Interaction of curcumin with erythrocytes: A microscopic study

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ABSTRACT

Studies report the interaction of curcumin nano-formulations with proteins and erythrocytes. However, no studies are reported for the microscopic interaction of free curcumin on erythrocytes. The objective of this study was to examine the interaction of curcumin with human red blood cells. The interaction of free curcumin with human red blood ghost cells was observed under light microscope, scanning electron microscope and fluorescence microscope. The binding of free curcumin on to the red blood ghost cells was confirmed by the presence of yellow colour vesicles when observed under light microscope. Scanning electron microscopic studies clearly revealed the binding of cuboidal shaped crystalline structures on the membrane surface of red blood cells. Free curcumin in aqueous environment is non-fluorescent. However, when curcumin is surrounded or interacting in hydrophobic medium fluoresces. Curcumin on binding with membrane of red blood cells becomes fluorescent at excitation wavelength of 430 nm and emission wavelength at 510 nm. Free curcumin appear to be interacting with red blood cell membrane *via* hydrophobic interaction, which suggests their use using an appropriate vehicle. These findings advance our understanding of the characteristic of curcumin, which could be implanted when synthesising the curcumin nano-formulations for therapeutic use.

Keywords: Curcumin; fluorescence; scanning electron microscope; nano-formulations; erythrocytes.

INTRODUCTION

Curcumin (diferuloly methane, Fig. 1) is a natural polyphenol isolated from the roots of turmeric. Turmeric is a widely used household traditional medicine in India and China. The medicinal property of turmeric is attributed to the presence of curcumin. Having neither side effects nor toxic effects curcumin is under several clinical trials for treatment of cancer, Alzheimer's disease, Crohn's disease, psoriasis and many skin diseases (1). Studies have demonstrated that curcumin is an efficient molecule for overcoming multidrug resistance and for inducing sensitization of tumour cells for chemotherapy and radiation (2-4). However, with all these potential as a powerful therapeutic agent free curcumin is short lived and unavailable in aqueous media. Efforts are being made to increase the bioavailability and stability of curcumin by preparing nano-formulations with various polymers like cellulose, β-cyclodextrin, dendrimers, polylactic-co-glycolic acid and protein based carrier systems using β-lactoglobulin, casein, albumin, sunflower protein (5-8). Many interaction-binding studies and their kinetics are reported using proteins and lipid carrier system. While a number of cell based carrier systems, using nano-formulations of curcumin are reported there are no reports to evaluate the direct binding of curcumin. Therefore, the aim of this study was to examine the microscopic studies on the binding of free curcumin with human red blood cells, which further provide evidence and advances our understanding towards the interaction of curcumin on cells while formulating the cell based carrier systems.

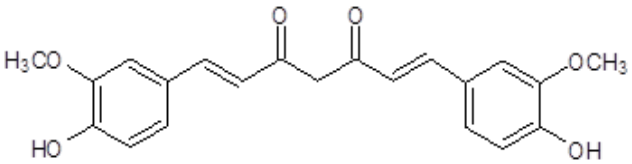

Fig. 1. Structure of curcumin

MATERIALS AND METHODS

Curcumin was procured from Sigma Aldrich, USA. All reagents were of analytical grade.

Preparation of RBC sealed ghosts

The protocol adopted for preparation of RBC sealed ghosts is as per Steck and Kant (9). 10 mL of venous blood were drawn from a volunteer and collected in tube containing sodium heparin as anticoagulant. The tube was centrifuged at 2,500 rpm for 8 minutes at room temperature. The clear supernatant and the buffy fraction were carefully removed leaving only the pelleted and packed RBC. The RBC pellet was washed twice with isotonic phosphate buffered saline (150 mM NaCl, 5 mM sodium phosphate, pH 8.0). Haemolysis of washed RBC pellet is initiated by rapidly mixing 1 mL of packed RBC with 40

volumes of 5 mM sodium phosphate, pH 8.0. The ghost cells were pelleted by centrifugation at 20,000 rpm for 30 minutes. The membranes are resuspended in 40 volumes of PBS and incubated 40 minutes at 37°C to induce resealing. The ghosts are then pelleted and washed twice in PBS.

