

*Opšti pregledi /
General reviews*

**BILE ACIDS ANTIVIRAL EFFECT:
ALONE AND IN COMBINATION WITH
ANTIVIRAL DRUGS**

**ANTIVIRUSNO DEJSTVO ŽUČNIH
KISELINA: SAMOSTALNO I U
KOMBINACIJI SA ANTIVIRUSNIM
LEKOVIMA**

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Ključne reči

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Abstract

Bile acids are organic compounds of significant physiological importance. They act as powerful biological detergents in the digestion of fats. The gallbladder stores approximately 4 grams of bile acid. These are activated through conjugation with amino acids or with coenzyme A. Bacterial flora metabolizes them into secondary bile acids when excreted into the small intestine. Bile acids also serve as carriers and enhancers of certain drugs. Antiviral drugs such as zidovudine, aciclovir and ribavirin are often found in pharmaceutical formulations in combination with bile acids and are extensively used. Bile acids themselves exhibit antiviral activity through several different mechanisms. They increase the absorption of the antiviral drug when taken orally in the same formulation.

INTRODUCTION

Bile acids are biologically significant organic compounds formed via various metabolic changes to the cholesterol ring. In addition to their role in fat metabolism, they also possess protective characteristics. These are evident in their ability to disrupt the lipid envelope of viruses, leading to their destruction. Bile acids not only inhibit the replication of certain viruses within cells but also participate in reducing the replication of particular viruses in specific cells⁽¹⁾. Derivatives of bile acids can interfere with the interaction between the virus and the proteins of the potential host cell. By directly affecting the virus, these derivatives disrupt the contact between the virus and the cells⁽²⁾. The protein responsible for bile acid transport in the liver is sodium taurocholate, which is also targeted by some hepatotoxic viruses with oncological potential. However, nearly all bile acids are transported into the liver via this protein⁽³⁾. Certain oncogenic viruses, such as Hepatitis C, can also cause severe kidney damage, eventually leading to irreversible renal failure⁽⁴⁾. Urodeoxycholic acid has a potent protective and therapeutic effect on hepatocytes. In one study where it was used in adequate concentrations, it demonstrated its potency in reducing liver enzymes, which were significantly elevated, thereby underlining its role in preventing

hepatocyte cell death⁽⁵⁾. Numerous metagenomic studies have proven a positive correlation between bile acids and a positive response in ulcerative colitis⁽⁶⁾.

Metabolism and pathophysiological characteristics of bile acids

The primary constituents of bile acids are deoxycholic and cholic acid. They can bind with amino acids to form new compounds, facilitating their storage in the gallbladder. During digestion, with the assistance of intestinal flora, these compounds are decomposed and reabsorbed into the bloodstream in significant quantities⁽⁷⁾. In addition to diet, these compounds are also produced, albeit in smaller quantities, by organs such as the ovaries. There are primary and secondary bile acids⁽⁸⁾. Certain liver diseases that cause disability can substantially decrease bile acid levels, thereby diminishing the quality of life⁽⁹⁾. Changes in the chemical structure of bile acids can indicate various diseases, including chronic liver disease, and can even point to certain oncological conditions^(10,11). Some studies underscore the importance of hygiene and dietary regimes in maintaining proper bile acid metabolism. They also highlight how specific diets can reduce secondary bile acids associated with rectal cancer⁽¹²⁾. A part of the primary bile acids re-enters

the circulation via the small intestine, while some are metabolised by bacterial flora into secondary bile acids⁽¹³⁾. In addition to influencing fat metabolism and absorption, these acids also facilitate the absorption of fat-soluble vitamins^(14,15). They play a role in mitigating the free radicals produced in the endoplasmic reticulum. Of all the known bile acids, tauroursodeoxycholic acid plays this role to the greatest extent⁽¹⁶⁾. The use of antibiotics can considerably disrupt bile acid metabolism because they reduce the intestinal bacterial flora responsible for the biological transformation of these acids⁽⁹⁶⁾.

