

Review Article

Melittin: the use of the main component of bee venom in the fight against lung tumor cell lines*Melitina: o uso do principal componente da peçonha de abelha no combate de linhagens de células tumorais pulmonares***Matheus de Barros Picolotto¹, Helder Silva Luna²**

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ABSTRACT: *Introduction:* Melittin is a peptide formed by 26 amino acids and the main component of the venom produced by honey bees. Preclinical studies have demonstrated the antineoplastic properties of the molecule and its effect on cancer cell lines. Lung cancer is the deadliest cancer in the world and nine out of ten cases of this disease are related to smoking history. Late diagnosis, often due to lack of clinical symptoms, directly affects treatment and prognosis, leading to low survival rates. In this context, melittin is a prospective therapeutic approach for lung cancer treatment. *Objective:* to conduct an integrative literature review on studies on the effect of melittin on lung tumor cells in humans published in the last five years. *Results:* a total of 15 publications were found, of which 10 were excluded for being duplicates or for not responding to the proposed objective directly, and five were selected for this review. *Discussion:* Melittin proved to be efficient in controlling lung tumor cell lines, corroborating the results already reported in other tumor cell lines. *Conclusion:* The use of melittin is a potential approach for the treatment of lung cancer.

Keywords: Antineoplastics; Peptides; Nanoparticles.

RESUMO: *Introdução:* Melitina é um peptídeo formado por 26 aminoácidos e principal composto da peçonha produzida por abelhas do gênero *Apis*. Estudos pré-clínicos têm demonstrado uma capacidade antineoplásica do composto, evidenciando seu efeito sobre linhagens celulares de câncer. O câncer de pulmão é o câncer que mais mata no mundo, sendo que 90% dos casos da doença estão relacionados a um histórico tabagista. O diagnóstico tardio muitas vezes devido à falta de sintomas clínicos compromete diretamente o tratamento e o prognóstico, gerando uma baixa taxa de sobrevivência. Neste sentido, a melitina surge como uma abordagem no tratamento de câncer de pulmão. *Objetivo:* Realizar uma revisão integrativa da literatura sobre as pesquisas publicadas nos últimos cinco anos sobre o efeito da melitina em linhagens de células tumorais pulmonares humanas. *Resultados:* Foram encontradas 15 publicações, 10 foram excluídas por não responderem diretamente ao objetivo proposto ou por se tratarem de artigos duplicados e cinco foram escolhidas para compor esta revisão. *Discussão:* Os artigos avaliados relatam o efeito anticancerígeno da melitina sobre linhagens celulares de câncer de pulmão *in vitro* e *in vivo*. *Conclusão:* Devido sua atividade citotóxica a melitina é uma candidata no desenvolvimento de novos tratamentos de câncer.

Palavras-chave: Antineoplásicos; Peptídeos; Nanopartículas.

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INTRODUCTION

Bees are a monophyletic group of Hymenoptera that evolved from wasps in the Cretaceous period, approximately 125 million years ago, shortly after the emergence of the first flowering plants. These animals play a key role in the process of pollination of a wide range of plant species¹, contributing substantially not only to world agricultural production², but also to the maintenance and recovery of the biodiversity of fauna and flora in threatened biomes³. More than 20,000 species of bees have been described, with a special focus on the bees of the genus *Apis*, which are responsible for a large part of the production of honey, propolis, royal jelly, and apitoxin for commercial purposes. These products are highly rich in bioactive compounds⁴ and recognized in traditional and modern medicine for their antimicrobial^{5,6}, antioxidant^{6,7}, anti-inflammatory⁷, antiviral^{8,9,10}, immunomodulatory⁹ and anticancer^{11,12,13} properties. Studies suggest that these products have great health benefits and that they can be used in the treatment of various human pathologies¹⁴.

Apitoxin, also referred to as “bee venom”, is the venom produced by two glands in the abdomen of bees of the genus *Apis*, for the purpose of individual or collective defense¹⁵. It is also one of the most recent products of these pollinators to be investigated in modern laboratories. Apitoxin is a mixture rich in peptides and its components include Apamin, Melittin, Histamine, Dopamine, Noradrenaline, Phospholipase (A2 and B), Hyaluronidase, in addition to various carbohydrates and lipids in small amounts^{16,17,18}. Among these components, melittin stands out for being a peptide with anticancer potential. It is formed by 26 amino acids¹⁰ and is the main active component of apitoxin¹⁹. Recent preclinical studies (*in vitro*, *in vivo* and *in silico*) have conducted experiments and demonstrated the anticancer effects of melittin through different mechanisms of action in cell cultures of osteosarcoma²⁰, gastric carcinoma^{21,22}, mammary carcinoma^{23,24,25}, urothelial carcinoma²⁶ and melanoma²⁷. Its high non-selective cytotoxic activity makes it a promising natural molecule for the development of new technologies and drugs for cancer treatment. However, it also casts a light on the biggest challenge faced by scientists in the development of a drug with peptide: safe delivery of melittin to specific targets, preserving the integrity of healthy cells^{27,28,29}.

