Antioxidant Activity And Stress-Protective Mechanisms Of Bile Acid Derivatives In Rat Aorta

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Abstract: This study investigates the effects of bile acid derivatives — ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — on the contractile activity of smooth muscles in the rat aorta. The research confirmed that both compounds induce dose-dependent vasorelaxation, and their mechanisms of action were analyzed in detail. The vasodilatory effect of UDCA was found to be associated with nitric oxide (NO) synthesis via the endothelium, while TCA's effect was attributed to the restriction of calcium ion influx into cells. The sensitivity of phenylephrine-induced smooth muscle contraction to these substances was assessed using myographic methods. The results suggest that UDCA and TCA may serve as potential natural therapeutic agents for cardiovascular diseases.
Keywords: bile acid derivatives, ursodeoxycholic acid, taurocholic acid, rat aorta, smooth muscle, vasorelaxation, nitric oxide synthesis, calcium channels, myography, antioxidant activity

Introduction

Cardiovascular diseases represent one of the most pressing challenges in modern medicine. According to global statistics, these pathologies are among the leading causes of mortality and disability worldwide. Recent scientific research emphasizes the central role of oxidative damage in the development of such conditions. Particularly, the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within cells damages cellular components — especially lipids, proteins, and genetic material — potentially leading to chronic vascular disorders.

Under physiological conditions, ROS are involved in essential processes such as signal transduction and immune responses. However, excessive levels of ROS result in molecular damage that the cells cannot adequately counteract. This leads to endothelial dysfunction, injury to vascular smooth muscle, inflammation, and apoptosis. The organs of the circulatory system are especially vulnerable to oxidative damage, which contributes to conditions such as atherosclerosis, hypertension, and ischemic heart disease.

The body's antioxidant defense system — which includes glutathione, superoxide dismutase, catalase, and other enzymes — works to neutralize such damage. Nevertheless, chronic stress, inflammation, and metabolic disturbances can impair these protective mechanisms, leaving cells under oxidative burden.

In recent years, growing scientific interest has been directed toward bioactive natural compounds — particularly bile acid derivatives — for their potential in neutralizing ROS. Once regarded solely as digestive agents, ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) have been shown in various studies to exhibit antioxidant, cytoprotective, anti-inflammatory, and cell signaling regulatory properties.

UDCA is known to stabilize the intracellular environment, preserve mitochondrial membrane integrity, and reduce apoptosis. TCA, in turn, stimulates the synthesis of cytoprotective proteins via interaction with nuclear or membrane-bound receptors, thereby enhancing the resistance of vascular walls to oxidative stress.

The aim of the present study is to investigate the protective mechanisms of UDCA and TCA against oxidative stress in rat aortic tissue, and to analyze the extent to which these compounds

safeguard smooth muscle and endothelial layers. The findings of this study may help uncover new therapeutic potentials of bile acid derivatives in the treatment of cardiovascular disorders.

Literature Review

Although bile acid derivatives — specifically UDCA and TCA — were initially studied as components of bile, they are now recognized as bioactive compounds that regulate various biological processes. Their antioxidant and cytoprotective properties are of particular interest in the context of cardiovascular health.

Numerous experimental studies have confirmed that UDCA reduces ROS levels. For example, Zhao et al. (2016) demonstrated that UDCA enhances nitric oxide (NO) synthesis in endothelial cells, thereby helping to mitigate oxidative stress. The NO signaling pathway promotes relaxation in vascular smooth muscle and regulates vascular tone — an indirect mechanism of antioxidant activity.

In addition, Kim et al. (2021) found that UDCA restricts intracellular calcium influx, which is significant because excessive intracellular calcium promotes ROS production and mitochondrial stress. This highlights another mechanism by which UDCA exhibits antioxidant effects.

TCA is primarily characterized by its direct action on cellular receptors. In a study by Lee (2018), TCA was shown to activate antioxidant genes via interaction with the farnesoid X receptor (FXR), a nuclear receptor. FXR activation leads to increased glutathione synthesis, higher NADPH levels, and inhibition of pro-oxidant enzymes. Rodríguez et al. (2020) also reported that TCA may activate signaling pathways through the membrane-bound TGR5 receptor, thereby inhibiting ROS signaling.

While both UDCA and TCA have antioxidant properties, their mechanisms differ. UDCA mainly works through mitochondrial stabilization, NO mediation, and apoptosis suppression, whereas TCA exerts its effects at the gene expression level by enhancing cellular defense pathways. This difference suggests they may be complementary when used together.

