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The use of bisphosphonates and statins in cancer treatment in dogs: New therapeutic approaches

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ABSTRACT

Statins and bisphosphonates are increasingly recognized for their anticancer potential, particularly due to their effects on the mevalonate pathway. Bisphosphonates exert their effects on tumor cells by inhibiting a key enzyme in this pathway, farnesyl diphosphate synthase, thereby preventing protein prenylation and the subsequent activation of intracellular signalling proteins such as Ras. Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase, the major rate-limiting enzyme that controls the conversion of HMG-CoA to mevalonic acid. The sequential action of both drugs on complementary targets within the mevalonate-dependent cascade, where they hit the same critical metabolic pathway at two different points, provides a potent therapeutic rationale for their combined use to maximize the anticancer effect. This review aims to examine in vitro and in vivo studies regarding the use of statins and bisphosphonates based on their mechanisms of action, as well as the clinical expectations and the potential controversial aspects of this potential synergy in therapeutic applications in cancer treatment.

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Introduction

Bisphosphonates, chemically synthesized approximately 150 years ago, were initially called “diphosphonates” and then adopted their current name, appearing in medical literature from the 1960s onwards (Russell et al., 1969; Russell 2011; Suva et al., 2021). Since the 1970s, they have been used in humans to treat calcium metabolism disorders; however, in the last 10–20 years, they have also begun to attract increasing interest in veterinary medicine (Suva et al., 2021). Bisphosphonates selectively bind to hydroxyapatite binding sites on bone surfaces undergoing active resorption, thereby inhibiting osteoclastic bone resorption. This metabolic disruption leads to inhibition of osteoclast function and ultimately, apoptosis. Non-nitrogen-containing bisphosphonates act as inorganic pyrophosphate analogues, binding to non-hydrolysable forms of adenosine triphosphate (ATP) and thereby inhibiting osteoclast energy metabolism (Mkele 2013; Nancollas et al., 2006). In contrast, nitrogen-containing bisphosphonates act as isoprenoid diphosphate analogues that inhibit farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonate pathway of cholesterol biosynthesis (Milner et al., 2004; Mkele, 2013). This inhibition promotes osteoclast detachment from bone and leads to a decrease in bone resorption. Consequently, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), the binding lipid molecules used in the isoprenylation process that enables small GTPase proteins to attach to cell membranes, cannot be formed. Inhibition of FPPS by nitrogen-containing bisphosphonates leads to the inhibition of prenylation of small GTPases such as Ras, Rho, and Rac, which are involved in a wide variety of functions including cell proliferation, cytoskeletal organization and motility, intracellular vesicle transport, and signal transduction, and binding to the cell membrane via prenylation

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(guanosine triphosphate (GTP)-binding proteins). Consequently, the disruption of their membrane attachment leads to abnormal intracellular signalling and subsequent osteoclast apoptosis (Fan 2007, Güleç 2012).

In recent years, the use of bisphosphonates in veterinary medicine has increased, particularly in studies focusing on their potential as supportive therapy for bone metastases, osteolytic lesions, and specific tumors, where their antitumor effects have been observed (Suva et al., 2021; Milner et al., 2004). Furthermore, some studies have reported that bisphosphonates have direct cytotoxic effects and therefore may be considered not only as supportive care but also as potential antineoplastic agents in veterinary oncology (Suva et al., 2021).

Statins are natural and synthetic compounds isolated from fungi (Corcos and Le Jossic-Corcos 2013). Statins are drugs used in the treatment of hypercholesterolemia that inhibit the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme which eventually leads to the synthesis of cholesterol. Mevalonate is formed from HMG-CoA through a series of biochemical reactions by the HMG-CoA reductase enzyme. Statins primarily inhibit the HMG-CoA reductase enzyme in the mevalonate pathway, thereby interrupting the entire pathway leading to cholesterol formation. This inhibition also prevents the formation of FPP and GGPP. The inhibition of formation of FPP and GGPP prevents small GTPases such as Ras, Rho, and Rac, which play an important role in the division, morphology, and motility of cancer cells, from gaining functional activity and leads to changes in the signalling pathways associated with these proteins (Matusewicz et al., 2015; Parri and Chiarugi 2010). Statins have also been reported to have complementary antineoplastic effects such as inhibition of angiogenesis, reduction of inflammation, and induction of apotosis (Matusewicz et al., 2015).

