*. All experimental protocols were approved by Marmara University Animal Care and Use Committee (63.2018.mar) according to Turkish law on the use of animals in experiments and guidelines of the New York Academy of Sciences.

Experimental design

The rats were divided randomly as control (C, n=6) and acute pancreatitis (AP, n=24) groups. The AP groups were subsequently divided into 3 subgroups as vehicle-treated (AP), astaxanthin (Sigma-Aldrich Co. LLC, Germany)-treated (ATX) and astaxanthin+ CoenzymeQ10 (Sigma-Aldrich Co. LLC, Germany) treated (ATX+Q10) group.

Acute pancreatitis was induced by the injection of cerulein (Sigma-Aldrich Co. LLC, Germany; 50 µg/kg) intraperitoneally twice, one hour apart. After the first cerulein injection the AP group was treated with vehicle olive oil, ATX group received 40 mg/kg astaxanthin, and ATX+ Q10 group (40 mg/kg astaxanthin + 1 gr/kg CoQ10) by orogastric gavage.

At the 6th hour following the final injection, the rats were sacrificed with cardiac puncture under anesthesia by thiopental sodium (50 mg/kg/ip). The pancreas tissue was removed for tissue analyses. Serum samples were collected for blood biochemical analyses.

Biochemical analyses

Serum amylase, lipase

Collected blood was centrifuged at 3,000g for 10 min at 4 °C. The serum amylase and lipase levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (eLabscience, San Diego, CA, USA) in accordance with the manufacturer's instructions and guidelines.

Malondialdehyde (MDA) and Glutathione (GSH) Levels

MDA level is a significant marker of lipid peroxidation. It is measured by a spectrophotometer at 532 nm by monitoring thiobarbituric acid reactive substance formation as previously described and expressed as nmol/g [17]. GSH was also measured by a spectrophotometric method via modification of the Ellman procedure [18], and a coefficient of 1.36 x 104 M-1 cm- was used to calculate GSH levels, which were expressed as µmol GSH/g tissue.

Myeloperoxidase activity

A heme protein, myeloperoxidase, is found predominantly in azurophilic granules and expressed by phagocytic cells. Tissue MPO activity is utilized to predict tissue PMN accumulation specially in inflamed tissues and correlates with the number of PMN determined histologically. By measuring the H2O2-dependent oxidation of o-dianizidine·2 HCl MPO activity is calculated. The activity of the enzyme was set as the amount of MPO that caused a change in absorbance tested at 460 nm by the spectrophotometric method. MPO activity was expressed as U/g tissue [19].

Chemiluminescence assay

To show reactive oxygen species (ROS) in the pancreatic tissue, we performed the chemiluminescence (CL) assay. It directly measures ROS where luminol and lucigenin can be used as enhancers. CL levels were tested with a Junior LB 9509 luminometer (EG&G Berthold, Germany). The results were calculated by the area under the curve and adjusted to the wet tissue weight. All results are expressed as relative light unit/ mg tissue (rlu / mg) [20].

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA post hoc Tukey test) with GraphPad Prism 6.0. Differences were considered significant if $P<0.05$. Values are expressed as mean ± SEM.

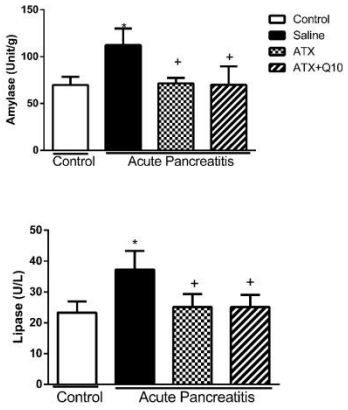
Results

Effect of compounds on serum amylase and lipase levels

When compared with the control group, serum lipase and amylase levels were higher in the vehicle-treated group ($P<0.05$),

and lower in the ATX and ATX+Q10 groups compared to the vehicle-treated group (P<0.05) (Figure 1).

Figure 1: Lipase and amylase levels in sera (Lipase (A)- and Amylase (B) levels in the pancreatic tissues of the control and vehicle, ATX and ATX+CoQ10 treated acute pancreatitis groups (n=6 per groups). Data are expressed as mean ± SEM; n=6 rats/group; **P<0.01 vs control group; +P<0.05 vs vehicle-treated group)



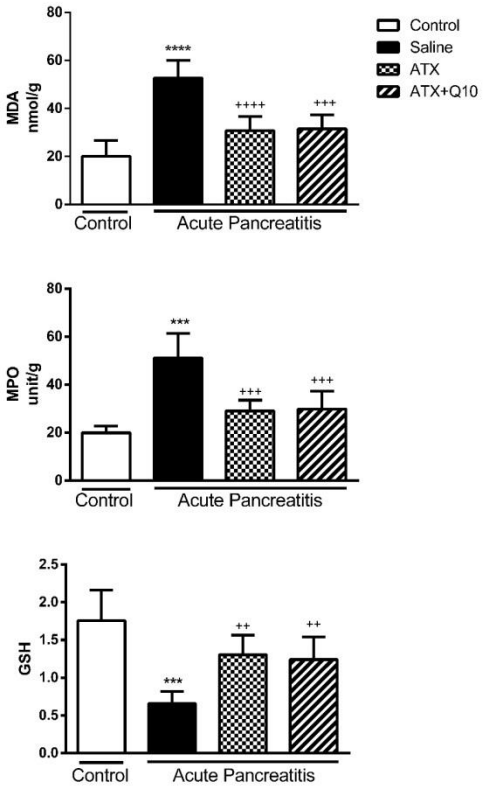
Effect of compounds on MPO activity and MDA level.