Special characteristics of bile acids

In addition to their ability to enhance the penetration of certain drugs into the bloodstream, bile acids can also be extremely hepatotoxic, and they possess the potential to damage other body cells as well⁽¹⁷⁾. Also, bile acids in higher concentrations can block the entry of hepatotoxic viruses into the liver, but elevated concentrations of these acids can have detrimental effects⁽¹⁸⁾. In patients who have undergone surgery on a part of their small intestine, or due to gallbladder removal or inability to reabsorb from the gut, these acids may induce diarrhoea, followed by increased secretion of water and minerals from the gut⁽¹⁹⁾. However, some studies show that bile acids aid viral replication⁽²⁰⁾. In the United States, randomized studies have been carried out in over 20 centres across the nation. The obtained results support the notion that bile acid formulations, particularly sodium tauroursodeoxycholate, increase survival rates from this disease⁽²¹⁾. In addition to affecting the metabolism of triglycerides and other fats, bile acids also affect glucose regulation. Their specific effect is a direct and indirect influence on certain drugs, which can considerably alter the progress of chronic diseases, as enabled by bile acid patents⁽²²⁾. Certain studies have investigated bile acids in mice, revealing that male mice tend to have higher concentrations of bile acids⁽²³⁾. Apart from their ability to bind to various receptors, bile acids also affect G protein receptors⁽²⁴⁾. Norucholic acid, derived from bile acid, is highly resistant to numerous metabolic processes, and possesses strong anti-inflammatory properties⁽²⁵⁾. It also has the ability to suppress immunity in animals and can be used in higher concentrations as a laxative in individuals struggling with bowel movements. Studies indicate that both primary and secondary bile acids have this effect⁽²⁶⁾. This process requires sodium and has bestowed significant pharmacological importance upon inhibiting the given receptor to prevent the return of bile acids into circulation. This action helps to prevent certain chronic and progressive diseases that could be caused by elevated concentrations of these acids⁽²⁷⁾. Misdiagnosis can occur when irritable bowel syndrome is inaccurately identified as the cause of diarrhoea, which in reality could be due to increased production and secretion of bile acids⁽²⁸⁾. Bile acids not only have potent detergent properties but also influence receptors. Indeed, an excess of ligands for bile acid receptors can lead to fatty liver disease⁽²⁹⁾. Specifically, mutations in the genes for these particular receptors to which bile acids bind are responsible for the appearance of the mentioned chronic diseases⁽³⁰⁾. The two main groups of cells from which bile acids can be isolated

are liver cells and enterocytes⁽³¹⁾. There are primary and secondary pathways for bile acid formation. The main pathway and site for bile acid formation is in the hepatocytes from cholesterol. This process involves several metabolic stages to protect the organism from potential hypercholesterolemia⁽³²⁾. A meta-analysis based on studies conducted in China determined that for the treatment of cholestasis, patented bile acids in combination with certain drugs yield more favourable results than treatment with ursodeoxycholic acid as monotherapy⁽³³⁾.

Antiviral characteristics of bile acids

In addition to the aforementioned functions and properties, bile acids serve an endocrine function, leading to gene expression that activates antiviral macrophages in the liver⁽³⁴⁾. Bile acids act as innate antiviral components that contribute to the antiviral effect by directly activating certain antiviral genes. They also indirectly stimulate the immune response through the activation of the gene responsible for producing type 1 interferon⁽³⁵⁾. Cohort studies conducted in Switzerland in patients with hepatitis C did not confirm the findings of some research, which suggests that bile acids can initiate viral replication; this was not corroborated in these studies⁽³⁶⁾.

Supplementation, interactions of bile acids

A study on bile acid supplementation, administered to the *Micropterus salmoides* fish species, demonstrated that bile acids can significantly mitigate the side effects of foods rich in starch and fat⁽³⁷⁾. These fishes exhibited numerous metabolic disturbances, and had notably shorter intestines, resulting in susceptibility to new and similar diseases⁽³⁸⁾. However, dexamethasone supplementation in humans led to the significant activation of certain genes and increased production of cholic bile acids⁽³⁹⁾. In contrast, hydroxycortisol greatly improved bile acid flow in dogs suffering from excessive bile accumulation, which caused damage to liver cells and the gallbladder. High-dose hydroxycortisol managed to completely alleviate the symptoms caused by excess bile acids⁽⁴⁴⁾. Some studies show that oral supplementation of bile acids in humans promotes the activation of brown adipose tissue, but not white adipose tissue⁽⁴⁰⁾. This can significantly reduce the production of these acids and increase their excretion from the body, leading to weight loss⁽⁴¹⁾. Polyphenols from strawberries can affect the production and recirculation of bile acids, as observed after ninety days of strawberry supplementation⁽⁴²⁾. Studies also confirm that calcium supplementation administered in a certain dosage and over a specific timeframe, can facilitate the elimination of secondary bile acids and simultaneously confer protective effects against malignant neoplasms of the large intestine⁽⁴³⁾. In randomized studies conducted across six centers in England and Italy, research was conducted with the aim of examining the dissolution effect on cholesterol stones. The monotherapy of ursodeoxycholic acid and chenodeoxycholic acid compared to the combined application of both acids at the same time did not show a significant statistical difference in the dissolution of gallstones. Only a three percent difference suggests that the application of only one of the two aforementioned acids could dissolve calculus, but it

