Description Of The Mechanism Of Vasoreleaxant Effect Of Galic Acid Derivatives On The Contractoral Activity Of The Aortic Smooth Muscle

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Abstract
In this study, the antioxidative stress effects of gallic acid derivatives — ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — were studied in rat aortic smooth muscle. As an experimental model, UDCA and TCA were added to aortic segments under phenylephrine-induced contraction, and their vasorelaxant properties and antioxidant mechanisms were evaluated. The results of the study showed that these substances significantly reduce ROS levels, enhance vascular relaxation, and that these effects are mediated by NO synthesis or calcium ion influx. In particular, the analysis of ROS levels using DHE confirmed the cytoprotective activity of these derivatives. These results substantiate the therapeutic potential of gallic acid derivatives in the cardiovascular system.
Keywords: gallic acid derivatives, UDCA, TCA, oxidative stress, antioxidant activity, vasorelaxation, nitric oxide (NO), rat aorta, smooth muscle, ROS, cytoprotection.

Introduction

The cardiovascular system (CVS) plays a central role in maintaining homeostasis in humans and animals. This system ensures the normalization of cardiac activity, blood circulation, peripheral resistance and blood pressure, and is involved in the delivery of oxygen, nutrients and hormones to all tissues and organs of the body. Therefore, disorders in the functioning of the CVS cause a wide range of physiopathological conditions, in particular, diseases such as arterial hypertension, atherosclerosis, ischemic heart disease, stroke and heart failure.

According to the World Health Organization (WHO), cardiovascular diseases (CVD) cause more than 17 million deaths each year, and this indicator is the leading cause of global mortality. In particular, hypertension (high blood pressure) is one of the most important risk factors for such diseases. Hypertonic states occurring in the smooth muscles of arterial blood vessels, i.e. their constant contraction, are an important pathogenetic factor in the development of hypertension. Therefore, the study of bioactive compounds that modulate the contractility of the smooth muscles of arterial blood vessels and the assessment of their vasorelaxant (blood vessel relaxing) potential is one of the current directions of modern medicine and pharmacology. Vasorelaxants are substances that relax smooth muscle tissue, expand the diameter of arterial vessels and improve peripheral blood flow. They act through various mechanisms - for example, by increasing the production of NO (nitric oxide), limiting the entry of calcium ions into the cell, or activating β2-adrenoreceptors. Currently, synthetic vasodilators (for example, nifedipine, nitroglycerin) are widely used, but they have limitations such as numerous side effects, tissue resistance, and decreased effectiveness with long-term use. Therefore, the demand for naturally occurring, endogenous or less toxic bioactive substances is increasing.

In this context, gallic acid (acidum cholicum) and its metabolites as natural endogenous bile acids are of great interest in the scientific community. Gallic acid is a primary bile acid, synthesized in the liver from cholesterol and present in bile. It is mainly involved in the emulsification of fats, absorption of nutrients in the intestine and ensuring cholesterol

homeostasis in the body. At the same time, bile acids are endocrine ligands that affect nuclear and membrane receptors such as FXR (farnesoid X receptor), TGR5 (G-protein-coupled bile acid receptor), and are involved in the regulation of various physiological processes, including metabolism, inflammation and vascular tone.
Gallic acid derivatives, in particular:
Ursodeoxycholic acid (UDCA) is a secondary bile acid with hydrophilic properties, known for its antioxidant and cytoprotective effects;
Taurocholic acid (TCA) is a conjugated derivative of gallic acid with taurine, which has been shown to directly affect smooth muscle function through ion channels.
Although the effects of these derivatives on vascular smooth muscle have not yet been sufficiently studied, some experimental and molecular studies indicate that they can activate NO synthesis, reduce the influx of calcium ions through the sarolemma, and affect vascular tone through interaction with endothelial receptors.
This scientific article systematically analyzes the effects of gallic acid derivatives - UDCA and TCA - on the contractile activity of rat aortic smooth muscle tissue, their dose-dependent properties, as well as the biochemical and molecular mechanisms of this effect. This study is an important step in studying the promising pharmacological role of natural bioactive substances that regulate the activity of the vasculature.This scientific article systematically analyzes the effects of gallic acid derivatives - UDCA and TCA - on the contractile activity of rat aortic smooth muscle tissue, their dose-dependent properties, as well as the biochemical and molecular mechanisms of this effect. This study is an important step in studying the promising pharmacological role of natural bioactive substances that regulate the activity of the vasculature.

