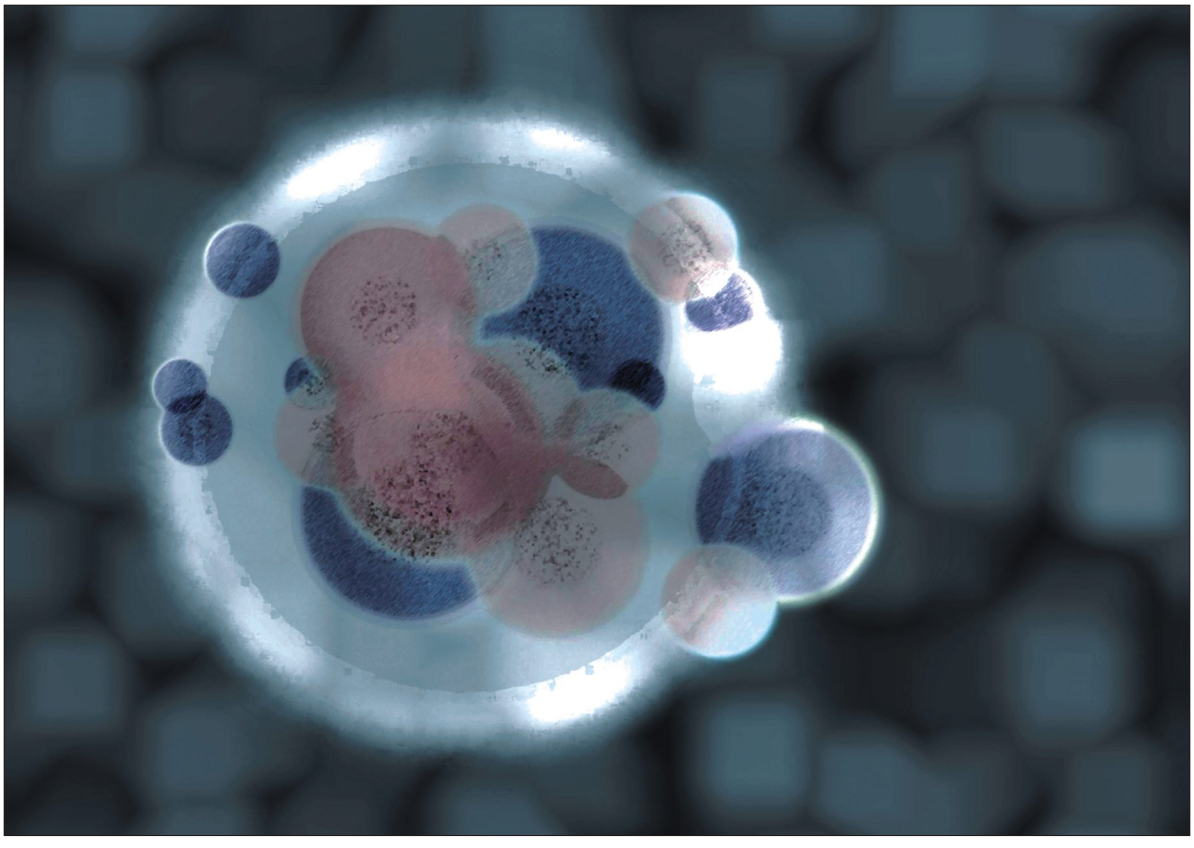

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Research Article
Anti-angiogenic Effects of Cadmium Chloride on the Process of Neovascularization