MPO activity and MDA level are elevated significantly in vehicle-treated group compared to the control group (P<0.001) (Figure 2a, b). Increased MPO activity and MDA levels were reduced by both treatments (P<0.001). The reduction amounts were similar between the two groups, and with the control group.

Effect of compounds on GSH level

When compared with the control group, GSH level decreased significantly in the vehicle-treated group (P<0.001). Depressed GSH level was elevated with ATX and ATX+Q10 treatments (P<0.01 for both) (Figure 2c).

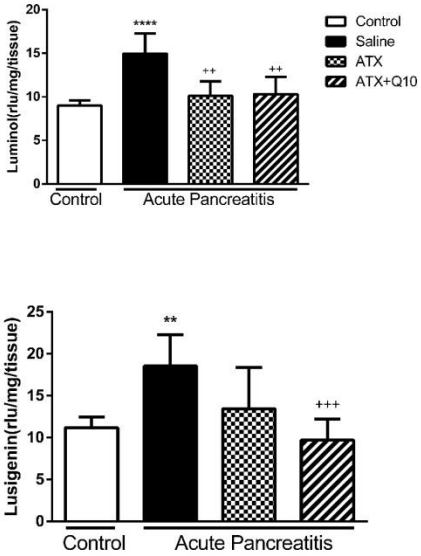
Figure 2: Pancreatic tissue, Malondialdehyde (MDA), Myeloperoxidase activity (MPO) and Glutathione (GSH) levels (Data are expressed as mean ± SEM; n=6 rats /group; **P<0.01, ***P<0.001 vs control group; +P<0.05, ++P<0.01 vs vehicle-treated group.)



Effect of Astaxanthin on CL values

Both luminol and lucigenin levels were elevated in the saline-treated group compared with the control group (P<0.001 and P<0.01, Figure 3). While elevated levels of luminol were reduced significantly (P<0.01), the decrease in lucigenin was not significant in ATX group. Significant decrease in luminol and lucigenin were observed in the Q10 group (P<0.01) compared to the AP group.

Figure 3: Pancreatic luminol-enhanced (A)- and lucigenin-enhanced (B) tissue chemiluminescence (CL) levels (Data are expressed as mean ± SEM; n=6 rats/group; *P<0.05, **P<0.01 vs control group; +P<0.05, ++P<0.01 vs vehicle-treated group.)



Discussion

Acute pancreatitis affects 13-45/100.000 individuals per year [21] and supportive treatment is the mainstay of management. We investigated the effect of astaxanthin and astaxanthin+Q10 combination, both of which are lipid soluble antioxidants, on a cerulein-induced rat acute pancreatitis model.

Serum lipase or amylase activities are the most used serum biochemical markers of AP. Our findings point out to a significant reduction in amylase and lipase levels in both groups. Similar to our results, a recent study reported a significant decrease on rising serum amylase and lipase with Q10 treatment [22].

Since the role of oxidative damage is defined in acute pancreatitis, scavenging efficiency of many natural products on ROS were evaluated [10, 23]. However, the antioxidant effect of plant-derived natural products on ROS species is still controversial [7-9, 24, 25]. A study reported that plant derived antioxidants affect free radicals [26]. ATX is a lipid soluble carotenoid [27] which inhibits cyclooxygenase 2 (COX2) and autoimmune reactions and activates T-cells [28]. Similar to ATX, coenzyme Q10 is a lipid soluble endogenous compound with antioxidant properties which is used to treat many diseases [29-32]. The role of Q10 in inhibition of ROS production is the blockage of NF-κB at the mitochondrial level [33, 34]. However, its antioxidant capacity is 800 times less than that of ATX.

Many studies showed synergistic effects of antioxidants by inhibition of lipid peroxidation [35]. Elevation in reactive oxygen species (ROS) and reduced antioxidant activity markedly influence inflammation [3, 4]. In this study, we investigated ATX's antioxidant capacity with or without coenzyme Q10. Our

results confirmed increased reactive oxygen species and decreased antioxidants in the cerulein-induced acute pancreatitis model, similar to previous reports. Our model reflects the early stage of acute pancreatitis, and as expected, MDA levels and MPO activity were increased while GSH was decreased [5, 10, 36-39]. GSH levels were later increased by ATX [36, 40]. However, Q10 addition was not effective, parallel with the data of the above-mentioned study [22].

Chemiluminescence is an assay for detecting reactive oxygen species. The luminol probe of this technique detects OH^-, H_2O_2, hypochlorite, peroxynitrite, and lipid peroxyl radicals, while lucigenin is specific for superoxide radicals. In conformity with the previous reports, our results showed an increased pancreatic production of reactive oxygen species. ATX decreased the luminol and lucigenin levels in acute pancreatitis just like in other inflammatory diseases [14]. Interestingly, while lucigenin decreased in ATX group, significant decrease was achieved in the ATX+CoQ10 group.

Limitations

These results must be considered in the context of the study limitations. First, although our results showed significant differences in the ATX+Q10 group, and the lack of Q10 group made it difficult to generalize our results. Moreover, we did not evaluate the underlying physiological mechanisms against acute inflammation. Despite these limitations, a strength of this study is that it compared inflammatory markers, enzymes, and reactive oxygen species indicators between the groups.

Conclusion

This study shows that antioxidant combination does not inhibit oxidative damage better than astaxanthin alone in a cerulein-induced rat model of acute pancreatitis. Astaxanthin, a carotenoid, ameliorates increased oxidative stress markers, and addition of Q10 is ineffective in this regard. It is probably a result of Q10's weak antioxidant capacity. Thus, astaxanthin can be used as a valuable therapeutic pharmacological agent in the treatment of acute pancreatitis.

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