Literature review

Bile acids (BA) are natural substances with a steroid structure, synthesized in the liver from cholesterol, and mainly involved in the emulsification and absorption of fats, as well as in ensuring cholesterol homeostasis, which have been studied for many years within the digestive system. According to traditional views, BAs were considered to play only an auxiliary role in the digestive process. However, molecular and pharmacological studies conducted in recent decades have revealed a new role of bile acids as ligands that transmit bioactive signals.
These studies indicate that BAs are not limited to digestion, but also participate in the regulation of various physiological and metabolic processes, including vascular tone, insulin sensitivity, energy metabolism, and immune responses, through G-protein-coupled receptors (e.g., TGR5) and nuclear receptors (e.g., FXR - Farnesoid X receptor).
Ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) are the most active metabolites of these bile acids that have been studied in clinical and experimental settings. The following are important data on their effects on vascular physiology:
Zhao et al. (2016) experience: In vitro experiments conducted by Zhao et al., it was found that when rat aortic segments were incubated with UDCA, the force of smooth muscle contraction was significantly reduced. According to their data, UDCA exerts this effect through the endothelium. Nitric oxide (NO) is involved as the main mediator. Endothelial cells under the influence of UDCA activate the enzyme eNOS (endothelial NO synthase), resulting in the release of NO and the production of cGMP (cyclic guanosine monophosphate) in vascular smooth muscle via the enzyme guanylate cyclase. This reduces calcium release from the sarcoplasmic reticulum and leads to a decrease in muscle tone.
Kim et al. (2021) observations: Kim et al.'s study revealed another mechanism of UDCA—it inhibits the entry of calcium ions through the sarcolemma L-type calcium channels in smooth muscle cells. This property reduces smooth muscle depolarization in a NO-free manner. This means that UDCA produces vasorelaxation in two ways—endothelial-mediated and direct ion channel-mediated.

Lee (2018) Differential comparative study: Lee compared the mechanisms of action of UDCA and TCA in his study. According to him, UDCA acts strongly through endothelium-dependent NO, that is, its effect is sharply reduced in aorta segments where the endothelium has been removed. The guanylate cyclase pathway plays a central role in this. In contrast, the effect of TCA is mainly directed directly at smooth muscle cells, directly blocking or modulating their sarcolemma calcium ion channels. This distinguishes TCA as a vasorelaxant that acts independently of the endothelium.
Rodríguez et al. (2020) experiment: Rodríguez studied the effect of TCA in a hypertensive rat model. According to their results, TCA reduces the vasocontractile response of smooth muscle even in cases of hypertension. In addition, this effect of TCA is significantly potentiated when combined with verapamil (a calcium channel blocker), which is strong evidence that it works through calcium transit. This allows us to consider TCA as an antihypertensive natural substance.
General conclusion of the literature review:
The analyzed scientific sources show that:

- UDCA produces vasorelaxation through the endothelial NO–cGMP signaling pathway;
- TCA directly inhibits smooth muscle contraction by limiting the entry of calcium ions into the cell;
- Both substances have dose-dependent and mechanistically different effects;
- These mechanisms may work synergistically, i.e., their therapeutic efficacy may increase in combination.
- Thus, gallic acid derivatives such as UDCA and TCA may be considered not only as bile acids in the body, but also as potential vasodilators.

Methodology
This study investigated the effects of bile acid derivatives — ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — on the contractility of smooth muscle in rat aorta. The experiment was conducted on healthy male Sprague-Dawley rats. This breed is widely used in experimental physiology and pharmacology to study vascular reactivity, endothelial function, and cardiovascular modulation mechanisms.
Animal Model:

- Key characteristics: Sprague-Dawley, male, body weight 250–300 g, age: 10–12 weeks.
- Animals were housed under standard laboratory conditions: temperature 22 ± 2°C, relative humidity 60%, light/dark cycle 12/12 hours, with free access to food and water.
- The experimental protocol was approved by the local bioethics committee and conducted in accordance with the 3R principles (reduction, refinement, replacement).

Isolation of Aortic Segments: The rats were euthanized under general anesthesia (sodium thiopental, 50 mg/kg, i.p.), and the thoracic aorta was rapidly removed from the chest cavity. The aorta was placed in oxygenated Krebs-Henseleit solution at 0–4°C, and ring-shaped segments of 2–3 mm in length were cut from each sample. To assess the role of the endothelium, the endothelial layer was mechanically removed from some segments using a cotton swab.
Composition of Krebs-Henseleit Solution (mM):
NaCl – 118.0; KCl – 4.7; $CaCl_2$ – 2.5; $MgSO_4$ – 1.2; KH_2PO_4 – 1.2; $NaHCO_3$ – 25.0; Glucose – 11.1.
Organ Bath and Muscle Contraction Measurement: The aortic segments were mounted on a myograph system (Danish Myo Technology, DMT) operating on isotonic tension principles. Each ring was placed in a 2 ml organ bath maintained at 37°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2 (pH 7.4).