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Abstract

Background and Objective: Angiogenesis (neovascularization) is an important process of forming new blood vessels, essential for embryonic development, reproduction, wound repair and growth. Present study reports the plausible angiogenic modulatory effects of the cadmium chloride ($CdCl_2$). Cadmium (Cd) is a toxic substance, which have many inhibitory effects but also medicinally important. There are several toxic materials which in low quantity can be used in therapeutic aspects. Keeping in view, the effects of $CdCl_2$ salt were investigated on the process of angiogenesis in chicken chorioallantoic membrane (CAM) model by assessing gross and histopathological alterations. **Materials and Methods:** The two groups of the embryonated chicken eggs (10 in each group, 1 control and 1 treated group) were taken and 200 µL of 0.5 M of $CdCl_2$ was directly introduced on to the CAM. The resealed eggs were incubated for 72 h in a humid incubator chamber at 37±1°C. Then the eggs were opened to observe the gross and histopathological alterations for angiogenesis modulation. **Results:** Gross examination of CAM revealed the reduction in the number of secondary and tertiary blood vessels amongst $CdCl_2$ treated group. Histopathological analysis revealed anti-angiogenic effects of $CdCl_2$ due to the less number of blood vessels and presence of breached mesodermal blood vessels, out of which RBCs were oozing out. Occasional abnormal thickening and accumulation of densely arranged cells at chorionic and allantoic sides was also observed. **Conclusion:** The anti-angiogenic properties of $CdCl_2$ can be explored in the therapeutics of cancer and tumor related disorders. However, to avoid any kind of side effects, further qualitative, quantitative analysis and critical dose determination is required before going for clinical trials. Present investigations revealed the anti-angiogenic effect of cadmium chloride salt on chicken chorioallantoic membrane.

Key words: Angiogenesis, chorioallantoic membrane, $CdCl_2$ salt, anti-angiogenic effect, therapeutics

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Angiogenesis is a vital process of forming new blood vessels[1,2]. It is fundamentally required in various biological processes viz., development, reproduction and wound repair, where it is a highly regulated process[3-5]. Numerous inducers of angiogenesis have been identified, including the members of the Vascular Endothelial Growth Factor (VGEF) family, angiopoietins, Transforming Growth Factors (TGF), Platelet Derived Growth Factor (PDGF), Tumor Necrosis Factor (TNF)-α, interleukins and members of the Fibroblast Growth Factor (FGF) family[6]. The VEGF-A is the most potent pro-angiogenic protein described till date[7]. It induces proliferation, sprouting and tube formation of endothelial cells. Angiogenesis is therefore a putative target for therapy. The prospective applications of different angiogenic inhibitors are currently under intense clinical investigations. A better understanding of the biology of angiogenesis may reveal new targets for treating many diseases that are associated with this complex process[8].

Over the past few decades, cadmium (Cd) has been recognized as an industrial, environmental and health hazard[9]. The Cd can damage several body organs including the kidney, lung, liver, testis etc., depending on the dose, route and duration of exposure[10]. Among humans, Cd exposure normally results from the inhalation of airborne Cd either in the workplace, cigarette smoke or from the ingestion of Cd-contaminated food or water[11]. Amongst the many organs affected, the kidney is one of the most important sites for Cd toxicity with chronic low level exposure. In addition, sub-chronic Cd exposure in the drinking water caused the proliferation of vascular endothelial cells, suggesting its role affecting endothelium[12]. This is an important aspect because angiogenesis plays an important role in the homeostatic responses to toxin exposure and the derangements might be involved in a range of toxin-induced patho-physiological processes[13]. Hence, the present study aimed to explicate the angiogenesis modulatory effects of cadmium chloride ($CdCl_2$) salt on chorioallantoic membrane (CAM) model of chicken.

MATERIALS AND METHODS

Experimental materials: Cadmium salt [Cadmium chloride ($CdCl_2$)] was used in present study to find out its effects on the process of angiogenesis using the chicken chorioallantoic membranes (CAM) model[14,15].

Sample size: The embryonated chicken eggs (n = 20, 61±3 g of 9-11 days of age) were procured from State Poultry Farm, Raisen Road, Bhopal, Madhya Pradesh.

Experimental groups: The eggs were divided into two experiments groups (10 eggs in each), comprising of one control group (Group I) and one treatment groups (Group II, treated with $CdCl_2$ salt).

Procedure: The egg surfaces were cleaned and wiped with 70% ethanol. Two hundred microliters of 0.5 M of $CdCl_2$ was directly introduced onto the CAM of respective group of embryonated eggs using 26 gauge hypodermic syringe along with antibiotics (Ampicillin 50 μg mL^{-1}, streptomycin 10 μg mL^{-1}) and antimycotic solution (Amphotericin 10 μg mL^{-1}). Phosphate Buffered Saline (PBS) with antibiotic and antimycotic solution was given to the control group. The eggs were resealed with cellophane tape and incubated for 72 h in a humid incubator chamber at 37±1°C.