Another important mechanism involves the modulation of apoptosis and inflammatory markers. The Nrf2 pathway, for instance, is a nuclear transcription factor activated in response to oxidative stress. It regulates the expression of several antioxidant proteins. Bile acid derivatives activate this pathway, increasing intracellular GSH levels and upregulating enzymes such as HO-1, SOD, and catalase.

In summary, the literature suggests that UDCA and TCA not only neutralize ROS directly but also enhance the body's antioxidant response, prevent mitochondrial dysfunction, and maintain the structural integrity of vascular walls, thus offering protection against oxidative damage in the cardiovascular system.

Methodology

This study aimed to evaluate the antioxidant and protective effects of bile acid derivatives — specifically ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — on oxidative stress in rat aortic tissue. The experimental design was based on a classical organ bath technique, assessing smooth muscle activity and ROS levels as primary indicators.

Animal Protocol

The study involved 24 healthy male Sprague-Dawley rats (250–300 g), housed in a controlled vivarium environment with a 12-hour light/dark cycle and free access to food and water. All procedures complied with international bioethical standards and were approved by the institutional animal ethics committee.

Aortic Segment Preparation

After anesthesia, the thoracic aorta of each rat was excised and immersed in Krebs-Henseleit solution (containing NaCl – 118 mM, KCl – 4.7 mM, $CaCl_2$ – 2.5 mM, $MgSO_4$ – 1.2 mM, KH_2PO_4

– 1.2 mM, $NaHCO_3$ – 25 mM, glucose – 11 mM). The aorta was cut into 2–3 mm ring segments and mounted in a myograph system (ex vivo smooth muscle tension analyzer).
The organ bath was maintained at 37°C and continuously aerated with a gas mixture of 95% oxygen and 5% carbon dioxide. Each aortic ring was preloaded with 1 g of passive tension and allowed to equilibrate before experimentation.
Experimental Design
1. Control Group: Incubation in Krebs-Henseleit solution only; baseline ROS levels measured.
2. Phenylephrine Stimulation (PE): 1 µM phenylephrine applied to induce contraction.
3. UDCA and TCA Exposure: PE-induced contractions were treated with various concentrations of UDCA or TCA (ranging from 10^{-7} M to 10^{-4} M).
4. Inhibitor Groups:
   - L-NAME (100 µM): to inhibit nitric oxide synthase (NOS)
   - Verapamil (1 µM): to block calcium channels

These inhibitors were used to elucidate the underlying mechanisms of UDCA and TCA action.
ROS Quantification
Reactive oxygen species levels were assessed using dihydroethidium (DHE) staining and visualized under a confocal microscope. In the presence of ROS, DHE is oxidized to ethidium, emitting a red fluorescence signal. Data were analyzed using ImageJ software.
Statistical Analysis
All results were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant.

Results

The results of the conducted study clearly demonstrated that bile acid derivatives — particularly UDCA and TCA — possess effective protective mechanisms against oxidative stress in rat aortic tissue. During the experiment, the administration of these compounds under phenylephrine-induced smooth muscle contraction led to pronounced vasorelaxation, indicating their vasodilatory potential.
One of the most notable observations was that UDCA, even at low concentrations (10^{-6}–10^{-5} M), significantly induced smooth muscle relaxation. This effect was markedly diminished in the presence of L-NAME, a nitric oxide synthase inhibitor, supporting the hypothesis that UDCA acts via nitric oxide (NO)-mediated pathways. These findings align with prior literature, particularly the studies by Zhao (2016) and Kim (2021), which also highlighted UDCA's capacity to enhance endothelial NO production, thereby mitigating the effects of ROS.
TCA, in contrast, exhibited a significant effect only at relatively higher concentrations (≥10^{-5} M). The inhibitory impact of verapamil, a calcium channel blocker, on TCA's activity suggests that this compound may exert its effect through direct modulation of calcium influx. By limiting calcium entry into the cell, TCA potentially reduces ROS production and exerts cytoprotective effects. These properties are consistent with previous findings — particularly those by Rodríguez (2020) — that link TCA's mechanisms to FXR and TGR5 receptor-mediated pathways.
ROS levels assessed via dihydroethidium (DHE) staining further supported these functional findings. Samples treated with UDCA and TCA showed markedly reduced fluorescence compared to the control group, indicating lower ROS accumulation. Notably, tissues treated with UDCA exhibited the lowest fluorescence intensity, underscoring its strong antioxidant activity.

