COMPARATIVE ANALYSES OF THE P450-DERIVED HPLC-METABOLOMES OF VERNONIA AMYGDALINA, PACLITAXEL, DOXORUBICIN AND VINCristINE

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Abstract: Vernonia amygdalina (VA) extract has potential use as an effective agent in the treatment of breast cancer without toxic side effects, whereas numerous undesirable side effects accompany use of Paclitaxel (TAXOL), Doxorubicin (DOX), and Vincristine (VIN), three commonly used anti-breast cancer agents. Our laboratory team previously observed VA 1) is anti-proliferative in MCF-7 cells, 2) influences cytochrome P450 expression and 3) VA synergizes with TAX, DOX, and VIN to inhibit MCF-7 breast cancerous cell growth. In this study, we performed in vitro HPLC-metabolomic analyses of these important cancer-killing agents. Specifically, we utilized P450-containing microsomes to generate metabolites from the anti-cancer agents. These metabolites were extracted and then fractionated on HPLC. Based on the comparison of the HPLC profiles of the control reactions with those of the drug-containing assays, an assessment was made based on disappearance of the parent peak and appearance of a novel peak. This simple metabolomic analysis yielded information suggesting which cytochrome P450 is involved in metabolism of each agent. Results indicate VA, TAXOL, and VIN undergo extensive P450 3A4 dependent metabolism. In this system, we demonstrated human liver microsomes convert each drug into the appropriate hydroxylated metabolites which are resolvable via HPLC. To confirm, we utilized P450 3A4-specific inhibitory monoclonal antibodies (Mabs) to demonstrate loss of metabolite formation. Similar experiments were conducted with P450 2E1, each drug, HPLC and inhibitory P450 2E1 MAbs, and were confirmed by Western blotting suggesting P450 2E1 is involved in metabolism of VA and TAXOL, not DOX or VIN. In addition to human liver microsomes, microsomes expressing only P450 2E1 were used to interrogate the metabolomic products of the extract, as compared to TAXOL. Thus, allowing a more definitive assessment of P450 2E1’s involvement and providing useful information about a very important anti-cancer agent, Vernonia amygdalina.

Keywords: Breast cancer, Paclitaxel, Doxorubicin, Vincristine, Vernonia amygdalina, CYP3A4, MCF-7 cells, HPLC.

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