would need to be administered over a sufficient period and at an adequate concentration⁽⁴⁵⁾.

Bile acids in different pharmaceutical formulations with antiviral effects

Esters of bile acids and three widely used antiviral drugs such as ganciclovir, penciclovir and their formulations have substantial application against Herpes Simplex Virus types 1 and 2⁽⁴⁷⁾. It is known that acyclovir is effective in treating herpes viruses, and that bile acids exhibit potent antiviral effects. The combination, specifically, the ester formulation of bile acid with acyclovir, provides an enhanced antiviral effect. This combination has also proven significant against the Epstein-Barr virus. In addition to their antiviral properties, bile acids serve as facilitators and enhancers of antiviral effects⁽⁴⁶⁾. Passive absorption of acyclovir was significantly lower compared to the association of the prodrug acyclovir with certain bile acids. It was shown that a significant improvement in the drug absorption into specific cells can be up to sixteen times more effective than passively absorbed acyclovir⁽⁴⁸⁾. A mouse study demonstrated that the combination of zidovudine and bile acid is much more effective than the use of zidovudine alone. Zidovudine is a drug that inhibits a certain enzyme involved in gene transcription. These ester compounds penetrated mouse macrophages more easily, releasing zidovudine and consequently exerting an antiviral effect⁽⁴⁹⁾. Lamivudine is an antiviral drug used against viral hepatitis and HIV. This drug, combined with ursodeoxycholic acid, reduces its cytotoxicity and increases its antiviral effect⁽⁵⁰⁾. Zanamivir is a very effective drug used against the influenza virus; it belongs to the group of drugs that inhibit the enzyme neuraminidase⁽⁵²⁾. Zanamivir disrupts the virus replication process and also inhibits the enzyme neuraminidase⁽⁵⁵⁾. Bile acids enhance the antiviral effect of this drug, acting as carriers for these smaller molecules. Oral administration of these conjugates has a much higher bioavailability than the drug itself⁽⁵¹⁾. Antiviral drugs that base their mechanism on the inhibition of protease enzymes are peptidomimetic molecules that block the active catalytic center of viral protein proteases. By doing so, they prevent the division of viral protein precursors. Two substances with notable antiviral effects are glycyrrhizic acid and glycyrrhetic acid⁽⁵⁴⁾. In certain randomized studies, patients on therapy with ursodeoxycholic acid were given zidovudine and lamivudine as an addition to their primary therapy to suppress the level of alkaline phosphatase in the blood below a certain threshold. However, none of the patients were able to lower their serum alkaline phosphatase level even after half a year of drug and bile acid administration⁽⁵⁶⁾. A randomized multicenter study included patients suffering from hepatitis C. One group received interferon and ribavirin, while another group received a triple therapy of interferon, ribavirin, and amantadine. Patients were also administered bile acids to improve drug absorption. Studies have shown that among all substances used with an antiviral effect, the concentration of ribavirin in the blood had the highest correlation with anaemia⁽⁵⁷⁾. Also, studies show that elevated concentrations of lipophilic bile acids did not provide adequate results in antiviral treatment in patients with the hepatitis C virus. High concentrations of ursodeoxy-

