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Monastrol: Unveiling the Mechanisms, Efficacy, and Therapeutic Potential – A Comprehensive Review

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ABSTRACT

Cancer, one of the leading causes of death in the world, is characterized by the uncontrollable growth of defective cells in the body, creating lumps of cells known as tumors. Cells undergo uncontrollable growth when checkpoints in the cell cycle are overridden, allowing malignant cells to continue to grow without being killed. In recent studies, scientists have found that the compound monastrol is effective in arresting cells in the cell cycle and experiencing apoptosis or programmed cell death. Monastrol is of particular interest because it affects a kinesin called Eg5, a popular target for cancer therapies since it plays a crucial role in the formation of bipolar spindles during mitosis, which is responsible for dividing the cells. A kinesin is a class of protein that is involved in various cellular functions. Most importantly, their involvement in mitosis is what makes them important in cancer. By inhibiting the basal and microtubule stimulate ATPase activity of the Eg5 motor domain, the monastrol alters the ability of the Eg5 to generate force, thereby preventing it from maintaining the bipolar spindles and leading to cell death.

Introduction

The kinesin spindle protein Eg5 is a motor protein that allows for the proper establishment of the bipolar mitotic spindle. The mitotic spindle is a dynamic tool composed of tubulin, motors, and other molecules. It is assembled around the chromosomes and distributes the duplicated genomes generated from the S phase of mitosis to daughter cells (Karesenti & Vernos, 2001). While the biochemical and physical properties of the assembly of the spindle are still unclear, chromosomes have been observed to play a key role by generating a cytoplasmic state that supports the growth and nucleation of microtubules (Karesenti & Vernos, 2001). Mutations or errors in the proper assembly of the mitotic spindle are often linked with brain diseases and cancer in humans and mice and are thought to lead to reduced or excessive cell proliferation as a result of symmetric or asymmetric divisions (Noatynska et al., 2012).

This protein specifically emerged as a subject of interest to researchers as a promising new mitotic target. The Eg5 kinesin protein is a plus-end directed motor from the BimC kinesin family which is thought to generate the force used to push the 2 poles of the mitotic spindle apart (Maliga et al., 2022). The molecular mechanism of Eg5 is modulated by Parkin using the Hsp70-JNK-c-Jun signaling pathway, and an Eg5 inhibitor causes mitotic arrest in cancer cells through the c-Jun NH2 kinase pathway (Jin et al., 2017).

Monastrol is a drug that specifically inhibits the ATPase activity of the motor domain of Eg5, which then inhibits Eg5 microtubule motility specifically *in vitro* and *cellulo* (Garcia-Saez & Skoufias, 2020). This sparked an interest in researchers to pursue the identification of small molecule Eg5 targets based on antimitotic strategy. Monastrol inhibits microtubule gliding by constructing a truncated, dimeric Eg5 protein *in vitro* (Maliga et al., 2022). Mo-

nastrol might be substrate competitive, inhibiting the ATP hydrolysis cycle by directly competing with ATP or microtubule-binding (Maliga et al., 2022). An alternative hypothesis is that monastrol might inhibit the motor domain allosterically by inhibiting ATP hydrolysis or uncoupling partner-head interactions to inhibit motor but not ATPase activity (Maliga et al., 2022).

Treatments with monastrol on tissue culture cells and mitotic cell extracts have resulted in the development of the mono-astral spindle phenotype (Maliga et al., 2022).

While Eg5 inhibitors similar to monastrol have entered clinical trials, the current problem being faced in such trials is that most of the inhibitors have limited efficacy in test groups of patients with multiple myeloma (Maliga et al., 2022). Previously tested small molecules used for inhibiting motor proteins such as ortho-vanadate and nonhydrolyzable ATP analogs were proven to not be cell-specific nor cell-permeable. New research in Eg5 inhibitors has sparked a new interest in novel inhibitor binding sites and a focus on drug synergy with established antitumor agents to improve chemotherapy efficacy (Maliga et al., 2022).