Despite all the significant advances in technology and knowledge on the diagnosis and treatment of cancer in recent decades, it is still one of the diseases with the highest mortality rates on the planet³⁰. Among the forms

of cancer, lung cancer is the deadliest, mainly because it is initially asymptomatic, which results in the late diagnosis of a tumor that may already be in a more advanced stage, jeopardizing treatment and worsening the prognosis³¹. Many of the cases are directly related to smoking (90%), which is its most important risk factor. Lung cancer can be classified into two subtypes, according to histopathological characteristics: non-small cell lung cancer (NSCLC), which comprises 80% of cases, and small cell lung cancer (SCLC) which comprises 20% of cases and has a 5-year survival rate after diagnosis³².

In this sense, studies that evaluate the potential of molecules in the development of new technologies and drugs for the treatment of lung cancer are extremely necessary.

OBJECTIVE

The objective of this study was to conduct an integrative literature review to analyze the most recent studies on the use of melittin in the control of lung tumor cells in humans.

METHOD

A literature survey in scientific databases was conducted to find preclinical studies that addressed the use of melittin in human lung tumor cell cultures. The following databases were used: Scientific Electronic Library Online (SciELO); National Library of Medicine National Institute of Health (PubMed); Latin American and the Caribbean Literature in Health Sciences (Lilacs). The descriptors used were “Melittin”; “Lung Cancer”, connected by the Boolean operator “AND” (e.g. Melittin AND Lung Cancer). The inclusion criteria were publications available online and in full, published in a period of five years (2017 to 2022), and available in Portuguese or English. Duplicates or articles that did not respond directly to the objective proposed were excluded.

RESULTS

The database search yielded a total of 15 articles, 13 in PubMed, and two in SciELO and Lilacs. After reading and evaluating the articles, two duplicates were removed and another 8 articles were excluded because they did not address the proposed theme. The five remaining articles met the eligibility criteria and were selected for this study, as shown in Figure 1.

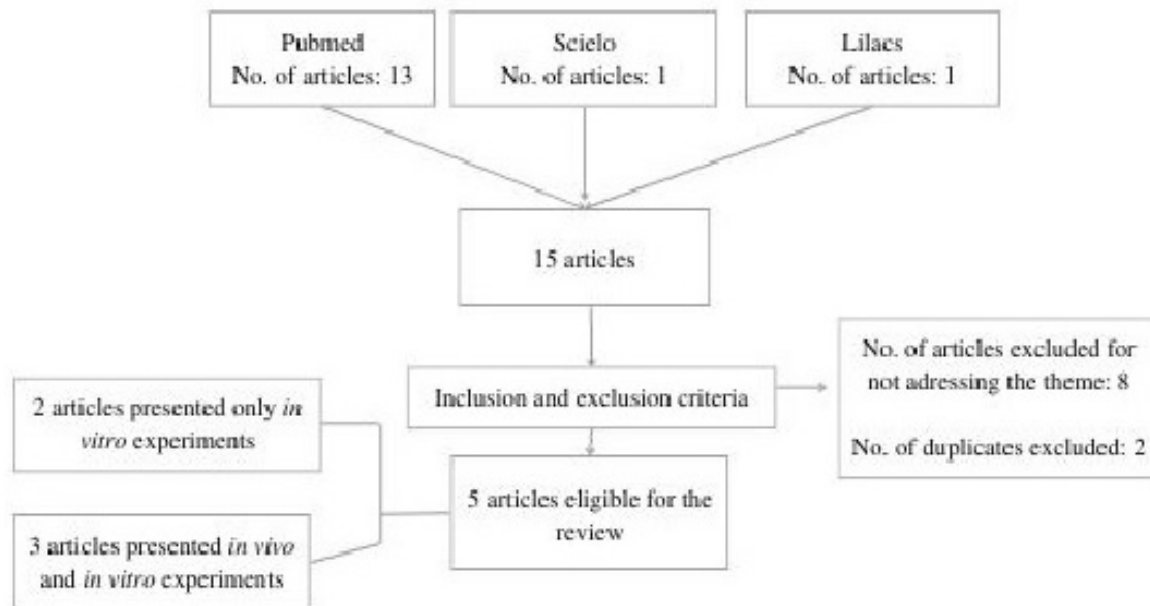


Figure 1. Methodology for article selection

A descriptive analysis of the results according to each study included in the review is presented in Table 1. The articles selected was performed, and a summary of