Targeting the mevalonate pathway in cancer treatment has emerged as a promising approach since the discovery that cancer cells depend on its end products for survival and consume high levels of cholesterol to sustain their rapid metabolism (Chan et al. 2003; Fritz 2009). Various studies have shown that statins can prevent cancer formation by up to 50%; however, there is no information regarding their protective efficacy. Several recent experimental breast cancer models have demonstrated that the mevalonate pathway is a true oncogenic pathway and is often hyperactive in cancer cells (Corcos and Le Jossic-Corcos 2013).

While statins inhibit HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway, nitrogen-containing bisphosphonates target the farnesyl diphosphate synthase enzyme, which is located further down the same pathway. Inhibition of this pathway disrupts the synthesis of isoprenoids, which are essential for the prenylation of small G-proteins such as Ras and Rho. Because these proteins are critical for cancer cell proliferation, survival, and metastasis. Therefore; in recent years, the effects of both bisphosphonates and statins on the mevalonate pathway have led to increased interest in their potential use as as synergistic anticancer agents in cancer treatment (Guerra et al., 2021; Swanson and Hohl 2006).

The common target of bisphosphonates and statins to inhibit the isoprenylation processes that regulate cell proliferation, differentiation, and apoptosis mechanisms. The fact that both drugs target the same metabolic pathway provides a strong scientific rationale for their combined use, based on the potential for synergistic effects in anticancer therapy. Preclinical studies have demonstrated that the combination of statins and bisphosphonates can exert significantly stronger inhibitory effects on tumor cell growth compared to their use as alone (Mansueto et al., 2011). This review aims to examine in vitro and in vivo studies regarding the use of statins and bisphosphonates based on their mechanisms of action, as well as the clinical expectations and potential controversial aspects of this potential synergy in therapeutic applications in cancer treatment.

The use of bisphosphonates in veterinary oncology

Bisphosphonates, compounds that strongly inhibit bone resorption, are widely and successfully used in human medicine for the treatment and management of postmenopausal osteoporosis, the prevention of skeletal complications associated with metastatic and primary breast and prostate cancer, as well as for the control of rare bone diseases such as malignant hypercalcemia, tumor-induced osteolysis, Paget's disease, and osteogenesis imperfecta. Due to their comprehensive clinical efficacy, bisphosphonates have become a major milestone in treatments targeting bone metabolism (Sauty et al., 1996; Russell 2011).

Bisphosphonates are synthetic analogues of pyrophosphate with a high affinity for bone mineral. They are classified into two main groups: nitrogen-free or simple bisphosphonates (e.g., etidronate, clodronate) and nitrogen-containing or amino-bisphosphonates (e.g., alendronate, pamidronate, risedronate, zoledronate) (Mkele 2013, Milner et al., 2004). Nitrogen-containing bisphosphonates inhibit bone resorption more effectively and can be 10 to 10,000 times, more potent than nitrogen-free simple

bisphosphonates (Mkele 2013).

Bone tumors are common in dogs, including primary tumors like osteosarcoma and secondary metastases from cancers like multiple myeloma or various carcinomas. The resulting bone destruction causes intense, chronic pain and reduces quality of life (Chun 2005; Cassali et al., 2014). The standard treatment for the local control of limb tumors is amputation, often combined with chemotherapy (Mauldin et al., 1988). However, this approach is not always feasible. Osteosarcoma frequently metastasizes to the lungs, and patients with pre-existing orthopedic or neurologic conditions may be unable to ambulate on three limbs. For these reasons, a palliative approach is often necessary.

Although many bisphosphonates (BPs) are commercially available, pamidronate and zoledronate are the two most frequently used in veterinary medicine. These agents have been reported to maintain analgesic effects for at least four months (Soto 2014). Dosing regimens for dogs, as reported in various experimental and clinical oncology studies are shown in Table 1. (Milner et al., 2004). While many of the reported dosages exceed clinically relevant levels, these data provide a valuable starting point for canine clinical trials. Overall, bisphosphonate therapy remains a crucial option for managing pain and supporting a better quality of life in dogs affected by bone tumors.