Table 1. ROS Levels in Aortic Segments (DHE Signal Intensity)
(Mean ± SD, n = 6; * p < 0.05 vs. control)

Group	DHE Signal (%)
Control	100%
PE (phenylephrine)	180%

PE + UDCA	110% *
PE + TCA	125% *
PE + UDCA + L-NAME	160%
PE + TCA + Verapamil	150%

These results indicate that bile acid derivatives not only modulate vascular tone but also provide potent cytoprotective defense against oxidative stress in smooth muscle by decreasing ROS levels. This protective action is mediated not merely through direct neutralization of free radicals, but also via regulation of NO-mediated signaling and ion-channel pathways.

In this light, the hypothesis that UDCA and TCA may serve as therapeutic agents for oxidative stress-related cardiovascular diseases (such as atherosclerosis and hypertension) gains strong scientific grounding. Moreover, their pharmacological safety and endogenous origin further enhance their potential as candidates for cardioprotective research and therapy.

Conclusion

Oxidative stress and its consequences are widely acknowledged as central factors in the pathogenesis of cardiovascular diseases. An excessive accumulation of reactive oxygen species (ROS) in the vascular system can lead to endothelial and smooth muscle dysfunction, inflammation, and the development of atherosclerosis. Therefore, strategies aimed at reducing ROS and enhancing the body's antioxidant defense — either through natural or pharmacological means — represent a vital component of current cardioprotective research.

This study confirms that bile acid derivatives — particularly ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — exert a dual influence on vascular smooth muscle and ROS production. UDCA facilitates muscle relaxation and reduces ROS levels via NO-mediated mechanisms, while TCA achieves cytoprotection by restricting calcium influx. Although these mechanisms differ, both compounds ultimately contribute positively to vascular health.

The experimental findings also provided insights into the synergistic and independent mechanisms of action of UDCA and TCA. The significant reduction in fluorescence intensity detected through DHE staining validates both the direct and indirect antioxidant actions of these bile acid derivatives. In particular, UDCA demonstrated a strong antioxidative profile, highlighting its potential therapeutic superiority.

In conclusion, bile acid derivatives — as naturally occurring, low-toxicity compounds — show promise as effective bioactive agents in the prevention and treatment of cardiovascular diseases. Their ability to improve endothelial function, regulate calcium dynamics, and suppress ROS formation opens new avenues for enhancing cardioprotective strategies. Therefore, further research into their molecular mechanisms, clinical applicability, and potential for combination with other therapeutic agents is warranted.

References:

Zhao, Y., Zhang, L., Wang, S., et al. (2016). Ursodeoxycholic acid stimulates nitric oxide production and protects endothelial function in rats. European Journal of Pharmacology, 789, 34–42. https://doi.org/10.1016/j.ejphar.2016.06.023

Kim, H. J., Lee, J. H., Park, E. J., et al. (2021). Inhibitory effects of ursodeoxycholic acid on calcium influx in vascular smooth muscle cells. Biomedicine & Pharmacotherapy, 138, 111504. https://doi.org/10.1016/j.biopha.2021.111504

Lee, M. J., Kim, S. H., Cho, H. J. (2018). Differential effects of bile acid derivatives on vascular relaxation in rat aorta. Vascular Pharmacology, 106, 16–23. https://doi.org/10.1016/j.vph.2018.02.003

Rodríguez, R. M., et al. (2020). Taurocholic acid modulates vascular tone via FXR-dependent pathways in isolated rat arteries. Frontiers in Physiology, 11, 124. https://doi.org/10.3389/fphys.2020.00124

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nature Reviews Molecular Cell Biology, 21(7), 363–383. https://doi.org/10.1038/s41580-020-0230-3

Bernardi, M., et al. (2011). Ursodeoxycholic acid in the treatment of liver diseases: a review of its pharmacological properties and clinical use. Drugs, 71(9), 1201–1228. https://doi.org/10.2165/11589410-000000000-00000

Lapenna, D., et al. (1998). Antioxidant properties of ursodeoxycholic acid. Biochemical Pharmacology, 56(3), 331–336. https://doi.org/10.1016/S0006-2952(98)00112-1

Halliwell, B., & Gutteridge, J. M. C. (2015). Free Radicals in Biology and Medicine (5th ed.). Oxford University Press.

Nrf2–Keap1 signaling pathway and cardiovascular diseases: role in redox homeostasis and inflammation – Zhang, D. D. (2017). Cardiovascular Research, 114(5), 653–665. https://doi.org/10.1093/cvr/cvx020.