Table 1. Characterization of the articles selected

Article	Method	Results	Mechanisms of action
Yu et al. ³³ 2021.	Lung cancer cell lines used: A549 and H358 (squamous cell carcinoma and adenocarcinoma, respectively). In vitro: Incubation of cell cultures with melittin. In vivo: Subcutaneous implantation of the cells in animals, followed by administration of injections of melittin.	In vitro: Melittin induced apoptosis and inhibited growth and migration of tumor cells. In vivo: Melittin inhibited growth and increased apoptotic activity in tumor tissues.	*Increased expression of pro-apoptotic genes caspase-3 and Apaf-1 *Decreased expression of TGF- β , ERK, and pERK
Zhang; Chen ³⁴ 2017	Lung cancer cell lines used: A549 (Squamous cell carcinoma) In vitro: Incubation of cell cultures with melittin. In vivo: Subcutaneous implantation of the cells in animals, followed by the administration of injections of melittin.	In vitro: Melittin induced apoptosis and inhibited growth and migration of tumor cells. In vivo: Melittin inhibited the growth of tumor tissues.	*Inhibition of EGF-induced effects *Decreased expression of HIF-1 α and VEGF
Gao et al. ³⁵ 2018	Lung cancer cell lines used: NCI-H441 (adenocarcinoma) In vitro: Incubation of cell cultures with melittin. In vivo: Subcutaneous implantation of the cells in animals, followed by oral administration of melittin.	In vitro: Melittin induced apoptosis and inhibited growth and migration of tumor cells. In vivo: Melittin inhibited growth and increased apoptotic activity in tumor tissues.	*Decreased expression of miR-183 *Increased expression of apoptotic proteins Bax and Caspase-2
Jeong et al. ³⁶ 2019	Lung cancer cell lines used: A549, H793 and H23. In vitro: Incubation of cell cultures with melittin.	In vitro: Melittin inhibited growth and migration of tumor cells.	*Inhibition of EGF-induced effects *Inhibition of transcription factors ZEB2 and Slug
Tipgomut et al. ³⁷ 2018	Lung cancer cell lines used: ChaGo -K1 In vitro: Incubation of cell cultures with melittin.	In vitro: Melittin inhibited tumor cell proliferation.	*Induction of cell cycle arrest.

DISCUSSION

Lung cancer is still one of the world's deadliest diseases, causing about 1.8 million deaths every year. Nevertheless, its prophylaxis, diagnosis and treatment are hampered by several obstacles. The popularization of tobacco (its most important risk factor) and the low global adherence to strong anti-smoking policies are the first barriers encountered in the attempt to reduce the number of people who are affected by and/or die from this disease every year. In most cases, the diagnosis is only made when the disease is already locally advanced or metastatic, which directly affects the survival rates. Furthermore, the current cancer treatment with drugs is not effective³⁸. Given these facts, safer and more effective therapeutic approaches for the treatment of lung cancer are extremely necessary. In this context, anticancer peptides have emerged as a new approach in the treatment of cancer. In the last two decades, natural peptides with anticancer properties have been isolated from several organisms, including mammals³⁹, fish⁴⁰, sponges⁴¹, molluscs⁴², amphibians⁴³ and algae⁴⁴. The present study, in turn, gathered evidence on the anticancer effect of the peptide melittin, from the venom of *Apis spp.*

All the articles evaluated carried out *in vitro* experiments with incubation with different doses of melittin, in an attempt to understand the action of this substance on human lung cancer cells. This allowed the researchers to observe the dose-dependent apoptotic effect of this molecule, which is responsible for inhibiting the growth and migration of cancer cells³³⁻³⁷.

Three of these articles^{33,34,35} also performed *in vivo* experiments with subcutaneous implantation of cancer cells in animals, followed a few days later by periodic application of a solution with melittin. The results demonstrate that melittin also induces apoptosis in tumors developed in animal models, substantially inhibiting growth and proliferation of these tissues.