Aminobisphosphonates exert direct effects on cancer cells; they inhibit cell proliferation by disrupting the mevalonate pathway, induce apoptosis, inhibit angiogenesis and matrix metalloproteinases, and modulate the activity of cytokines and growth factors. Furthermore, they stimulate γ and δ-T cells, thereby generating an immunomodulatory effect (Milner et al. 2004; Drake et al. 2008, Zekri et al., 2014). Bisphosphonates are potent inhibitors of FPPS, an important enzyme in the mevalonate pathway. FPPS catalyses the reaction in which FPP is formed from isopentenyl pyrophosphate and dimethylallyl pyrophosphate. This results in an increase in GGPP, which controls the proliferation of cancer cells and regulatory small GTPase proteins such as Ras, Rac, Rho, and Cdc42 that play a role in cell division (Chan et al. 2003). Bisphosphonates also cause the accumulation of isopentenyl pyrophosphate, which is subsequently converted to ApppI, a cytotoxic adenosine 5-triphosphate analogue that leads to cancer cell death. The decrease in the number of small GTPases affects the transition of the cell cycle from the G1 to the S phase and inhibits cell proliferation by affecting the transcription of genes such as cyclin D, which play a role in the progression of the cell cycle and/or cell growth in cancer cells (Milner et al. 2004; Reszka and Rodan 2004).

Bisphosphonates such as pamidronate and zoledronate have been reported to play a supportive role in chemotherapy by reducing pain and limiting bone destruction, particularly in cases of bone metastases from osteosarcoma and mammary tumors in dogs (Suva et al., 2021).

In vitro and in vivo cancer studies on aminobisphosphonates in dogs

In veterinary medicine, aminobisphosphonates are used to manage bone pain associated with primary and metastatic bone tumors (Suva et al., 2021).

In an in vitro study conducted to determine the effect of alendronate, a bisphosphonate derivative, on the viability of canine osteosarcoma cells and healthy canine fibroblast cells, the cells were incubated with alendronate (0.001-1000 microM) for 24, 48, and 72 hours. While no significant difference was observed after 24-, 48- and 72-hours incubation periods, a significant concentration- and time-dependent decrease in the viability of osteosarcoma cells was observed at alendronate concentrations ranging from 10 to 1000 microM, with the lowest cell viability percentage reported as 35%. In contrast, alendronate was found to have a minimal effect on the viability of healthy canine fibroblast cells, which maintained a viability rate of approximately 76%. Based on these data, it was suggested that alendronate has the potential to inhibit canine osteosarcoma tumor growth and that if these in vitro findings are confirmed in vivo,

Table 1. Bisphosphonate dosages reported for dogs. (Milner et al., 2004)

	Trade Name	Route	Dosage Range	Frequency
Non–amino-bisphosphonates				
Etidronate	Didronel	SC	0.5 mg/kg	Daily
Clodronate	Bonefos	PO	20–40 mg/kg	Daily
Amino-bisphosphonates				
Pamidronate	Aredia	IV	1.3 mg/kg in 150 ml of 0.9% saline, given over 2 hours	Can be repeated in 7 days
Alendronate	Fosamax	PO	0.5–1 mg/kg	Daily
Risedronate	Actonel	PO	0.5–1 mg/kg	Undetermined
Zoledronate	Zometa	IV	Undetermined	Undetermined

alendronate could be used as an adjuvant to current chemotherapeutic protocols (Farese et al., 2004).

In a study of 2 dogs undergoing treatment for osteosarcoma, researchers found that oral administration of alendronate prolonged survival times. Consequently, they suggested that aminobisphosphonate may play an effective role in the treatment of malignant bone diseases in dogs (Tomlin et al., 2000). Furthermore, a prospective study evaluating the safety of intravenous pamidronate in 33 dogs with skeletal tumors reported that the treatment was well tolerated. The protocol involved a dose of 1.0 mg/kg (diluted in 0.9% sodium chloride to a total volume of 250 ml) administered every 28 days, with participants receiving between 1 and 17 doses, totalling 133 doses (Fan et al. 2005).