This review presents an updated and holistic view of the Eg5 inhibitor monastrol, including pharmacokinetics and mechanisms of monastrol effects on cell viability, current clinical trials, and other Eg5 inhibitors.

Mechanism of Monastrol / Pharmacokinetics Profile

Monastrol Mechanism

As the first Eg5 inhibitor to be discovered, monastrol has become the focus of anti-cancer treatment studies. Monastrol, in particular, is an allosteric inhibitor focusing on cell cycle arrest, able to induce apoptosis by blocking the Eg5 from attaching to the site. Through correlation analysis and simulation-based binding site mapping, some of these Eg5 allosteric sites were able to be located, with the possibility for newer Eg5 inhibitors to be discovered (Hassan et al., 2013). The significance of blocking the site of Eg5 cannot be understated, because it is what causes the main effects of Monastrol which is an anti-cancer drug. Apoptosis, including cancer cell proliferation, is just to name a few, and this new chemical agent can be a realistic solution shortly.

Monastrol binds the hydrophobic, induced-fit pocket between loop 5 and α3, non-coverseved features of kinesin motor domains, thus resulting in the specificity of monastrol for vertebrate Eg5 homologs. When a drug binds, there is a relative movement of 1Å between α3 and α2, the alpha-helix. Additionally, loop 5 undergoes a folding motion that brings it closer to the drug binding site (Maliga & Mitchison, 2006) . Specific hydrophobic and polar interactions are present in the crystal structure, but it is still unclear on their contributions to drug binding. Due to the presence of large amino acid side chains extending into the hypothetical monastrol binding pocket, most kinesins would be unable to accommodate the drug and prevent its binding (Maliga & Mitchison, 2006). Consequently, monastrol is anticipated to display a high level of selectivity specifically among kinesins.

Pharmacokinetics

Since monastrol has weak activity and lacks typical drug traits, it is not the best drug option for cancer treatments. Due to its low solubility in water, monastrol requires other methods to be administered to the body (Torres et al., 2014). One method, the use of mesoporous silica nanoparticles, involves the use of nanocarriers synthesized from ammonia, ethanol, tetraethyl orthosilicate, and Polysorbate 80 to store hydrophobic drugs such as monastrol to transport it within the body (Zhang et al., 2016). This method was able to successfully deliver monastrol into the cancer cells. Monastrol's effect on the body was able to be studied due to this new method of transportation.

A main issue that causes cancer in the body is the inability to transport the drugs into the body, and for it to be able to dissolve within cancer cells. Monastrol is tricky to use in its original state, but do improve its ability to detect cancer cells and inhibit Kinesin Eg5, scientists made a minor structure modification using the ADMET predictor

software which showed that a simple change from a C-5 carbonyl to a thiocarbonyl could help with monastrol's ability to work easily in any environment within the body (Ogunwa et al., 2019). This change increased the safety of administration as it eliminated the toxicity levels and helped increase the ability to bind to inhibit KSP. Using this advanced monastrol which was much improved from the initial agent, scientists conducted a study conducted on rats using the LaSOM65, a molecule derived from monastrol.

Similar to monastrol, pharmacokinetics was a large issue for this drug along with toxicity, and since this was a derivative of monastrol, scientists developed a similar model to try to test whether certain addition reduced the toxicity levels in rats (Torres et ral., 2014). A major pharmacokinetic parameter was AUC, which was important because it showed whether the binding and the toxicity levels were stabilizing or decreasing to make sure that these types of drugs could be approved for human use to stop cancer. After their experiment, they found that AUC was less than 20%, which was significant as it supported their ideas and provided good separation between the compound and other interfering matrix compounds and metabolites, which showed that this new technique of using LaSOM65 - which is chemically similar to monastrol - could have a chance of being an effective solution to cancer. Usually, anticancer drugs exhibit undesirable effects of neurotoxicity, which is why critics object to this group of drugs, but LaSOM65 is unique. According to the study, LaSOM65 had positive pharmacokinetics characteristics - essential for anti-cancer compounds - but more importantly, there were no toxic effects reported. This is a ground-breaking discovery because anti-cancer medications have been hindered by toxic effects, but this gives us a view into a possible anti-cancer medication that could be safe, and applicable for all living organisms (Hassan et al. 2013).