cholic acid, however, which is prescribed at a specific dosage for patients with this disease, demonstrated a significant antiviral effect⁽⁵⁸⁾. Recent research has indicated that some bile acids have a significant antiviral effect on cell cultures infected with rotavirus. By activating signal molecules, they reduced virus replication within the cell. Here deoxycholic acid showed a particularly favorable effect. In a study with mice, it was found that mice infected with this virus that received chenodeoxycholic acid significantly reduced the percentage of virus excretion in feces. This research shows that bile acids would potentially serve as a component of a disinfectant for this type of virus in the future⁽⁵⁹⁾. Today, the use of pharmaceutical mixtures of bile acids and appropriate medications is increasing. These mixtures have shown their ability to influence molecules that are difficult to penetrate through the membrane, thus making these drugs more penetrative. Apart from this absorption-enhancing effect, they also serve as carriers for certain drugs that would not be able to penetrate the brain barrier or the placenta on their own⁽⁶⁰⁾. That is why it is very important to emphasize that the bioavailability of a drug is determined by its physical and chemical characteristics, which can change in relation to the appropriate carrier. However, when separated from the carrier, the active substance remains unchanged⁽⁶¹⁾. Heparin is a drug that is poorly absorbed orally. However, with the use of bile acids, a formulation was created that enabled the micro and nanoparticles of heparin to have significantly better absorption than orally administered heparin⁽⁶²⁾. Over the past decade, emphasis has been placed on pharmaceutical formulations containing steroid hormones⁽⁶³⁾. Newer formulations of the bile acid ursodeoxycholic acid have resulted in very favorable effects for the pediatric population as it is pharmacologically bioequivalent⁽⁶⁴⁾. Norursodeoxycholic acid is a water-soluble bile acid that has recently been significantly utilized in the clinical treatment of cholestasis. This is due to its hydrophilic properties and the fact that it does not undergo the conjugation process⁽⁶⁵⁾. Bile acids also have an indirect antiviral effect through the two previously described receptors. They can affect macrophages in the liver, intestines, T cells and dendritic cells, thereby guiding the strength of the immune response^(66, 67). In a study conducted on rats the formulation of the drug Gliclazide with bile acids showed incomparably better absorption of the drug than the change of the drug itself, which was used as monotherapy⁽⁶⁸⁾. A randomized study revealed that fat storage and hyperinsulinemia were negatively correlated with the beneficial effects of antiviral therapy. The study examined the effect of various antiviral drugs such as rosinavir, but observed that the effectiveness of antiviral drugs depended on the intestinal flora and bile acids, which significantly contribute to the absorption of drugs through the intestines⁽⁶⁹⁾. Bile acids also showed a neuroprotective effect, particularly hydrophilic bile acids^(70, 90). New discoveries in the successful treatment of Herpes Simplex Virus type 1 are directed towards pharmaceutical formulations based on a homogeneous mixture of acyclovir with the bioactive polymer polycaprolactone⁽⁷¹⁾. Acyclovir in the form of three esters with three different bile acids forms a pharmaceutical formulation that has shown success in the treatment of Epstein Barr virus⁽⁴⁶⁾. Moreover, the formula-

tion of acyclovir with ethyl cellulose provides a gradual release of the drug aciclovir, which increases its absorption by more than 98 percent⁽⁷²⁾. Acyclovir without a carrier has a very low oral bioavailability, the maximum bioavailability without a carrier is up to 20 percent⁽⁷³⁾. Ganciclovir is an antiviral drug used in the treatment of infections caused by the Epstein-Barr virus and Cytomegalovirus. It inhibits DNA polymerases and thus prevents virus replication. However, the bioavailability of this drug is only 5-6% of the total administered drug orally. The pharmaceutical formulation of this drug with cyclodextrin significantly increased the absorption of the drug⁽⁷⁴⁾. In addition to antiviral effects, bile acids also have proviral effects⁽⁸⁰⁾.

