NMR based pharmacometabolomics for evaluating the drug response of polyherbal formulations

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Introduction

Cancer is a leading cause of death worldwide and poses a huge health challenge. Many of the chemotherapy drugs used in cancer treatment are derived from natural products. Although single molecule drugs continue to be the focus of drug development, polyherbal formulations are evoking interest since these are considered to have synergistic activity, low toxicity and multitargeting potential[1]. NMR metabolomics can play an important role in evaluating the therapeutic efficacy of such formulations without the need to fractionate them. The present study has evaluated the response of chick Chorio-Allantoic Membrane (CAM) to polyherbal formulations using high resolution NMR and has also assessed their antiangiogenic potential using CAM assay.

Materials and Methods

Evaluation of antiangiogenic potential: Two polyherbal formulations (labeled VK and GTK) in aqueous form were purchased from a certified pharmaceutical company. Antiangiogenic potential of the formulations was evaluated and compared with that of thalidomide (positive control) using CAM assay. Fertile White Leghorn Chicken eggs (n = 180) were divided into 3 groups of 60 eggs each, for studying three concentrations (50, 75 and 100 µg/ml) of the formulations. For each dose, the 60 eggs were divided into 4 groups of 15 eggs each. Group 1 was administered Vascular Endothelial Growth Factor (VEGF) (10 ng per coverslip); Group 2 served as the positive control (synthetic antiangiogenic drug thalidomide - 10 µg/ml); Groups 3 and 4 were administered VK and GTK, respectively. Data was acquired in six replicates for each concentration. The following protocol was followed: Day 1- incubation of eggs; Day 3 - aspiration of albumen; Day 8 - treatment; Day 13 - morphometric analysis of area of vascularization using digital imaging system. Angioquant toolbox in MATLAB programming platform (Mathworks Inc., USA) was used for further analysis. The data is presented as HeatMap using R statistical programming language v.2.15.1.

NMR studies: The treated chick chorio-allantoic membranes were removed from the chick embryos and extracted using perchloric acid. Samples were lyophilized and redissolved in 600 µl of 100 mM phosphate buffer (pH 7.0) prepared in 90% H_2O-10% deuterated trimethylsilyl propionate (TSP). Six replicates were prepared per treatment. Water suppressed 1D proton spectra of the metabolic extracts were acquired on a 700 MHz NMR spectrometer (Agilent, USA) with the following parameters: spectral width - 12 ppm, relaxation delay - 4s, no. of scans - 64, data points - 32K. Peaks were assigned using 2D NMR and BML-NMR library.

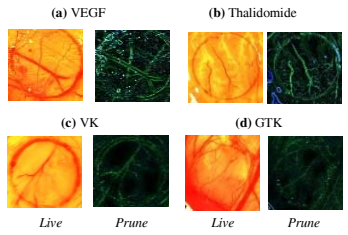

Fig. 1. *Live (yellow-red) and prune (green for capillary visualisation) images of vascularization on 13th day after treatment with* ***(a)*** *VEGF* ***(b)*** *thalidomide and 100 µg/ml of formulations* ***(c)*** *VK* ***(d)*** *GTK.*

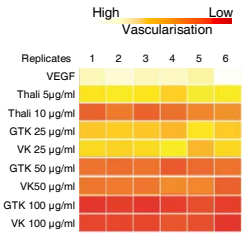

Fig.2. *Comparative analysis of area of vascularization shown as HeatMap for six replicates per sample.*

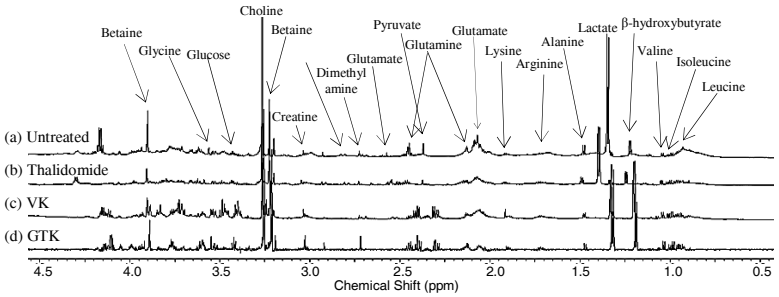

Fig. 3: *Proton NMR spectra of chick chorio-allantoic membrane extracts:* ***(a)*** *untreated and treated with* ***(b)*** *thalidomide and polyherbal formulations* ***(c)*** *VK and* ***(d)*** *GTK*

Results

CAM assay: Of the 3 doses (50, 75 and 100 µg/ml), maximum antiangiogenic activity was observed at 100 µg/ml. The data is therefore presented for 100 µg/ml only. The area of vascularization measured from the morphometric images (Fig. 1) showed significant treatment induced reduction. Area of vascularisation / % inhibition : GTK (33.2 ± 4.3 mm^2 / 63%), VK (36.6 ± 3.5 mm^2 / 60%), and thalidomide (36.4 ± 3.6 mm^2 / 60%). Both the formulations showed significant antiangiogenic activity comparable to that of thalidomide. Figure 2 shows the area of vascularization after treatment as HeatMap for all the 6 replicates in each group. These results show that the polyherbal formulations have the potential to block signaling pathways involved in angiogenesis.

NMR metabolic profiling: Figures 3a-3d show respectively, proton spectra (0.4 - 4.6 ppm) of untreated CAM extracts, and those after treatment with thalidomide and the two formulations. In addition to the peaks shown in Fig 3a, Adinosine phosphates (ATP/ADP), Uridine phosphates, Nicotinamide adinine phosphates (NADP/NAD), tyrosine and formate were seen in the region 4.6-9 ppm. Although treatment induced changes were observed in peaks such as alanine, creatine, ATP/ADP and NADP/NAD, significant reduction was seen in choline, pyruvate, and lactate after treatment with thalidomide (Fig. 3b), VK (Fig. 3c), and GTK (Fig. 3d). The reduction in choline compared to that from untreated CAM is: thalidomide - 50%, VK - 60% and GTK - 40%. Treatment induced decrease in lactate (thalidomide- 90%, GTK - 40% and VK - 30%) and pyruvate (thalidomide - 60%, GTK - 80% and VK - 90%) suggest reduction of anaerobic glycolysis in CAM and reflects its positive response to treatment.

Conclusion

This study shows that inhibition of neovascularization by the two formulations comparable to that of the synthetic drug thalidomide could be seen both by NMR drug response and the bioassay. It is pointed out that in addition to their antiangiogenic potential, these formulations also have apoptotic activity in human hepatocellular carcinoma (Hep-G2) cells[2] proving that the polyherbal formulations are multitargeting. NMR based pharmacometabolomics offers a quick and convenient method to evaluate the drug response of polyherbal formulations without the need to fractionate them.

References

1. Lee KW, Bode AM and Dong Z. Molecular targets of phytochemicals for cancer prevention. *Nat Rev Cancer*. 11, 211-218, 2011.
2. Sharma G, Jayasundar R, Velpandian T et al. NMR metabolomics of drug response to antineoplastic polyherbal formulations studied in human hepatocellular carcinoma cell lines. *Proc Intl Soc Mag Reson Med*. 21, 3430, 2013.