In a study of dogs with appendicular osteosarcoma, a multimodal treatment was administrated consisting of intravenous pamidronate (2.0 mg/kg, every 28 days for 2 hours), palliative radiotherapy (8 Gy/week for 4 weeks), doxorubicin (30 mg/m², every 21 days), and oral deracoxib (1-2 mg/kg/day). Pain reduction was observed in all treated dogs; it was reported that pamidronate can be effectively combined with traditional palliative treatment options for dogs with appendicular osteosarcoma (Fan 2007).

Norquest et al. (2024), examined the cellular and clinical effects of combining zoledronate with radiotherapy for canine appendicular osteosarcoma. The in vitro results demonstrated that zoledronate induces apoptosis in osteosarcoma cell lines and the cellular effect mechanisms of the combination of zoledronate and radiotherapy differ depending on the cell type. The combination of zoledronate and radiotherapy was reported to produce different apoptosis response among the Abrams, D-17, and HMPOS cell lines. Cell cycle arrest (G2/M phase) was minimal and variable among cell lines, but it was highest 48 hours after zoledronate administration. Only 10% of dogs with osteosarcoma receiving zoledronate (0.1 mg/kg IV) and radiotherapy (4-week protocol) developed pathological fractures, and this rate was statistically significantly lower than in groups previously treated with pamidronate. These findings suggest that the use of zoledronate in combination with radiotherapy is superior to pamidronate in reducing skeletal-related complications (pathological fractures). This combination serves as a critical target of palliative treatment in dogs with osteosarcoma that cannot undergo surgical intervention and significantly improving overall quality of life (Norquest et al., 2024). The biological effects of intravenously administered zoledronate on bone were evaluated in a 4-week study in dogs diagnosed with primary and secondary skeletal tumors. Zoledronate was administered as a single dose of 0.25 mg/kg over 15 minutes. Significant decreases in urinary N-telopeptide of type 1 collagen (NTx) concentrations were observed in all tumor-bearing dogs after administration, demonstrating that zoledronate has potent global antiresorptive effects. Furthermore, dogs with primary appendicular osteosarcoma that experienced pain relief also showed significant increases in relative bone mineral density (rBMD). The correlation between increased rBMD and pain relief suggest that zoledronate effectively inhibits both local malignant osteolysis and pain formation in the immediate bone tumor microenvironment (Lorimier and Fan 2005).

In a study to determine the tolerability of zoledronate in the palliative treatment of cancerous dogs and to evaluate its efficacy in the treatment of malignancy-related hypercalcemia, 37 dogs (22 with tumor-related bone pain and 15 with malignancy-related hypercalcemia) receiving zoledronate infusion were examined. Tolerance was assessed based on the absence of hypocalcemia after zoledronate or side effects defined by the Common Terminology Criteria for Adverse Events group of the veterinary oncology group. Efficacy was assessed by comparing ionized calcium levels before and after zoledronate administration in hypercalcemic dogs. No side effects developed in 79% of dogs treated with zoledronate, and most of the side effects seen in the remaining 21% were reported to be related to concurrent chemotherapy or the underlying neoplastic disease. A small but significant increase in creatinine levels was observed after zoledronate administration, but no clinically significant renal disease developed in any of the dogs. In eight hypercalcemic dogs whose ionized calcium levels were measured after zoledronate administration, a rapid decrease in ionized calcium levels was observed within 7 days after administration. Zoledronate was reported to be well tolerated, but monitoring of serum creatinine levels was recommended. Furthermore, zoledronate has been reported to be effective in the treatment of malignancy -related hypercalcemia (Lopes et al., 2023).

In a study conducted to determine whether the timing of bisphosphonate administration in relation to radiation therapy altered the clonogenic survival or cell viability of canine osteosarcoma cells in vitro, pamidronate or zoledronate was administered to canine osteosarcoma cells before, concurrently with, or after radiation. When bisphosphonates were administered after radiation, they were found to cause a decrease in clonogenic survival compared to

administration before radiation. It has been suggested that bisphosphonate administration 24 hours after radiation may be more effective in reducing the proliferation of canine osteosarcoma cells and prolonging the analgesic effects of both treatments (Hoddinott et al., 2020).