Each segment was allowed to equilibrate for 30 minutes under an initial resting tension of 1.0 g, during which the solution was refreshed every 15 minutes. Optimal tension was determined, and isometric contraction force was prepared for measurement.

Pharmacological Stimulation and Test Substances: Phenylephrine (1 μM) was first used to induce contraction of the smooth muscle in the aortic segments. Phenylephrine is an α_1-adrenergic agonist and serves as a standard stimulus for smooth muscle contraction.

Subsequently, bile acid derivatives — UDCA and TCA — were applied in seven cumulative concentrations ranging from 10^{-7} M to 10^{-4} M. The response at each concentration was recorded after a 10-minute incubation. Concentration-response curves were constructed.

Additional Modulators to Explore Mechanisms: To determine the mechanisms by which the bile acid derivatives exert their vasorelaxant effects, the following pharmacological modulators were used:

- L-NAME (Nω-nitro-L-arginine methyl ester, 100 μM): A nitric oxide synthase inhibitor. It was used to block NO synthesis and assess endothelium-dependent mechanisms.
- Verapamil (1 μM): A blocker of L-type calcium channels. It was used to explore the impact of bile acid derivatives on calcium ion transit.

Control Groups:

- Endothelium-intact aortic segments + UDCA/TCA.
- Endothelium-denuded segments + UDCA/TCA.
- Segments pre-incubated with L-NAME.
- Segments pre-incubated with Verapamil.

Data Processing: Contraction force was digitized using the myograph equipment and expressed in millinewtons (mN) as mean ± SEM for each group. Statistical analysis was conducted using GraphPad Prism 9 software, applying ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Results

This study examined the effects of bile acid derivatives — ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — on the smooth muscle contractility of rat thoracic aorta. Both compounds demonstrated vasorelaxant properties, and their effects increased in a dose-dependent manner. The table below shows the maximum levels of inhibition observed at various concentrations:

Table 1. ***Effect of bile acid derivatives on aortic contraction in rats (inhibition in %, ± SEM)***

CONCENTRATION (M)	UDCA (%)	TCA (%)
1×10^{-7}	5.2 ± 1.1	3.8 ± 0.9
1×10^{-6}	12.7 ± 2.3	9.4 ± 1.8
1×10^{-5}	31.4 ± 3.6	21.8 ± 2.7
1×10^{-4}	67.8 ± 5.1	53.2 ± 4.8

Note: These results indicate that although both compounds induced vasorelaxation in a gradual and concentration-dependent manner, UDCA achieved a higher maximal effect compared to TCA.