Observation methods: The eggs were then opened to observe the effects of $CdCl_2$ on vascularisation. The CAM was grossly examined, harvested and subjected to histopathological analysis using hematoxylin and eosin stain.

RESULTS AND DISCUSSION

The group I (control) and group II (treated with $CdCl_2$) resealed embryonated chicken eggs were unfasten the cellophane tape and examined following 72 h of incubation after treatment. Modulatory effects of $CdCl_2$ salt on the process of angiogenesis were observed by visualizing the gross and histopathological alterations in chorioallantoic membranes (CAM).

Gross examination of control group I revealed well arbourized vasculature with major and minor blood vessels (Fig. 1a) where the treated group II eggs showed reduction in number of secondary and tertiary blood vessel (Fig. 1b).

Histopathological analysis also corroborated the gross examinations of the CAM, which revealed normal thickness of CAM among controls (Group I) with concordant dispersed Mesodermal Blood Vessels (MBV) with loose connective tissue (Fig. 1c). However, in $CdCl_2$ salt treated CAM, mesodermal blood vessels were found to be breached out of which RBCs were oozing out. Also occasional abnormal thickening and accumulation of densely arranged cells at chorionic and allantoic sides was observed (Fig. 1d).

At present, chemotherapeutic drugs are being mostly used to treat cancer as well as other diseases. Unfortunately, many compounds showed limited efficacy with impaired delivery, penetration and specificity for the cancerous cells, hence causing deleterious side effects[16]. The activity of these compounds is mainly restricted by the drug resistance towards tumor cells. Pre-clinical studies and clinical trials