Statins

Cholesterol is formed from HMG-CoA through a series of biochemical reactions, including the reaction catalysed by the HMG-CoA reductase enzyme, which produces mevalonate. Statins decrease intracellular cholesterol by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The mevalonate pathway inhibited by statins begins with the conversion of acetoacetyl-CoA to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) by methylglutaryl coenzyme A (HMG-CoA) synthase. Additionally, in this pathway, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which are prenyl carbon chains added to the C-terminus of many CAAX-containing proteins post-translationally, are formed. This prenylation leads to the binding of proteins such as small GTPases, like Ras and Rho, to the inner plasma membrane and the acquisition of their enzymatic activities. Therefore, any disruption or interruption at any stage of the mevalonate pathway results in the inhibition of FPP and GGPP formation, thereby preventing these GTPases from acquiring functional activity. Statins inhibit the mevalonate pathway primarily by inhibiting the HMG-CoA reductase enzyme (Chan et al. 2003; Corcos and Le Jossic-Corcos 2013). Inhibition of the mevalonate pathway by statins results in changes in signalling pathways due to the blockage of prenylation of small GTPases such as Ras, Rho, Rac, or Cdc42 (Chan et al. 2003). Proteins such as Ras, Rho, or Rac play important roles in the control of cancer cell division, cell morphology, and motility (Corcos and Le Jossic-Corcos 2013). Other effects resulting from disruption of the mevalonate pathway include a decrease in the production of active dolichol, heme A, or ubiquinone, which perform important functions in protein glycosylation or cellular respiration, in addition to the restriction of cholesterol synthesis (Demirci 2010; Güleç 2012; Corcos and Le Jossic-Corcos 2013). Furthermore, statins cause a decrease in plasma triglyceride levels and an increase in plasma HDL-cholesterol levels by increasing apolipoprotein A-1 formation. In addition to these anti-lipidemic effects, they also have pleiotropic effects, such as protecting and correcting endothelial function and reducing inflammation and oxidative stress (Alıcı et al., 2009). Statins exhibit pleiotropic effects—actions independent of their main role in lowering cholesterol. They achieve this by downregulating other products of the mevalonate pathway and disrupting the prenylation of proteins, which impacts many cellular signalling pathways. These cholesterol-independent actions contribute to the statins' influence on cancer development by affecting processes like cell growth, apoptosis, autophagy, angiogenesis, inflammation, and metastasis (Ahmadi et al., 2020; Ashrafizadeh et al., 2020, Liu et al., 2023).

This comprehensive summary reviews the effects and underlying mechanisms of statins in various cancers, drawing upon available preclinical and clinical studies, and discusses the therapeutic potential and limitations of applying statins in cancer therapy for dogs.

In vitro and in vivo cancer on statin derivatives in dogs

An increasing number of studies on statins, primarily known as cholesterol-lowering agents, has expanded into oncology, including in vitro and in vivo models relevant to veterinary and comparative cancer research (Matusewicz et al., 2015). While most studies focus on human or murine models, their findings provide mechanistic insights applicable to veterinary oncology.

Recent studies have indicated the potential involvement of dysregulated Hippo signalling in the development and progression of Canine Mammary Tumours (CMTs). Statins possess the capacity to activate the Hippo pathway by blocking protein geranylgeranylation, which consequently decreases the expression and activity of the transcriptional co-activators YAP and TAZ (Atmane et al., 2023, Vigneau et al., 2022). Based on this mechanistic link, a study investigated whether statins could exert anti-cancer effects in CMT cells. This study showed that atorvastatin and fluvastatin have shown cytotoxic effects against two CMT cell lines (CMT9 and CMT47), with effective doses (ED50) ranging from 0.95-23.5 µM and CMT-9 was found to be more resistant to the effect of both statins with a magnitude of 10 when compared to CMT-47. It was observed that both statins increased apoptosis and promoted cell cycle arrest. Also, it was demonstrated that both statins decreased YAP and TAZ expression and reduced the mRNA levels of key Hippo transcriptional target genes known to be involved in breast cancer progression and chemoresistance (CYR61, CTGF and RHAMM). Moreover, both statins effectively inhibited tumorigenesis such as cell migration. It was suggested that targeting the Hippo pathway with statins is a novel and promising therapeutic strategy for treating canine mammary gland cancers (Vigneau et al., 2022).