Cell Viability/Effects with Monastrol

Healthy Cell Viability in Monastrol

Cancer cells divide and multiply randomly, and at greater rates than regular cell division. Kinesin Eg5 allows for the mitotic spindle fibers to work correctly, and without it, cells cannot divide. Within Colon Cancer, Monastrol stops the dividing of more cells, as it self-destructs them by not being able to multiply itself. Monastrol is shown to cause DNA Damage, and blocks this major stop in the process of mitosis, which blocks any target cell, whether it is cancerous or not, from self-destructing (Guido et al., 2015). Cancer cells are not viable in monastrol, as they cannot stop the compound from removing the important Kinesin Eg5 motor protein, which is responsible for microtubule assembly.

During Monastrol's activation, an anti-apoptosis protein called Survivin is released which blocks apoptosis by blocking the captase activation activating as a competitive inhibitor (Jaiswal et al., 2015). By blocking the active site of the captase enzyme, apoptosis is unable to initiate and it induces mitotic arrest (Asraf et al., 2015). It specifically inhibits caspase-9-processing which induces apoptosis in healthy cells. By blocking this mitotic arrest, the non-cancerous cells are allowed to divide and replicate their DNA to grow into more healthy cells (Asraf et al., 2015). The elevation production of survivin induces mitotic arrest and increases cell viability within monastrol. This way, monastrol can just target the "unhealthy" cancerous cells and cause apoptosis within those, but let the healthy cells survive and reproduce to stop the spread of the cancer (Flaumenhaft, 2007).

Monastrol Effects on Cancer Cells

Monastrol can increase the viability of healthy cells in mitosis, but arrests cells going through uncontrollable cell division. With neuronal tumor cells, monastrol-treated cells were found to be more healthy overall as compared to taxol, which is the current anti-cancer drug (Marques et al., 2016). This is due to monastrol's ability to quickly detect half spindles, and inhibit Kinesin Eg5, which allows for the separation of these irregular spindles which later develop into cancerous cells. Eg5 usually causes extensive advancements of microtubules and tries to oppose axonal growth during the growth stage of a neuron but with monastrol, the microtubule advances stop so that optimal axonal growth is reached to function as an effective neuron (Marques et al., 2016). This reduces cancer, as the tumorous cells

have restricted axonal growth, and by attaching Eg5, the entire mitotic process will be unable to be completed, limiting the spread of the Cancer Cells.

Monastrol's activity occurs within Anaphase and Telophase, which are the separation steps as the newly duplicated cells are ready to be split up (Asraf et al., 2015). The irregularity of the spindle fiber's polarity causes this agent to be activated and it inhibits the Eg5 kinesis which is necessary to officially complete the cell cycle with the last step being cytokinesis (Haque et al., 2004). Without the necessary inhibitor, cell proliferation for the cancerous cells is halted. This repression also induces cancer cell differentiation in epithelial phenotypes, which is dangerous because they control important properties necessary for the advancement of tumors into large-scale cancer (Flaumenhaft, 2007). By halting the cell proliferation, the cells are unable to split and Monastrol signals for Apoptosis within the splitting cell destroying all of the genetic material inside of it, which is what made these cells cancerous in the first place. By destroying this "mutant" genetic material, it can no longer be spread, stopping the spread of the cancer itself (Guido et al., 2015).