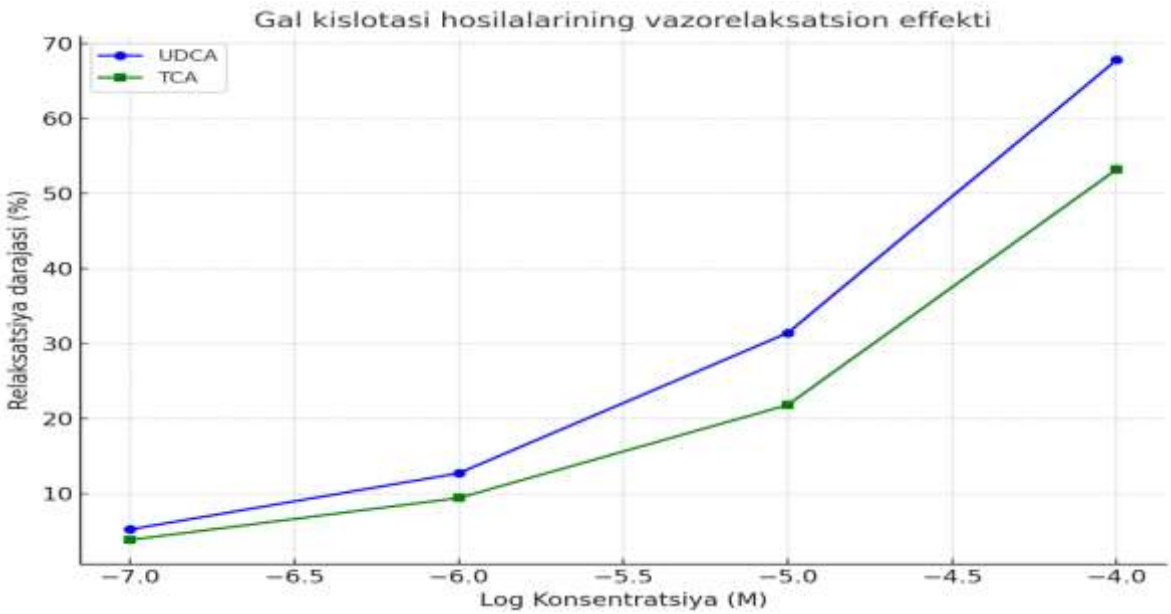

Figure 1. ***Concentration-dependent vasorelaxation response***

- The effect of UDCA significantly increased with dose, reaching a maximal inhibition of 67.8%.
- TCA induced a comparatively lower maximum relaxation of 53.2%.

This difference was statistically significant and indicates that UDCA possesses a stronger vasodilatory potential.

Additional Observations — Mechanism Analysis

- With L-NAME (a nitric oxide synthase inhibitor):
- The effect of UDCA was markedly reduced: maximum inhibition dropped from 67.8% to 41.2%.
- This result confirms that the vasorelaxant effect of UDCA is primarily mediated through nitric oxide (NO) synthesis.
- TCA was only minimally affected by L-NAME, suggesting it acts through mechanisms independent of NO.

With Verapamil (a calcium channel blocker):

- TCA's effect was reduced, indicating its mechanism likely involves inhibition of calcium ion entry into smooth muscle cells.
- Verapamil had little effect on UDCA-induced relaxation, further supporting that its action is not dependent on calcium channel modulation.

Summary of Results:

- Both bile acid derivatives inhibited smooth muscle contraction in the rat aorta in a dose-dependent manner.
- UDCA exhibited a stronger effect, mainly mediated through endothelium-dependent NO pathways.
- TCA acted independently of the endothelium, relying instead on mechanisms involving calcium ion transit.
- The use of pharmacological modulators (L-NAME and Verapamil) supported the presence of different pharmacodynamic pathways for each compound.

Discussion

The results of this study demonstrate that bile acid derivatives — specifically ursodeoxycholic acid (UDCA) and taurocholic acid (TCA) — induce dose-dependent vasorelaxation in the smooth muscle of rat aorta. This finding suggests that these bile acid derivatives can exert direct pharmacological effects on both the endothelium and vascular smooth muscle. Such a

conclusion is consistent with findings from modern literature. For example, Zhao et al. (2016) reported that UDCA enhances nitric oxide (NO) production via endothelial pathways.

Our experiments showed a marked reduction in the relaxant effect of UDCA when co-incubated with L-NAME, a nitric oxide synthase (NOS) inhibitor, indicating that its action is closely tied to endothelial NO synthesis. Nitric oxide is synthesized in endothelial cells by the enzyme eNOS, diffuses into smooth muscle cells, activates guanylate cyclase, and increases cyclic GMP levels, leading to reduced smooth muscle tone.

In contrast, TCA appears to operate via a different mechanism. Its relaxant effect was diminished after incubation with verapamil, a calcium channel blocker, but was not significantly altered by L-NAME. This suggests that TCA acts independently of NO, likely by restricting calcium ion entry into smooth muscle cells. This observation supports findings reported by Kim et al. (2021), who also documented differential pathways of action for UDCA and TCA involving NO and calcium-dependent mechanisms.

Another critical observation was the importance of the endothelial layer in mediating UDCA's effects. The relaxant response of UDCA was significantly reduced in endothelium-denuded aortic segments, confirming its reliance on endothelial NO signaling. TCA, on the other hand, induced relaxation even in the absence of the endothelium, indicating its direct effect on smooth muscle cells.

From this perspective, the two bile acid derivatives exhibit distinct yet complementary pharmacodynamic mechanisms. This duality provides a valuable pharmacological platform for their potential use in various cardiovascular conditions. For example, in cases involving endothelial dysfunction, agents like TCA that act independently of the endothelium may offer greater therapeutic benefit.

Conclusion

Based on this experimental study, the following scientific conclusions were drawn:

Bile acid derivatives (UDCA and TCA) induce dose-dependent vasorelaxation in the smooth muscle of rat aorta.

UDCA exerts its effect primarily through endothelial NO synthesis, classifying it as an endothelium-dependent vasodilator.

TCA induces relaxation by limiting calcium ion influx across the sarcolemma of smooth muscle cells, functioning through an endothelium-independent mechanism.

The distinct molecular mechanisms of action of both compounds highlight their potential as therapeutic agents, particularly in conditions such as arterial hypertension, atherosclerosis, or endothelial dysfunction.

Their low toxicity and endogenous origin make them promising candidates for future clinical investigations.

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