Bile acids, apoptosis, toxic bile acids

Six death receptors leading to apoptosis are identified on hepatocytes. Receptors for apoptosis are also found on cell surfaces. Bile acids can initiate this process by binding to one of these receptors, triggering a cascade of reactions that lead to induced cell death. These studies confirm that high concentrations of bile acids are highly toxic to the organism⁽⁷⁵⁾. Bile acids also interact with extracellular receptors as well as receptors on the cell nucleus itself, resulting in various cellular effects. The activation of these receptors is contingent on the quantity of bile acids; therefore, it is crucial to maintain an optimal ratio of bile acids in the body⁽⁷⁶⁾. One significant function of bile acids through receptors on adipose cells is the conversion of white adipose tissue to brown adipose tissue, which directly impacts thermoregulation⁽⁷⁷⁾. All these properties of bile acids provide a scientific basis for the discovery of new therapeutic treatments⁽⁷⁸⁾. Exposure to tocilizumab, an immune-modulating drug, led cells to oxidative stress, induced changes in the hepatitis membrane, and resulted in decreased secretion of bile acids⁽⁷⁹⁾. The interaction of bile acids with drugs and the administration of pharmaceutical formulations of bile acids with drugs via nano and microparticles contribute to the treatment of many diseases. An antioxidant effect of bile acids has also been discovered⁽⁸⁰⁾. Vitamin D is a liposoluble vitamin with numerous functions in the body⁽⁸¹⁾. Receptors for vitamin D are responsible for many physiological functions in the body, but one important function is the conversion of toxic lithocholic acid into a non-toxic form of this bile acid. This bile acid is a poisonous bile acid that originated from primary bile acid^(82, 83, 84). It should be noted that lithocholic bile acid is hepatotoxic and one of the causes of colon cancer, especially if it is present in high

doses that lead to strong inflammation and necrosis of the intestinal epithelium⁽⁸⁵⁾. It was observed that after supplementation in the diet of mice with chenodeoxycholic acid, there was an increase in the excretion of bile acids in the mice's urine⁽⁸⁶⁾. Bile acids are also used therapeutically. They can be administered orally, parenterally and subcutaneously. They have the ability to cross the blood-brain barrier and stimulate macrophages to respond more aggressively to microorganisms, whilst also exerting an anti-inflammatory effect⁽⁸⁷⁾. Globally, the hepatitis A virus is a primary factor in the development of acute hepatitis⁽⁸⁸⁾. In the treatment of the hepatitis A virus, pharmaceutical formulations of antiviral substances isolated from plants are also used, one of these substances being an aglycon belonging to the class of flavones⁽⁸⁹⁾. Studies examining cells infected with human sapoviruses found that virus replication in intestinal tract cells was impossible without the presence of bile acids, suggesting a correlation between bile acids and virus replication⁽⁹¹⁾. The strength of therapy to be used in people with chronic hepatitis B is also being researched⁽⁹²⁾. More than 800,000 people die annually from hepatitis B virus⁽⁹⁵⁾. Over a decade ago, the hepatitis B receptor, a polypeptide found on hepatocytes that transports bile acids, was discovered⁽⁹⁴⁾. Ritonavir, together with bile acids, blocks the site of the hepatitis B virus into the hepatocyte cell via the receptor for virus entry into the cell⁽⁹³⁾. This increases the effectiveness of the drug⁽⁹⁷⁾. Taurodeoxycholate is a bile acid that strongly binds to hepatocyte receptors, thereby directly inhibiting the entry of hepatitis B and C viruses. The higher the concentration of this acid, the greater the direct interference with the binding of the virus to the hepatocyte receptor⁽⁹⁸⁾.

CONCLUSION

Today, bile acids represent a significant area for new scientific discoveries. In addition to their primary functions, modern scientific research continues to unveil new physicochemical properties of bile acids. One such novel discovery is the ability of bile acids to function as desiccants, in addition to the development of broad-spectrum pharmaceutical formulations. Bile acids can affect numerous diseases, both directly and indirectly. Pharmaceutical formulations of drugs and bile acids hold substantial public health significance.

Sažetak

Žučne kiseline su organska jedinjenja koja imaju veliki fiziološki značaj. Deluju kao moćni biološki deterdženti u varenju masti. Žučna kesa skladišti oko 4 grama žučne kiseline. One se aktiviraju konjugacijom sa aminokiselinama ili sa koenzimom A. Bakterijska flora ih metabolizuje u sekundarne žučne kiseline kada se izluče u tanko crevo. Žučne kiseline takođe služe kao nosioci i pojačivači određenih lekova. Antivirusni lekovi kao što su zidovudin, aciklovir i ribavirin se često nalaze u farmaceutskim formulacijama u kombinaciji sa žučnim kiselinama i intenzivno se koriste. Žučne kiseline same po sebi pokazuju antivirusnu aktivnost kroz nekoliko različitih mehanizama. Oni povećavaju apsorpciju antivirusnog leka kada se uzimaju oralno u istoj formulaciji.

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