Complex formation of curcumin with RBC sealed ghosts

To prepare complexes between sealed erythrocyte ghost cells and curcumin, 1 mL cells (5 x 10^5 erythrocytes) were incubated for 30 min at room temperature with curcumin (10 mM). The cells were collected by centrifugation (20,000 rpm for 30 min) and then washed three times by centrifugation at 8000 rpm for 10 min to remove unbound free curcumin. To collect the cells the samples were again subjected to centrifugation. The sedimented cells were re-suspended in PBS and were subjected to microscopic visualization.

Microscopic visualization

The prepared samples were observed under the light microscope using 100x objective lens (Olympus).

Scanning electron microscopy

The sealed ghost cells were observed using scanning electron microscopy (S3400 EVO 60). The samples were sputtered with gold and analysed with a 20.0 kV operating voltage and scanned at different magnification.

Fluorescence microscopy

The sealed ghost cells were viewed under a fluorescent microscope (Carl Zeiss, Germany, AXIO Imager A2) at the wavelength range of 425 to 525 nm.

RESULTS AND DISCUSSION

Previous studies have shown the interaction and binding studies of curcumin complexed with various proteins, liposomes and polymers (6-9, 10, 11). In this report as an initial study, attempts are made to observe the interaction of curcumin with the erythrocyte by microscopy.

Figure 2 shows the light microscopic view of curcumin bound on to the sealed erythrocyte cells. The view clearly shows the binding of erythrocyte membrane with curcumin, which is yellow in colour. To further explore the mode of binding of curcumin on erythrocyte ghost cells, scanning electron microscopic studies were done. Figure 3a shows the scanning electron microscopic view of the curcumin on sealed erythrocyte ghost cells at 100x magnification. The curcumin are appearing as cuboidal crystalline structures and the sealed erythrocyte cells are ovoidal in shape. Along with free curcumin molecules, careful observation of the image reveals that a few curcumin molecules are bound on the surface of the membrane and from the image; it appears that the curcumin molecules are surface bound. Figure 3b shows only the image of erythrocyte ghost cells without the addition of curcumin. Figure 3c shows that the curcumin molecules are bound on surface of erythrocyte membrane at 300x magnification. It is clearly observed that curcumin is embedded on the surface of erythrocyte membrane.

Further, to confirm the binding of curcumin on the erythrocyte sealed cells fluorescent microscopy was done. Curcumin when bound to hydrophobic material emits fluorescence (12). Curcumin shows strong absorption spectra with the maxima at 430 nm and emits fluorescence at 510 nm. Figure 4 shows the fluorescent microscopic images confirming the binding of curcumin on sealed erythrocytes.