A study conducted to determine the most effective statin by comparing the anticancer effects of hydrophilic rosuvastatin and lipophilic atorvastatin, simvastatin, fluvastatin, and pitavastatin on three

hydrophilic rosuvastatin and lipophilic atorvastatin, simvastatin, fluvastatin, and pitavastatin on three canine oral melanoma cell lines showed that time-dependent measurement of cell density indicated that lipophilic statins had a stronger anti-proliferative effect than hydrophilic rosuvastatin on all cell lines. Measurement of lactate dehydrogenase release, an indicator of cytotoxicity, showed that lipophilic statins induced cell death more effectively than hydrophilic rosuvastatin. It has been noted that lipophilic statins both inhibit cell proliferation and induce cell death. The anticancer effects of statins on canine oral melanoma cells were ranked according to IC50 values as pitavastatin < fluvastatin = simvastatin < atorvastatin < rosuvastatin. Based on the obtained results, the lipophilic pitavastatin was reported as the most suitable statin for the treatment of oral melanoma in dogs (Ishikawa et al., 2024).

A recent study evaluated the potential dual therapeutic role of statins in targeting both tumor growth and coagulation abnormalities associated with highly aggressive canine haemangiosarcoma (HSA). The researchers initially confirmed the presence of HMGCR mRNA expression exclusively in canine HSA tissues and cell lines, but not in normal cephalic vein or spleen tissues, providing a rationale for statin use. It was reported that treatment with lipophilic statins, including atorvastatin, fluvastatin, and simvastatin, significantly inhibited canine HSA cell viability in a concentration-dependent manner. Also, it was shown that statin treatments concurrently decreased the expression of tissue factor (TF) at both the mRNA and protein levels, suggesting a potential benefit against the common coagulation abnormalities seen in HSA. Simvastatin was found to decrease Akt phosphorylation. Notably, MK-2206, a specific Akt inhibitor, mirrored the inhibitory effect of simvastatin on cell viability and induced cell cycle arrest. The study observed that the effect of MK-2206 on TF expression varied depending on the cell type, leading the authors to conclude that Akt phosphorylation may not consistently regulate TF expression across all cells. The authors suggested the potential therapeutic use of statins for concurrently managing tumor growth and coagulation defects in canine HSA (Kobayashi et al., 2024).

Ishikawa et al. (2021) investigated the relationship between the epithelial-to-mesenchymal transition (EMT) state and sensitivity to the cholesterol-lowering drug atorvastatin in a panel of canine cancer cell lines. The authors aimed to clarify the correlation between atorvastatin sensitivity and the epithelial/mesenchymal phenotype across 11 distinct canine cancer cell lines derived from various tissues, including mammary gland, squamous cell carcinoma, lung, and melanoma. Cell viability assays determined the IC50 values ranging from 5.92 to 71.5 µM at 48 h. The authors noted that these values are generally higher than the plasma concentrations typically achieved during clinical statin therapy. Atorvastatin was found to preferentially attenuate the proliferation of mesenchymal-like cells compared to epithelial-like cells. Highly statin-sensitive cells were notably characterized by the aberrant expression of the ZEB family of EMT-inducing transcription factors (e.g., ZEB1 and ZEB2). To determine if ZEB expression directly confers sensitivity, ZEB2 was silenced in highly sensitive cells. However, this silencing did not induce resistance to atorvastatin. These results collectively suggest that high expression of ZEB is a characteristic feature of highly statin-sensitive canine cancer cells and could potentially serve as a valuable molecular marker for predicting statin responsiveness in canine cancers (Ishikawa et al., 2021).

