International journal of biological sciences (ISSN: 2693-3608)
Volume 03, Issue 01, 2023
Published Date: -11-09-2023 Page No: 13-19

CADMIUM CHLORIDE'S ANTI-ANGIOGENIC EFFECTS: MECHANISTIC INSIGHTS INTO NEOVASCULARIZATION INHIBITION

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ABSTRACT

Cadmium chloride, a well-known toxic metal compound, has recently emerged as a potent agent with anti-angiogenic properties. This study delves into the mechanisms underlying the inhibitory impact of cadmium chloride on the neovascularization process. Employing in vitro and in vivo models, we investigate the intricate interactions between cadmium chloride and angiogenic pathways. Our findings reveal that cadmium chloride effectively hinders the formation of new blood vessels through multiple anti-angiogenic mechanisms, shedding light on its potential therapeutic applications in angiogenesis-related disorders.

KEYWORDS

Cadmium Chloride; Anti-angiogenic Effects; Neovascularization Inhibition; Mechanistic Insights; Angiogenesis; Toxic Metal Compound; Therapeutic Applications; Angiogenic Pathways

INTRODUCTION:

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, plays a pivotal role in various physiological processes, such as wound healing, embryonic development, and tissue repair.

However, dysregulated angiogenesis is also implicated in the pathogenesis of numerous pathological conditions, including cancer, diabetic retinopathy, and inflammatory diseases. Therefore, the inhibition of angiogenesis has emerged as a promising therapeutic strategy for combating these disorders.

Cadmium chloride, a well-known toxic metal compound, has recently garnered attention for its unanticipated potential as an anti-angiogenic agent. While cadmium is traditionally associated with detrimental health effects due to its toxicity, recent research has revealed its ability to effectively hinder the process of neovascularization. This unexpected discovery has sparked interest in unraveling the mechanistic insights behind cadmium chloride's anti-angiogenic effects and exploring its potential therapeutic applications.

This study aims to delve into the intricate interactions between cadmium chloride and angiogenic pathways, shedding light on the underlying mechanisms through which cadmium chloride exerts its inhibitory impact on neovascularization. Utilizing in vitro and in vivo models, we explore the multifaceted roles that cadmium chloride plays in impeding the formation of new blood vessels. By elucidating these mechanisms, we aspire to contribute to the growing body of knowledge surrounding anti-angiogenic agents and their potential applications in the treatment of angiogenesis-related disorders. This investigation holds promise not only for our understanding of cadmium chloride's effects but also for the broader field of angiogenesis research and therapeutics.

METHOD

The research conducted in this study aimed to elucidate the anti-angiogenic effects of cadmium chloride and uncover the mechanistic insights into its inhibition of neovascularization. To achieve this, a series of well-established methods were employed.

In the laboratory setting, human umbilical vein endothelial cells (HUVECs) were used as a model system to investigate the impact of cadmium chloride on angiogenesis. These cells were cultured and exposed to varying concentrations of cadmium chloride, and their responses were monitored over different time intervals. Key in vitro angiogenesis assays, such as the tube formation assay and cell proliferation assay, were performed to assess the effects of cadmium chloride on the ability of HUVECs to form new blood vessel-like structures and their proliferation, which are critical aspects of angiogenesis.

To complement the in vitro findings, an in vivo murine angiogenesis model was employed. Mice were subcutaneously injected with Matrigel plugs containing vascular endothelial growth factor (VEGF), with or without cadmium chloride. This model allowed for the examination of cadmium chloride's effects on angiogenesis in a more complex, physiologically relevant environment.

Immunohistochemical staining for CD31 was conducted on Matrigel plugs and tissue sections to evaluate microvessel density, providing insights into the impact of cadmium chloride on blood vessel formation in vivo. Western blot analysis and real-time polymerase chain reaction (PCR) were used to explore the expression levels of angiogenesis-related markers and genes in response to cadmium chloride treatment.

Statistical analysis of the data generated from these experiments was carried out to determine the significance of the findings. The collective results from the cell culture, in vitro assays, animal model, and molecular analyses contribute to our understanding of how cadmium chloride exerts its anti-angiogenic effects and provide mechanistic insights into the inhibition of neovascularization. This study's findings hold promise for both advancing our knowledge of anti-angiogenic agents and their potential therapeutic applications in angiogenesis-related disorders.

1. Cell Culture and Treatment:

Human umbilical vein endothelial cells (HUVECs) were cultured in EGM-2 medium (Lonza) and treated with varying concentrations of cadmium chloride ($CdCl_2$) for different time points. Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

2. In Vitro Angiogenesis Assays:

a. Tube Formation Assay: HUVECs were seeded on Matrigel-coated 96-well plates after $CdCl_2$ treatment. Tube formation was monitored, and the number of branch points and tube length were quantified.

b. Cell Proliferation Assay: The effect of $CdCl_2$ on HUVEC proliferation was assessed using the MTT assay.

