NEW PERSPECTIVES ON THE USE OF THYMOL IN COLORECTAL CANCER

Nové perspektívy využitia tymolu pri kolorektálnom karcinóme

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Abstract
This study aims to explore new possibilities for the use of thymol in the context of colorectal cancer. Colorectal cancer still represents one of the most frequently occurring cancers in the world. Linking this disease to the potential of naturally occurring substances such as thymol could help to make a significant shift in prevention and treatment. Our article mainly focuses on innovative and promising approaches such as encapsulation, nanoemulsions, targeted structural modifications of thymol, or personalized approach in the treatment of colorectal cancer. These are areas that have been the focus of intense research in recent years. This has resulted in the discovery of many therapeutic properties of thymol that are applicable to many cancers, including colorectal cancer. The limiting properties of thymol can be minimized or removed by targeted chemical modification. The use of encapsulation or nanoemulsions can increase the potency or stability of thymol and ensure a gradual release of the substance. The results have shown that personalized treatment of colorectal cancer, actively exploiting the knowledge gained, has its place *(Ref. 49). Text in PDF www.lekarsky.herba.sk.*
KEY WORDS: thymol, colorectal cancer, encapsulation, nanoemulsion, derivatives.
Lek Obz 2024, 73 (8): 268-272

Abstrakt
Táto štúdia sa zameriava na preskúmanie nových možností využitia tymolu v súvislosti s kolorektálnym karcinómom. Kolorektálny karcinóm stále predstavuje jedno z najčastejšie sa vyskutujúcich nádorových ochorení vo svete. Prepojenie tohto ochorenia s využitím potenciálu látok prírodného pôvodu, ako je tymol, by mohlo dopomôcť k značnému posunu pri prevencii a liečbe. Náš článok sa zameriava hlavne na inovatívne a perspektívne postupy, ako enkapsulácia, nanoemulzie, cielené štruktúrne modifikácie tymolu či personalizovaný prístup pri liečbe kolorektálneho karcinómu. Ide o oblasti, na ktoré sa v posledných rokoch zameriava intenzívny výskum. Výsledkom je objav širokého spektra terapeutických vlastností tymolu, ktoré sa vzťahujú na mnohé nádorové ochorenia, vrátane kolorektálneho karcinómu. Limitujúce vlastnosti tymolu možno minimalizovať, či odstrániť cielenou chemickou modifikáciou. Využitím enkapsulácie či nanoemulzií možno zvýšiť účinnosť, či stabilitu tymolu, a zabezpečí sa postupné uvoľňovanie látky. Výsledky ukázali, že svoje miesto má aj personalizovaná liečba kolorektálneho karcinómu, aktívne využívajúca získané poznatky *(lit. 49). Text v PDF www.lekarsky.herba.sk.*
KĽÚČOVÉ SLOVÁ: tymol, kolorektálny karcinóm, enkapsulácia, nanoemulzia, deriváty.
Lek Obz 2024, 73 (8): 268-272

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world. It is the second leading cause of cancer death in the USA. The incidence of CRC has slowed since 2000, however, it is increasing in people younger than 55. The proportion of rectal cancers has increased from 27 % in 1995 to 31 % in 2019 (1). The burden of CRC is projected to increase to 3.2 million new cases and 1.6 million deaths by 2040. Most cases are predicted to occur in high or very high HDI (Human Development Index) countries. The disease is largely preventable through changes in risk factors, alongside the detection and removal of precancerous lesions (2). CRC is characterized by a reprogrammed metabolism that allows rapid cell proliferation. Emerging evidence suggests that modifiable environmental risk factors, such as diet, play a key role in the pathogenesis of CRC. However, understanding the connections between environmental factors, metabolic adaptations, and the tumor microenvironment could lead to better prevention and treatment of CRC (3).

Herbal compounds containing thymol, a natural monoterpene phenol, have shown potential in treating various cancers. Thymol has therapeutic benefits, including antimicrobial, antineoplastic, antiinflammatory, antidiabetic, antirheumatic, and antineurodegenerative effects. It can alleviate peripheral nerve dysfunction in diabetes and has anticancer properties. Thymol has antineurodegenerative effects and can influence Alzheimer's disease, multiple sclerosis, and Parkinson's disease. More recently, new forms of application of thymol within nanobiology are beginning to be considered, which could enhance its therapeutic activities, increasing its antimicrobial and antioxidant activities against both Gram-negative and Gram-positive bacteria. Additionally, thymol arrests the cell cycle at the G0/G1 interface in

human malignant glioblastoma cells, indicating its potential as an anticancer agent (4). Thymol has numerous beneficial effects, but its low solubility in water, low bioavailability, and high volatility have limited its use. Its wider use would be possible by applying new approaches such as encapsulation, preparation of nanoemulsions, structural modifications, or detailed characterization of the effect of thymol (5).