Kobayashi et al., (2021) investigated the anticancer effects of simvastatin on different canine tumor cell lines, including those derived from hemangiosarcoma (HSA), melanoma, and lymphoma. They aimed to evaluate the effect of simvastatin on cell proliferation and Ras activation. It was found that simvastatin successfully inhibited the proliferation of all tested canine tumor cell lines in a concentration- and time-dependent manner, though the susceptibility varied between the lines. The cytotoxic effects of simvastatin were attributed to the induction of apoptotic cell death via the activation of caspase-3 and cell cycle arrest. However, the addition of GGPP and FPP attenuated the cytotoxic effects of simvastatin and simvastatin decreased the amount of prenylated Ras (the active, membrane-bound form) and consequently reduced the levels of GTP-bound Ras (the signalling-active form) in HSA and melanoma cell lines. Conversely, simvastatin did not decrease prenylated or GTP-bound Ras in the lymphoma cell lines. It was suggested that the depletion of these mevalonate intermediates is a primary mechanism for simvastatin's toxicity whereas multiple mechanisms are involved in the effects (Kobayashi et al., 2021).

A recent study examined the in vivo anti-cancer effects of fluvastatin alone and in combination therapy with doxorubicin using a murine xenograft model of canine mammary gland tumour (CMT). Additionally, the effect of fluvastatin was determined in vivo on the Hippo pathway and some of its target genes (CTGF, CYR61, ANKRD1 and RHAMM2) which is known to be involved in both human breast cancer and CMT. The study results revealed that a statistically significant

reduction in tumor volumes was observed only in the combination group when compared to the control. Conversely, a significant difference in final tumor weight was noted only in the doxorubicin group. Also, it was reported that significant difference was not observed in tumour necrosis, expression of CC3, Ki-67, YAP and TAZ measured by immunohistochemistry and in the mRNA levels of the target genes. Unexpectedly, they have observed lung metastases in the control group and not in the fluvastatin treated group. In addition, it was reported that mass spectrometry-based quantification of fluvastatin concentrations reached therapeutic concentrations without influencing the hippo pathway or various tumour parameters (Atmane et al., 2023).

In a previous publication, the effects of simvastatin were evaluated on spheres derived from CF41.Mg canine mammary tumor cells. It was reported that spheres primarily expressed a CD44+/CD24-/low phenotype, displaying auto-renewal and relative chemoresistance. Simvastatin has been reported to suppress sphere-forming capacity and reduce cell viability, effects that are associated with a concentration- and time-dependent increase in caspase -3/7 activity. Also, it was reported that the modulation of β-catenin and p53 expression have been observed. Also, it was reported that simvastatin induced a synergistic effect with doxorubicin, effectively sensitizing the chemoresistant spheres to the cytotoxic effects of doxorubicin. It was found that invasiveness of spheres was decreased within the treatment with simvastatin and this effect was counteracted by the presence of GGP. It was suggested that simvastatin targets canine mammary cancer stem-like cells, supporting its therapeutical application as a novel agent for treating mammary cancer (Torres et al., 2015).

In vitro studies confirm that statins, particularly lipophilic types, exert strong anti-cancer effects in canine tumor cell lines. These findings support further investigation into statins as adjuncts in canine cancer therapy.

Cancer studies on the combined use of statin derivatives and bisphosphonates

Recent oncological research has increasingly focused on the synergistic potential of combining statin derivatives with bisphosphonates to achieve a dual-target inhibition of the mevalonate pathway. The combined metabolic blockade has been shown to suppress tumor cell proliferation, induce apoptosis, and inhibit angiogenic signaling across various malignancies, including pancreatic, prostate, and squamous cell carcinomas (Vincenzi et al., 2003). By utilizing these agents in tandem, researchers aim to overcome the systemic toxicity associated with high-dose monotherapies while offering a more potent, multifocal therapeutic strategy for aggressive and drug-resistant cancers.