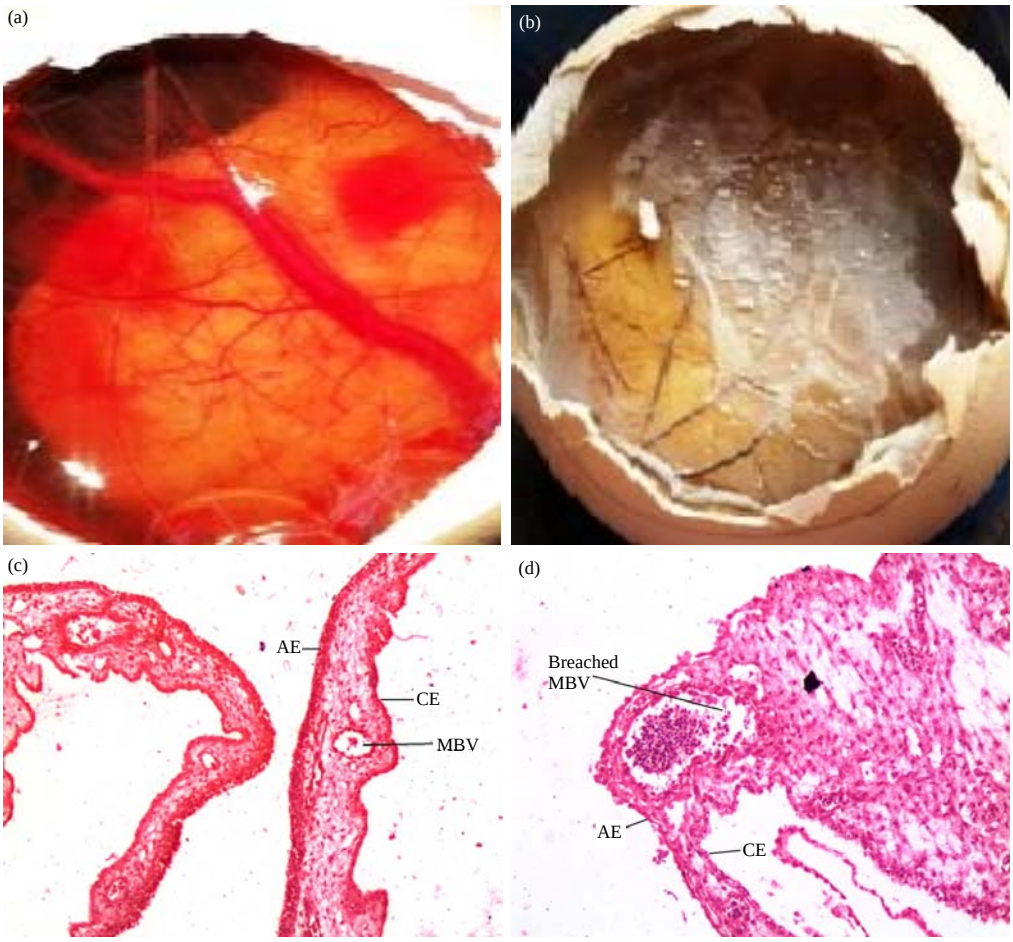

Fig. 1(a-d): (a) Gross examination of CAM of control eggs (Group I) with well arborized vascular system, (b) CAM of $CdCl_2$ treated eggs (Group II) showing primary blood vessel with less visible secondary and tertiary blood vessels, (c) Histopathology of CAM of controls (Group I, 100X) showing AE and CE of uniform thickness and (d) $CdCl_2$ treated (Group II, 400X) CAM showing abnormal thickening and accumulation of densely arranged cells at chorionic and allantoic sides with and occasional bleeding; AE: Allantoic epithelium, CE: Chorionic epithelium, MBV: Mesodermal blood vessels

suggest that angiogenesis-based therapy may be useful in the future care of patients[17]. In particular, anti-angiogenic therapy is a unique approach to destroy tumor cells, since it inhibits the growth of blood vessels[18], it does not target cancer cells directly. So far, anti-angiogenic agents are not likely to result in bone marrow suppression, gastrointestinal adverse symptoms or hair loss like other anti cancer therapies. This approach to slow the growth of blood vessels may require several months to a year, thus, the administration of the agents at lower doses and longer uninterrupted periods than the usual doses and periods of conventional cytotoxic agents should be considered in the design, several of these are under clinical trials[19,20]. The development of resistance to angiogenic inhibitors has not been a big problem so far, furthermore, a combination of anti-angiogenic therapy and conventional therapy may be more effective than either therapy alone[21,22], which may provide a novel, selective, safe and reasonable treatment in future medicine.

Cadmium (Cd) exposure brings changes in the blood vessel architecture and can cause pathologiocal conditions. It induces changes in morphological features and is through altering the mRNA and protein level expression. The results of present observations are in concordant with the findings of the Woods *et al.*[23] and Ma and Waxman[22], that it inhibits the tube formation. In this results too, it is demonstrated that number of secondary and tertiary blood vessels are reduced in number. With Cd, c-Jun N-terminal kinases (JNKs)[24] and VEGF[25] are activated, which can be related to the increased angiogenesis, actually it reduce angiogenesis by virtue of inhibition in tube formation, which indicate that Cd is involved

in anti-angiogenesis using diverse molecular pathways. Also cadmium (II) complex with selenosemicarbazone potentially inhibit NO production in human endothelial cell leading to anti-angiogenesis[25].

Hence, due to the great anti-angiogenic effect all are reduces the number of blood vessels also affected the endothelial layer, therefore it has great role in therapeutics of cancer and tumor related disease.

CONCLUSION

The $CdCl_2$ activate extracellular signal-regulated kinases (ERK) and AKT signaling with induction of hypoxia-inducible factor-1 (HIF-1), a key pro-angiogenic molecule. Also it induce vascular endothelial growth factor expression, however despite inducing angiogenic factors, reduction in endothelial tube formation and NO production, exhibits its complex impact on process of angiogenesis. However, its anti-angiogenic property shown in this study, as exhibited by reduced number of secondary and tertiary blood vessels with occasional breaching of blood vessels indicated its therapeutic value in cancer therapy but it require further dose optimization and safety testing. Present study may pave road for further study in direction of exploitation of antiangiogenic property of Cd.

SIGNIFICANT STATEMENT

The effects of $CdCl_2$ were found to be anti-angiogenic on CAM evident by reduction in the number of secondary and tertiary blood vessels and revealed detrimental effects by inducing inflammation. Therefore, it may have potential to be explored as a therapeutic material in anti-angiogenic and wound repair medicines.

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