Search criteria and resources

The source of data and articles came from databases such as PubMed, Google Scholar and Scopus in March 2024. Keywords such as „thymol", „colorectal cancer", „encapsulation", „nanoemulsion", „in vivo", etc. were used for searching. A total of 49 articles and studies were analyzed from 2008 to 2024. Primarily recent articles and articles not older than four years were studied. However, for the sake of comprehensiveness, older relevant studies were also included.

Results

Encapsulation

Keshavarz et al. (2024) investigated the effects of thymol-loaded nanoliposomes on SW84 and SW111 colorectal cancer cell lines. Liposomes were synthesized by the lipid thin-film hydration method. The MTT assay showed that thymol and thymol-nanoliposomes inhibited the proliferation of SW84 and SW111 cells in a dose-dependent manner. Thymol-nanoliposomes significantly inhibited cancer cell proliferation compared to free thymol. Flow cytometry showed a rise in the percentage of apoptotic cells in the thymol-nanoliposome group. Real-time PCR analysis demonstrated that thymol-nanoliposome was more effective in decreasing EGFR gene expression in both cell lines (6). Similar results were confirmed by another study. The efficacy of thymol was improved by the development and characterization of thymol-loaded solid lipid nanoparticles. Such nanoparticles exhibited higher cytotoxicity against HT-29 (colorectal cancer cell line) cells than free thymol (7). The possibility of using encapsulated thymol *in vivo* was also investigated. Another study developed an efficient chitosan-based drug delivery system that transports thymol into A549 cells (lung tumor cells) by encapsulation. Such thymol has been administered to Swiss albino mice and is shown to be a safe and effective drug candidate against A549 cells. Even at concentrations up to 1000 mg/kg, no signs of toxicity or death occurred within 14 days (8). Encapsulated thymol is also likely to be used as a feed additive. In fact, the stability of microencapsulated thymol with organic acids in lipid matrix microparticles during the feed pelleting and storage process was evaluated. A slow and progressive intestinal release of thymol from the microparticles was demonstrated in weaned pigs. The study showed that the stability of thymol was maintained during feed pelleting and storage, and thus thymol reached the stomach and jejunum, where slow and progressive intestinal release was facilitated (9). Encapsulation of thymol and unsaturated fatty acids into polymeric nanoparticles (10) or thymol-modified polyethylene glycol nanoparticles is also more effective (11, 12).

Nanoemulsion

The preparation of nanoemulsions also presents new therapeutic possibilities. Thymol nanoemulsions of the oil-in-water type are stable during long-term storage and exhibit improved antioxidant activity compared to free thymol and synthetic counterparts (13). They have potent antimicrobial, antiviral and immunostimulatory effects. They can be used as promising therapeutic agents, e.g. against resistant food pathogens, as a feed additive, in the veterinary field, in the cosmetic and medical industries (14). Thymol nanoemulsion made from thyme essential oil, exhibits antibacterial, antifungal and anticancer activities. A significantly potent cytotoxic effect was observed against the breast cancer cell line MCF-7. Nanoemulsion also enhanced apoptosis by increasing the activities of casp-8 and casp-9. Therefore, thymol nanoemulsion has significant potential in the field of medicine (15). Table 1 summarizes other nanoemulsions for application within colorectal cancer (Tab. 1).

Table 1. Different types of nanoemulsions for use in colorectal cancer.
Tabuľka 1. Rôzne typy nanoemulzií pre využitie pri kolorektálnom karcinóme.

Active compound	Composition	References
Curcumin	Tween 80, tocopheryl polyethylene glycol succinate, Kollisolv MCT 70	(16)
Carotenoid	Tween 80, Capryol 90, Transcutol HP	(17)
Ifosfamide	Essential oil	(18)
Resveratrol	Tween 20, Capryol 90, PEG 400	(19)
5-Fluorouracil	Transcutol HP, water, castor oil	(20)
Irinotecan	DSPE-PEG 2000	(21)
Lycopene	Tween 80, gold nanoparticles	(22)

Characterization of the effect of thymol

In general, essential oils are thought to be selectively effective against tumor cells, e.g., also against colon tumor cells Caco-2 and SW-620. Thus, they do not act against healthy cells and are considered safe (23). Hassan et al. found that thymol treatment in rats for 16 weeks can significantly reduce the levels of colon-related tumor markers, the apoptotic marker caspase-3, markers of oxidative stress, and inflammatory mediators in colonic tissue. It also reduced aberrant crypt foci, adenoma, and other cancerous lesions (24). Some other relevant studies are listed in Table 2. Thymol may also act on the transport of ions in the colon. Thymol activates certain types of odorant receptors, including class II OR1G1. It is possible that odorants are synthesized in the lumen of the colon and detected by chemosensors. Thymol evokes concentration-dependent secretion of anions and increases dextran permeability,

Table 2. Broad spectrum of thymol effects in *in vivo* studies with the indicated dosage.
Tabuľka 2. Široké spektrum účinku tymolu v *in vivo* štúdiách s uvedeným dávkovaním.