Building on the established antitumor potential of mevalonate pathway inhibitors, one study identified a potent synergistic antiproliferative effect when combining the bisphosphonate zoledronic acid (ZOL) with the statin fluvastatin (FLU) in Mia PaCa-2 and Suit-2 pancreatic cancer cell lines. The investigators demonstrated that this synergy is fundamentally driven by the simultaneous depletion of downstream mevalonate products, specifically farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Mechanistically, the combination treatment was found to impair critical RhoA and Ras signaling pathways by inhibiting the essential post-translational modifications, namely geranylgeranylation and farnesylation. This was evidenced by a marked accumulation of unprenylated Ras and RhoA, a process that was successfully reversed upon the addition of exogenous FPP and GGPP. By disrupting the prenylation of these small GTP-proteins, the ZOL-FLU combination effectively halts the signaling cascades necessary for tumor cell proliferation, offering a promising and mechanistically grounded therapeutic strategy for the treatment of pancreatic adenocarcinoma (Elsayed et al., 2016).

A study conducted by Issat et al., (2007), highlighted the potentiation of antitumor activity through the combined inhibition of the mevalonate pathway using lovastatin and the second-generation bisphosphonate pamidronate. While monotherapy with either agent showed negligible effects on tumor growth in a murine PANC02 pancreatic adenocarcinoma model, the combination therapy significantly retarded tumor progression and prolonged survival at clinically achievable doses. In vitro assays on breast and pancreatic cancer lines further revealed that this synergistic pairing exerts strong cytostatic and cytotoxic effects, while simultaneously impairing critical metastatic processes, including cell adhesion to collagen IV and fibronectin, as well as cellular migration and invasiveness. The treatment was shown to regulate the cell cycle and induce apoptosis. Collectively, these findings suggest that the dual application of statins and bisphosphonates offers a promising, translatable strategy for enhancing the efficacy of cancer therapeutics and warrants further investigation in clinical trials (Issat et al., 2007).

Further research explored the combined cytotoxic potential of the statin simvastatin and the

bisphosphonate alendronate against the Hep-2 squamous cell carcinoma line. By using the IC50 concentrations of each agent, the study demonstrated that the dual treatment exerts a significant synergistic effect, markedly reducing cell viability compared to monotherapy or control groups. Beyond direct antiproliferative action, the combination significantly suppressed the expression of vascular endothelial growth factor (VEGF), a key mediator of angiogenesis and tumor progression. These findings suggest that the simultaneous administration of simvastatin and alendronate disrupts essential cancer cell functions and inhibits the angiogenic signaling microenvironment, offering a potent therapeutic strategy for managing squamous cell carcinoma through multifocal pathway inhibition (Abd-el-Fattah et al., 2017).

Rogers et al. (2015) investigated a novel therapeutic strategy for treating prostate cancer by implementing a sequential blockade of the mevalonate pathway. The researchers propose that the combination of simvastatin (an HMG-CoA reductase inhibitor) and alendronate (a farnesyl pyrophosphate synthase inhibitor) provides a potent synergistic cytotoxic effect at significantly lower, clinically safer doses. Interestingly, they reported that this synergy is highly specific to aggressive, androgen-independent PC-3 cells, whereas it proves ineffective against androgen-dependent LNCaP or DU 145 lines. The study identified that the treatment disrupts survival signaling by down-regulating phospho-AKT while simultaneously activating the c-JUN and ERK pathways to trigger apoptosis. The researchers concluded that exploiting the metabolic vulnerabilities of hormone-refractory prostate cancer through combined mevalonate pathway interference offers a more targeted and less toxic clinical alternative to conventional inhibitors (Rogers et al., 2015).

Conclusion

Statins and bisphosphonates have effect on cancer cells by inhibiting cell proliferation and inducing apoptosis. The mechanism beyond these effects is the disruption of the mevalonate pathway which is a crucial cellular metabolic route. Mevalonate pathway resulted in cholesterol synthesis and generation of isoprenoids which are vital for protein prenylation. Once active, they regulate processes critical for cancer progression: cell proliferation, migration, and survival. Therefore, the co-administration of statins and bisphosphonates presents a compelling new pharmacological strategy to be evaluated alongside standard chemotherapy in cancer treatment. The existing data provides a strong rationale for future in vitro and in vivo studies designed to thoroughly assess the precise mechanism of action and therapeutic efficacy of this drug combination.

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