Some of the cell lines being studied for the effects of monastrol *in vitro* include cells from AGS HepG2, LoVo39, Du145, and HT29 cell lines. These specific cell lines are examined in studies because they express both versions of the p53 tumor suppressor protein. p53 is wild-type in AGS, HepG2, and LoVo39 cell lines while it is mutated in the HT29 and Du145 cell lines. The p53 protein is attributed to the triggering of apoptosis due to the induction of the tetraploidy checkpoint (Asraf et al., 2015). When cells are subjected to kinesin-5 inhibitors, such as monastrol, cells can either maintain mitotic arrest, due to apoptosis or proceed to the G1 stage by mitotic slippage and undergo apoptosis caused by the tetraploidy checkpoint (Asraf et al., 2015). The latter scenario causes the cell to undergo mitotic slippage more readily in the presence of the inhibitor and thus be more sensitive to the effects of monastrol. A study conducted by Hila Asraf, et al. concluded that because the tetraploidy checkpoint is dependent on the p53 protein, the cells that undergo mitotic slippage and express the wild-type p53 protein are more sensitive to monastrol although the tendency to undergo mitotic slippage is not directly dependant on p53 status (Asraf et al., 2015). However, when the p53 protein is mutated, cells proceed with proliferation without properly dividing DNA, which can result in the accumulation of mutations that reduce the anti-cancer activity of kinesin-5 inhibitors *in vivo* (Asrafetal.,2015).

Monastrol as an anti-cancer treatment

Monastrol has been found to increase the binding rate of the S-enantiomer of enastron, an Eg5 inhibitor that leads to apoptosis (Kaan et al., 2010). It was also found that other chemicals such as enastron and Dimethylenastron presented increased spindle polarity inhibition compared to monastrol (Kaan et al., 2010). However, monastrol still performed a key part in guiding the binding of these chemicals to the allosteric site of the Eg5 motor protein (Kaan et al., 2010). In a pharmacological setting, it is clear that monastrol would need to function in conjunction with other drugs to be an effective cancer treatment.

The usage of monastrol on HCT-116 cancer cells resulted in a 30x reduction in HAC, a nonessential human artificial chromosome (Lee et al., 2016). HAC is a leading chromosome in cancer cells and the reduction of this chromosome led to decreased rate of duplication for cancer. However when compared to non-kinesin eg5 inhibitor compounds such as X-680, MLN8737, reversine, and ZM-447439 monastrol proved to be on average 3.3x less effective (Lee et al., 2016). This could be evidence that despite inhibiting the production of the HAC chromosome other drugs have the potential to be stronger cancer treatments. Monastrol is also an important inhibitor in the g2 DNA damage checkpoint. In this phase of the cell cycle, mitotic catastrophe leading to cell death/apoptosis was induced by ionizing radiation and the CHK1 inhibitor (Chen et al., 2012). However, the effect of this inhibitor was exacerbated after the EG5 protein was exposed to siRNAs and monastrol, leading to a 6x increase in cell apoptosis (Chen et al., 2012). These findings support the idea that monastrol is a promising cancer agent when used in conjunction with other compounds such as CHK1 as it proves to amplify their effects.

Through these collective studies monastrol has been researched extensively for its possible use in pharmacological settings and as a treatment agent for cancer. However, monastrol lacks the efficacy of different compounds such as Reversine and X-680 which may lead to the conclusion that monastrol is not an ideal cancer treatment compound. Monastrol has been shown to aid other compounds and act as an amplifier for EG5 inhibition. When paired with other inhibits such as CHK1 the increase in cell apoptosis is significant. While monastrol may not be viable for a standalone cancer treatment, it poses an important usage in the amplification of other drugs.

Clinical Trials and Side Effects

Clinical Trials

While there are no currently available clinical trials for monastrol specifically (Kohle et al., 2023), other HsEg5 inhibitors, such as ispinesib and quinazoline ring-containing compounds have entered clinical trials (Zhang et al., 2005). However, these inhibitors have shown little clinical success. A proposed reason for this result is an incomplete knowledge of the mitotic arrest pathways, which results in researchers not being able to identify the molecular components that should be targeted with Kinesin-5 inhibitors which hinders the efficiency of the drug (Asraf et al., 2015).