Yu et al.³³ investigated the antitumor effect of melittin in NSCLC cell lines and observed *in vitro* that melittin (2 µg/ml) promoted apoptosis of tumor cells, which, according to the author, is related to the increase in the expression of pro-apoptotic genes Caspase-3 and Apaf-1 (responsible for encoding proteins that are fundamental for the process of cell death). In addition, the results also show that melittin inhibited the growth, migration and invasion of tumor cells, which was associated with decreased expression levels of transforming growth factor beta (TGF-β) (a protein directly involved in the differentiation/cell proliferation processes) mediated by the ERK pathway. *In vivo* experiments, in turn, demonstrated that melittin inhibited tumor growth in animal models and downregulated TGF-β and ERK expressions when compared to the control group.

Zhang and Chen³⁴ investigated the anti-angiogenic actions of melittin using NSCLC cell lines and observed *in vitro* that melittin (2 µg/ml) inhibits the migration and

invasion of tumor cells (processes induced by epithelial growth factor - EGF), which, according to the authors, occurs because the molecule is able to suppress the expression of HIF-1α and, consequently, the secretion of vascular endothelial growth factor (VEGF) (protein with an active role in the process of angiogenesis) in tumor cells. *In vivo* experiments, in turn, demonstrated that melittin inhibited tumor growth in animal models.

Gao et al.³⁵ investigated the participation of miRNA-183 (a gene strongly associated with lung cancer) in the anticancer effect of melittin using NSCLC cell lines and demonstrated *in vitro* that melittin (2 µg/ml) inhibits the migration and invasion of tumor cells and induces apoptosis through the activation of pro-apoptotic proteins such as Caspase-2 and Bax, which, according to the author, is a result of the reduced miRNA-183 expression in tumor cells. *In vivo* experiments, in turn, demonstrated that melittin inhibited tumor growth in animal models.

Jeong et al.³⁶ investigated the antimetastatic properties of melittin on EGF-induced epithelial-mesenchymal transition (EMT) in NSCLC cell lines and demonstrated *in vitro* that melittin (2 µg/ml) inhibits TEM by blocking the phosphorylation of ERK, FAK and mTOR, resulting in the suppression of transcription factors essential for this process (ZEB2 and Slug).

Tipgomut et al.³⁷ investigated the antiproliferative effect of melittin using a lung cancer cell line and demonstrated that melittin (2.5 µM) induces apoptosis and inhibits tumor cell proliferation, an effect that, according to the author, is caused by cell cycle arrest, especially during the G0/G1 phases. Furthermore, the presence of melittin upregulated the expression of the pro-apoptotic protein Bcl-2.

The results of the experiments described here reiterate the anticancer properties already observed in studies that used other human tumor cell lines and indicate some of the mechanisms of action of the molecule. Experiments that used gastric carcinoma cell lines, for example, had already indicated the relationship between the treatment of tumor cells with melittin and the increase in the expression of pro-apoptotic proteins, corroborating the results of Yu et al.³³ and Gao et al.³⁵. Furthermore, the use of melittin in these same strains has already been shown to inhibit the epithelial-mesenchymal transition of tumor cells⁴⁵, corroborating the findings of Jeong et al.³⁶. Experiments using cervical carcinoma and mammary carcinoma cell lines also showed a relationship between the suppressive action of melittin on the expression of VEGF and HIF-1α^{46,47} and its inhibitory effect on migration and invasion of tumor cells, confirming the findings of Zhang and Chen³⁴.

Although these results highlight the potential of this peptide as a possible new approach for the treatment of lung cancer, there is still a long way to go in research before starting clinical trials. Its high non-selective cytotoxic

activity is a characteristic that demonstrates the risk and benefits of the development of a safe and effective drug with melittin for the treatment of cancer, as it causes damage to cells, regardless of whether they are carcinogenic or not. Therefore, even though it can be used to control lung cancer, the delivery of melittin to internal organs of animal models while preserving the integrity of healthy tissues is still a challenge, which researchers are now trying to respond to

with the help of nanotechnologies^{48,49}.

CONCLUSION

Melittin has a high cytotoxic activity against lung tumor cells, which makes it a promising candidate for the development of new safer and more effective drugs for the treatment of lung cancer.

Participation of the authors: *Matheus de Barros Picolotto*: Development of the text; data collection and characterization. *Helder Silva Luna*: Orientation and organization of the data

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