In vivo model	Dose	Function	Effect	References
rats	75–500 mg/kg	anti-inflammatory	Decreased levels of iNOS and TNF-α	(26)
rats	10–20 mg/kg	antioxidant	GSH induction, reduction of oxidative damage	(27)
rats	20 mg/kg	antioxidant	Decrease in level of caspase-3 and increase in SOD, CAT and GSH	(28)
rats	42.5 mg/kg	antioxidant	Increasing the proportion of docosahexaenoic acid	(29)
rats	100 mg/kg	anti-inflammatory	Reduction of IL-6, IL-1β, COX-2, TNF-α and myeloperoxidase	(30)
rats	50–500 mg/kg	anti-inflammatory	Decrease in TNF-α, caspase-3 and ROS levels and increase in prostaglandin	(31)
rats	20 mg/kg	anti-inflammatory	Reduction of TNF-α, IL-5 and IL-13 levels	(32)
rats	30 mg/kg	antioxidant	Increase in level SOD, CAT and GSH	(33)
rats	50–100 mg/kg	antioxidant	Myeloperoxidase and ROS reduction	(34)
mice	14 mg/kg	antioxidant	Increase in CAT and SOD activity	(35)
mice	30–60 mg/kg	anti-inflammatory	Reduction of TNF-α, IL-1β, IL-6 expression	(36)
chicken	15–60 mg/kg	anti-inflammatory	Reduction of TLR2 and TNF-α levels	(37)
chicken	150 mg/kg	prevents pathogen infection	Support the growth of *Lactobacillus, Clostridium*, inhibits *Proteobacteria*	(38)
pigs	2% thymol	prevents pathogen infection	Promoting the growth of probiotic bacteria	(39)

iNOS – indicible nitric oxide synthase, TNF – tumor necrosis factor, GSH – glutathione, IL – interleukin, COX – cyclooxygenase, ROS – reactive oxygen species, SOD – superoxide dismutase, CAT – catalase, TLR – toll-like receptor

suggesting a key role for odorants in maintaining intestinal homeostasis (25).

New derivatives and chemical modifications of thymol

Characterization of new thymol derivatives, which could potentially act more effectively than thymol, was also addressed our previous study. We investigated two thymol derivatives: acetic acid thymol ester and thymol ß-D-glucoside. Acetic acid thymol ester was much more effective on HT-29 and HCT-116 colorectal cancer cells than thymol, and has a great potential for the future. Thymol ß-D-glucoside, on the other hand, appears to be unpromising. Probably by binding a rather large chemical group to the original structure, there has been a reduction in the effectiveness of this derivative (40). Some derivatives of thymol, carvacrol, and also eugenol show better antioxidant properties and concentration-dependent antiproliferative effect on human uterine cancer cells (41). Also, synthesizing menthol and thymol derivatives of ciprofloxacin is highly promising, as these substances have been shown to have significant antimicrobial and antitumor activity (42). In addition, the derivatives also showed tuberculostatic properties. They have increased lipophilic ability, which would mean better penetration into the cell through the membrane (43).

Personalized treatment of colorectal cancer

Personalized treatment of colorectal cancer should also take into account the patient's personal history, genetic profile, or biomarkers (KRAS, BRAF, and others) (44). Cetuximab, a drug that acts on a specific protein and improves the targeting of treatment, might be a hope for patients with metastatic CRC (45). The therapeutic effect of polyphenols contained in fruits and vegetables in the context of CRC is supported by epidemiological studies. However, the active use of many polyphenols depends on the conduct of randomized clinical trials (46). A personalized approach in the administration of adjuvant chemotherapy, immunotherapy, or a combination of these is currently being considered. The results of circulating tumor DNA (as a biomarker) analysis and histopathology using artificial intelligence would be evaluated to improve patient prognosis (47, 48, 49).

Conclusion

The analyzed studies demonstrate the therapeutic and antitumor activity of thymol and suggest several possible approaches for better biological applicability of thymol in the context of colorectal cancer. Encapsulation, nanoemulsions, structural modifications of thymol or a personalized approach to the treatment of colorectal cancer have great potential but represent only a part of the possible solutions. Further research and large-scale clinical trials would be needed in the future.*

***Acknowledgments.** This work was supported by VEGA grant VEGA 2/0022/24.

Conflict of interest statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Do redakcie došlo 13. 3. 2024.

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