

USING EX OVO CHICK CHORIOALLANTOIC MEMBRANE (CAM) ASSAY AS MODEL TO COMPARE PRO- AND ANTI-ANGIOGENIC PROPERTIES OF VITAMIN B12 AND CURCUMIN FOR INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

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HYPOTHESIS / AIMS OF STUDY

Interstitial Cystitis/ Bladder Pain Syndrome (IC/BPS) is a chronic pain syndrome of unknown cause that carries a substantial psychosocial burden. IC/BPS is characterized by constant pain and discomfort around the bladder region and some lower urinary tract symptoms such as frequent voiding and pain during urination.

Abnormal angiogenesis, characterized by increased and disorganized vessel formation, plays a critical role in the pathogenesis of IC/BPS. Curcumin is known for its anti-inflammatory and anti-angiogenic properties, and vitamin-B12, implicated in endothelial function modulation, may significantly affect angiogenic processes (1).

The chicken chorioallantoic membrane (CAM) assay has traditionally been used to test pro- and antiangiogenic response to drugs, to study many aspects of tumor angiogenesis and test biomaterials (2).

This preliminary study aimed to assess the modulatory effects of commercially available vitamin-B12 and curcumin on angiogenesis using the ex-ovo CAM assay, evaluating their potential therapeutic relevance in relation to angiogenesis in IC/ PBS.

STUDY DESIGN, MATERIALS AND METHODS

All CAM procedures conformed to institutional and national guidelines and performed according to Hamburger and Hamilton classifications (3). The CAM assays were performed in three groups: Gr1:control, Gr2:vitamin B12 application (1000 µg), and Gr3:curcumin application (1000µg), where each group (n=20) were conducted twice. Fertilized chicken eggs were cleaned with 70% ethyl alcohol, checked for embryo viability under beam light, and incubated for 3 days at 37°C in an air-ventilated incubator. On embryonal day 4 (ED4), the eggs were cracked using the ex-ovo cultivation method as previously described and incubated further until ED7. The CAMs were checked daily for the presence of a heartbeat and angiogenesis.

On ED7, commercially available, sprayable vitamin-B12 supplement (without vitamin-B-complexes) was pipetted onto sterile filter paper discs. Similarly, a commercially available micelle-based curcumin supplement, (without additives: pepper), was pipetted onto sterile filter paper discs. Each disc was then immediately placed between a vascular Y-branch.

Treatments were repeated daily by adding new filter papers on the CAM, and angiogenic changes were monitored until no heartbeat was visually observed (2). The percent vascularization effect of the drugs were assessed using Python analysis software.

RESULTS

The control CAMs showed vascularization throughout the experiments and generally survived up to ED10-14, with an average mean %vascularization area of 51,51 + -0,51 at ED8.

Vitamin-B12-treated CAMs displayed enhanced vessel growth and statistically increased %vascularization area (mean:53,35 + -0,34) and thickness on ED8 in comparison to control and curcumin counterparts (p < 0,05), and some of the embryos survived until ED14.

CAMs treated with curcumin showed a visually visible reduction of %vascularization area (mean: 47,94 + -0,32) and vascular density by ED8, and were not viable on ED9 (Fig.1, Fig.2). The CAMs, thus received curcumin only on ED7 and ED8, and did not survive for a third application on ED9. Curcumin had a statistically decreased %vascularization area on ED8 in comparison to control and B12 counterparts (p < 0,05).

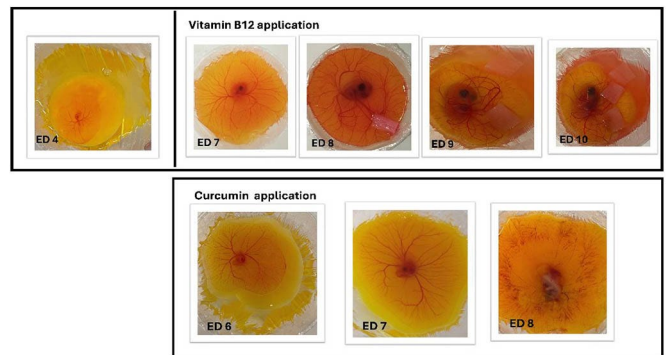
INTERPRETATION OF RESULTS

This preliminary study specifically aimed to evaluate over the counter commercially available pure forms of vitamin-B12 and curcumin supplements. This study highlights its therapeutic potential for angiogenesis-driven disorders like IC/BPS. The pro-angiogenic response of B12 may facilitate endothelial regeneration in certain contexts; however, it must be administered cautiously in inflammatory conditions due to its potential to exacerbate pathological angiogenesis. Curcumin consistently showed an anti-angiogenic effect.

CONCLUDING MESSAGE

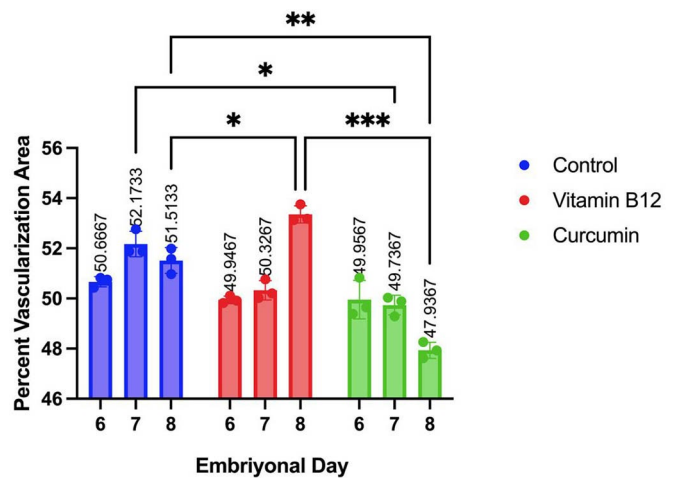
Despite the presence of sophisticated nanoparticle covered curcumin trial for IC/BPS, the CAM assay presented herein is the first to investigate and compare over the counter supplement usage for angiogenesis, which is one of the pathophysiologies for IC/BPS. Curcumin may serve as a promising anti-angiogenic candidate for future IC/BPS research. Conversely, the angiogenesis-promoting effect of vitamin-B12 suggests a need for careful dosage consideration in IC/BPS patients. These preliminary findings offer insights into the vascular modulation potential of these compounds in IC/PBS.

FIGURE 1



The effects of Vitamin B12 and curcumin on angiogenic growth of the CAM, observed daily during embryonic development. Following the second dose, curcumin decreased and expedited the decomposition of angiogenesis, while B12 promoted angiogenic growth.

FIGURE 2



REFERENCES

1. Inal-Gültekin G, Janssen D, Mangir N, Cruz F, Charrua A. Current clinical status of IC/BPS and what the future holds in basic & translational science. *Continence*, 2024, 11: 101338.
2. Mangir N, Dikici S, Claeysens F, MacNeil S. Using ex Ovo Chick Chorioallantoic Membrane (CAM) Assay To Evaluate the Biocompatibility and Angiogenic Response to Biomaterials. *ACS Biomater Sci Eng*. 2019;5(7):3190-3200.
3. Schomann T, Qunneis F, Widera D, Kaltschmidt C, Kaltschmidt B. Improved method for ex ovo-cultivation of developing chicken embryos for human stem cell xenografts. *Stem Cells Int*. 2013;2013:960958.

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