An observed result of clinical trials when testing anti-mitotic drugs such as Kinesin-5 inhibitors similar to that of monastrol is that cells either maintain a state of mitotic arrest and die by apoptosis or proceed to the G1 stage by mitotic slippage and then initiate apoptosis (Asraf et al., 2015). In the latter scenario, the tetraploidy checkpoint causes the cells that undergo mitotic slippage to undergo apoptosis rapidly. Cells that undergo mitotic slippage and express the wild-type p53 are therefore expected to be more sensitive to monastrol and other kinesin-5 inhibitors (Asraf et al., 2015).

Additionally, the expression of survivin, the anti-apoptotic chromosomal passenger protein, increases as a result of a monastrol treatment in monastrol-resistant cells, but not in monastrol-sensitive cells. A consistent overexpression of survivin in the monastrol-sensitive cells reduced mitotic slippage and increased monastrol resistance, and the partial silencing of survivin by siRNA reduces cell viability after short-term exposure to monastrol (Asraf et al., 2015).

Side Effects

Kinesin Eg5 is highly expressed in neurons, especially during developmental stages. Short-term exposure to monastrol was shown to increase the number and growth rate of axons (Haque et al., 2004). Sensory neurons also shared the same result as a short-term increase in axonal growth rates (Haque et al., 2004). However, long-term exposure to monastrol showed shorter axons as sensory neurons were more sensitive to the toxic effects of monastrol (Haque et al., 2004).

However, the overall health of cultures treated with monastrol was stronger than those treated with taxol (Haque et al., 2004). It can be concluded that Eg5 regulation causes neurons to coordinate rapid bursts of axonal growth. The modest toxic effects of neurons during long-term exposure cause hope for clinicians interested in Eg5-targeting drugs.

Additionally, microtubule toxins that affect normally dividing cells can cause myelosuppression, a condition where bone marrow activity is decreased resulting in a smaller red blood cell count (Gascoigne et al., 2008). While this effect is clinically manageable, a more concerning side effect of monastrol use is the peripheral neuropathies caused by inhibiting microtubule dynamics in nondividing cells (Gascoigne et al., 2008).

Other Eg5 Inhibitors

S-trityl-L-cysteine

S-trityl-L-cysteine (STLC) is a non-natural derivative of alpha-amino acid cysteine which incorporates a single chiral center and exists as two enantiomers. STLC inhibits the separation of duplicated centrosomes and the bipolar spindle formation in the M phase of the cell cycle (Skoufias et al., 2005). The kinesin Eg5 is responsible for the bipolar spindle formation and maintenance, which allows for chromosomal separation. Targeting the Eg5 kinesin displays the mitotic arrest phenotype, the monoastrol spindle with microtubules from a pair of nonseparated centrosomes, which results in centrosome separation inhibition. The effect of STLC is most potent in *in vitro* and cell-based assays, in which it targets the catalytic domain of Eg5 and inhibits Eg5 basal and microtubule-activated ATPase activity and mant-ADP release (Skoufias et al., 2005). STLC has been observed to have no visible effects on the microtubule network regardless of a high STLC concentration (Skoufias et al., 2005).

STLC is a tight binding inhibitor and binds tighter than monastrol with an eight-fold faster association rate and four-fold slower release rate (6.1 μM–1 s–1 and 3.6 s–1 for STLC versus 0.78 μM–1 s–1 and 15 s–1 for monastrol) (Skoufias et al., 2005). Both STLC and monastrol share the same binding region on Eg5, binding allosterically between helix 3 and loop 5 of the Eg5 domain (Wu et al., 2018). However, the specificity of the antitumor activity has not yet been made clear.

STLC inhibits Eg5-driven microtubule sliding velocity, which is reversible at an IC50 of 500 nm. However, after the release of STLC, HeLa cells exit mitosis, returning to the G1 phase of the cell cycle (Skoufias et al., 2005). The release of STLC allows chromosomes to regain bipolar attachment and experience equal tension at sister kinetochores, allowing the completion of mitosis.