3. Animal Model:

An in vivo murine angiogenesis model was employed. Male C57BL/6 mice were subcutaneously injected with Matrigel containing vascular endothelial growth factor (VEGF) with or without CdCl2. After two weeks, Matrigel plugs were harvested for analysis.

4. Immunohistochemistry:

Matrigel plugs and tissue sections were subjected to immunohistochemical staining for CD31 to assess microvessel density.

5. Western Blot Analysis:

Protein extracts from HUVECs and Matrigel plugs were subjected to Western blot analysis for angiogenesis-related markers, including VEGF, VEGFR2, and phosphorylated-VEGFR2.

6. Real-Time Polymerase Chain Reaction (PCR):

Total RNA was extracted from HUVECs, and the expression of angiogenesis-related genes, such as angiopoietin-1, angiopoietin-2, and angiostatin, was quantified by real-time PCR.

7. Statistical Analysis:

Data were analyzed using appropriate statistical tests, and results were presented as mean ± standard deviation. A p-value of less than 0.05 was considered statistically significant.

The methods outlined here were employed to investigate the anti-angiogenic effects of cadmium chloride and uncover the mechanistic insights underlying its inhibition of neovascularization in both in vitro and in vivo settings.

RESULTS:

The results of this study demonstrated that cadmium chloride ($CdCl_2$) exerts significant anti-angiogenic effects both in vitro and in vivo. In HUVEC cell culture, $CdCl_2$ treatment led to a substantial reduction in tube formation, as evidenced by a decreased number of branch points and tube length. Moreover, $CdCl_2$ effectively inhibited HUVEC proliferation, highlighting its ability to impede an essential step in angiogenesis.

In the in vivo murine angiogenesis model, the presence of $CdCl_2$ in Matrigel plugs significantly reduced microvessel density compared to the VEGF-only control group. Immunohistochemical staining for CD31 confirmed this reduction in blood vessel formation in the presence of $CdCl_2$. Further investigation revealed that $CdCl_2$ treatment downregulated key angiogenesis-related markers, such as VEGF, VEGFR2, and phosphorylated-VEGFR2, both in vitro and in Matrigel plugs.

Real-time PCR analysis also showed that $CdCl_2$ treatment led to altered expression levels of angiogenesis-related genes, with a significant decrease in angiopoietin-1 and an increase in angiostatin. These findings suggest that $CdCl_2$ affects angiogenic signaling pathways, potentially contributing to its anti-angiogenic effects.

DISCUSSION:

The anti-angiogenic effects of cadmium chloride observed in this study offer intriguing insights into its potential applications in angiogenesis-related disorders. The inhibition of tube formation and cell proliferation in HUVECs suggests that $CdCl_2$ disrupts critical steps in the process of neovascularization. This

may be attributed to its impact on key angiogenic signaling pathways, including the VEGF/VEGFR2 pathway, which plays a central role in angiogenesis regulation.

The downregulation of VEGF and VEGFR2, along with the reduced phosphorylation of VEGFR2, in response to $CdCl_2$ treatment further supports its anti-angiogenic potential. $CdCl_2$ appears to interfere with VEGF-mediated angiogenic signaling, which is crucial for endothelial cell activation and blood vessel formation.

The alteration of angiogenesis-related gene expression, such as the decrease in angiopoietin-1 and increase in angiostatin, highlights the broader impact of $CdCl_2$ on the angiogenic process. These changes may disrupt the intricate balance of pro- and anti-angiogenic factors, ultimately hindering blood vessel formation.

CONCLUSION:

In conclusion, this study has provided mechanistic insights into the anti-angiogenic effects of cadmium chloride. Through a combination of in vitro and in vivo experiments, we have demonstrated that $CdCl_2$ effectively inhibits neovascularization by interfering with key angiogenic pathways, such as the VEGF/VEGFR2 axis. The reduction in microvessel density and altered expression of angiogenesis-related genes further emphasize the potential therapeutic value of $CdCl_2$ in angiogenesis-related disorders.

These findings open doors for further exploration of $CdCl_2$ and similar compounds as potential anti-angiogenic agents, which could have applications in cancer therapy, retinopathy, and other diseases characterized by abnormal angiogenesis. The mechanistic understanding gained from this study not only contributes to our knowledge of anti-angiogenic mechanisms but also underscores the significance of further research in this field to harness the full therapeutic potential of cadmium chloride and related agents.

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