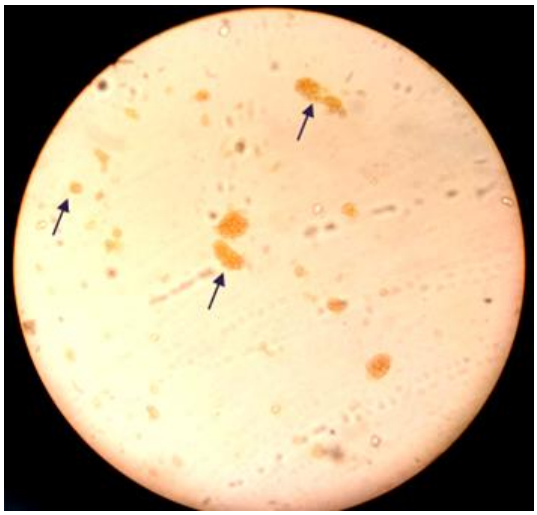

Fig. 2: Light microscopic image of curcumin bound to sealed erythrocyte cells

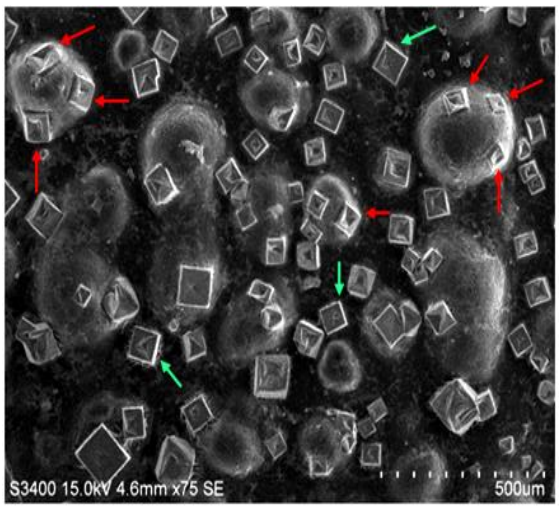

Fig. 3a: Scanning electron microscopic image of curcumin bound with sealed erythrocyte cells (red arrow) and free unbound curcumin (green arrow)

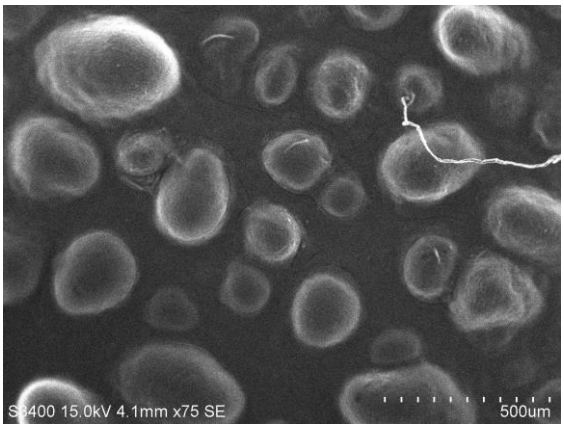

Fig. 3b: Scanning electron microscopic image of sealed erythrocyte cells without added curcumin

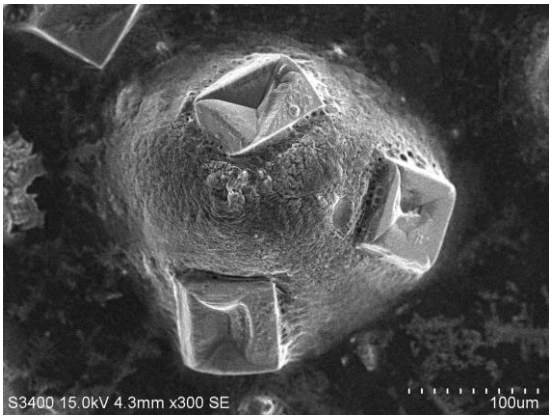

Fig. 3c: Scanning electron microscopic image of curcumin bound with sealed erythrocyte cells at 300x magnification

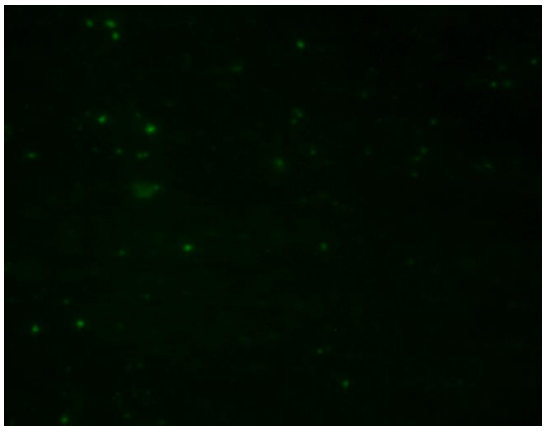

Fig. 4: Fluorescent microscopic image of curcumin fluorescence when bound with sealed erythrocyte

CONCLUSION

The present communication offers a new feasible way to study and confirm the binding of curcumin on the cells. However, further studies using different cells and with other self-luminescent, compounds are required for its wide approval.

CONFLICT OF INTEREST: None.

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