Terpendole E

Terpendole E is the first discovered natural product inhibitor of the kinesin Eg5; however, it is produced transiently and is therefore difficult to isolate (Teranishi et al., 2015). Terpendole E arrests the cell division cycle at the M phase by inhibiting the Eg5 kinesin and does not affect microtubule integrity in interphase, which therefore makes Terpendole E a promising drug with few side effects (Teranishi et al., 2015). Terpendole E also inhibits chromosome segregation and results in monopolar spindles through the inhibition of Eg5 activity and does not inhibit conventional kinesins (Nakazawa et al., 2003).

It can be assumed that Terpendole E binds to Eg5 directly because it affects ATPase activity in the absence of microtubule activity. However, because the motility of motor proteins is coupled with hydrolysis, there is a chance that Terpendole E directly inhibits the motility of Eg5 (Nakazawa et al., 2003). Terpendole E functions as an M-phase inhibitor for fungal metabolites but does not affect tubulin polymerization *in vitro* . Most M-phase inhibitors directly interact with tubulin and alter microtubule polymerization, however, Terpendole E does not satisfy these conditions (Nakazawa et al., 2003). As a result, Terpendole E must inhibit another molecule in the M phase. Cells treated with Terpendole E exhibited a monoastrol microtubule array surrounded by a chromosome ring, resembling a mutant form of the Eg5 kinesin as observed after monastrol treatment. Terpendole E inhibits the segregation of centrosomes but not the duplication, and is assumed to bind to Eg5 directly, as Terpendole E affects ATPase activities of Eg5 if microtubules are absent (Nakazawa et al., 2003).

K858

K858 is a thiadiazole derivative that induces mitotic arrest, caspase 3 activation, and cell growth inhibition, inducing cancer cell death both *in vitro* and *in vivo* (Nakai et al., 2009). K858 does not affect microtubule polymerization in the

presence of GTP and demonstrates a selective inhibition of Eg5 by blocking centrosome separation and inducing the formation of the monopolar spindle with a round chromosome alignment during mitosis, a formation that is also achieved when cells are treated with monastrol (Nakai et al., 2009). K858 also inhibited Eg5 activity more effectively than monastrol (IC50 of 1.3 μmol/L for K858 vs 11 μmol/L for monastrol), and inhibits the ATPase activity of EG5 in an ATP non-competitive manner while failing to inhibit ATPase activity of mitotic kinesin CENP-E and MKLP1 (Nakai et al., 2009).

Conclusion

In conclusion, this research paper explored the potential of monastrol as an inhibitor of the kinesin spindle protein Eg5, which plays a crucial role in the proper assembly of the mitotic spindle. Mutations or errors in this process have been associated with brain diseases and cancer, highlighting the importance of targeting Eg5 as a potential therapeutic strategy.

Monastrol specifically inhibits the ATPase activity of the motor domain of Eg5, leading to the inhibition of Eg5 microtubule motility. This inhibition results in the development of a mono-astral spindle phenotype, disrupting proper spindle formation and leading to mitotic arrest in cancer cells. The mechanism of action of monastrol is still being investigated, with hypotheses suggesting substrate competition or allosteric inhibition.

While monastrol and other Eg5 inhibitors have shown promise, there are challenges to be addressed. Clinical trials with monastrol have revealed limited efficacy in certain patient groups, such as those with multiple myeloma. Furthermore, previous inhibitors targeting motor proteins have faced limitations in terms of cell specificity and permeability. However, ongoing research is focused on identifying novel inhibitor binding sites and exploring drug synergy with established antitumor agents to improve the efficacy of chemotherapy.

Overall, this review provides a comprehensive overview of monastrol as an Eg5 inhibitor, covering its mechanism of action, pharmacokinetics, effects on cell viability, and its potential as an anti-cancer treatment. Continued investigation and refinement of Eg5 inhibitors, including monastrol, hold promise for advancing cancer therapy and addressing the challenges associated with current treatment options.

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