ETHYL ACETATE FRACTION OF ANDROGRAPHIS PANICULATA NEES INCREASES CYTOTOXIC EFFECT OF 5-FLUOROURACIL ON HUMAN CANCER CELL LINES

SUKARDIMAN*, HERRA STUDIAWAN, ABDUL RAHMAN, MULJA HADI SANTOSA AND FIRSA ARDI PRATAMA
Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya Indonesia.
Email: maman_ht@yahoo.com

ABSTRACT

Objective: The objective of the present investigation was to examine whether ethyl acetate fraction from Andrographis paniculata Nees (EAA) synergizes the therapeutic potential of 5-fluorouracil against different human cancer cell lines (HeLa, Widr and T47D).

Methods: MTT assay was used to measure the growth inhibitory effect of the combination. Synergistic efficacy was subjected to median effect analysis with nonexclusive model as previously described by Chou and Talalay [1].

Results: IC₅₀ of EAA were 18.486 mg/ml in HeLa cells line, 13.467 mg/ml in Widr cells line and 21.618 mg/ml in T47D cells line. EAA and 5-FU combined as a cocktail, synergistically inhibited the growth of cancer cells in vitro, with Combination Index value (CI) ranging from 0.20 to 0.07 in HeLa cells line, 0.97 to 0.35 in Widr cells line and 0.004 to 0.001 in T47D cells lines.

Conclusion: EAA and 5-FU, combined as a cocktail, showed strong synergism in inhibiting the growth of human breast cancer cells (T47D) in vitro.

Keywords: Andrographis paniculata Nees, 5-FU, Ethyl Acetate Fraction, Human Cancer Cell Lines

INTRODUCTION

Cancer is a multifactorial disease that requires a multi-targeted therapeutic approach [2,3]. Chemotherapy has undergone a gradual transition from mono-therapy to multidrug therapy, and drug cocktails strategy has become widely adopted. Properly formulated drug combinations are believed to enhance synergism and the interactions of chemical components within the combination may improve therapeutic efficacy over single drugs [4] and in many cases plant extracts are thought to be therapeutically superior to their single isolated constituents [5,6]. Therefore, herbal medicines are increasingly combined with chemical medicines in anticancer drug cocktails, especially in countries where herbal medicines are well accepted [7,8]. Some studies have suggested that for cancer treatment, drug cocktails combining herbal and chemical medicines may exhibit enhanced efficacies with diminished side effects and complications [9,10].

Andrographis paniculata Nees (Acanthaceae) is a traditional medicinal herb, grown as shrub in the moist soil, shady areas of India, China, Indonesia and throughout Southeast Asia. It has been used as immunostimulant [11], for myocardial ischemic [12], pharyngotonsillitis [13], respiratory tract infections [14] and common cold [15]. It also possesses antimicrobial effect [16], anti-inflammatory [17], hypotensive effect [18], antihyperglycemic [19,20], oxygen radical scavenging [17], atherosclerotic [21], antimalarial activity [22], anti-HIV [23], antiplatelet aggregation [24], hepatic lipid peroxidation protective [25], hepatoprotective [26], choleretic effect [27], and anticancer effects [28,29,30,31]. One of the major constituents of A. paniculata Nees is diterpene lactone andrographolide which has anticancer activity in vitro in many tumor cell lines including leukemia, myeloma, HeLa, colon (HT-29), human peripheral blood lymphocytes (HPBLs), and human breast cancer MCF-7 [32]. It was found that andrographolide possessed inhibitory effect of DNA Topoisomerase II [33]. Satyanarayana et al. [32] reported that andrographolide inhibition of cell cycle from human breast cancer cell MCF-7 by induction of cell-cycle inhibitory protein p27 also decreased expression of cyclin-dependent kinase. Andrographolide isolated from Andrographis paniculata Nees induced apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increase expression of p53 bax, caspase-3 and decrease expression of bcl-2 [34]. 5-Fluorouracil (5-FU) is one of the most commonly used drugs for treatment of breast, digestive tract, and other cancers [35,36]. It is often used clinically in combination with other agents such as paclitaxel, docetaxel, and cisplatin [37,38]. A few studies have shown synergistic effects of combinations of 5-FU with herbal medicines or components thereof. For example, oroxylin A, a bioactive Scutellaria baicalensis Georgi flavonoid, has a synergistic effect with 5-FU on HepG2 human hepatocellular carcinoma and on H22 transplanted [39]. Chan-Yu-Bao-Yuan-Tang, a herbal medicine formula, induced apoptosis synergistically with 5-FU in lung and cervical cancer cells [40]. Though herbal medicines and 5-FU are both commonly used in clinical practice, there have been far fewer studies combining 5-FU and herbal medicines than on 5-FU or herbal medicines alone.

The aim of this paper is to evaluate the efficacy of the ethyl acetate fraction of Andrographis paniculata Nees (EAA) as a source of useful anticancer agents and the co-efficacy at the cellular level of a cocktail combining EAA and 5-FU.

MATERIALS AND METHODS

Plant Material
Andrographis paniculata Nees herb were obtained from Mojokerto, East Java area, which was then determined by the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya Indonesia.

Preparation of Ethyl Acetic Fraction (EFA)
The extract was prepared by macerating dried aerial part powder in 95% ethanol. The macerate was then concentrated under vacuum rotary evaporator. The concentrated fraction was then prepared in DMSO (Sigma) for treatment. The final DMSO concentration was set not higher than 0.1%.

Cell Lines
Widr, T47D and HeLa cells were cultured in RPMI Medium containing Fetal Bovine Serum (FBS) 10% (v/v) (FBS qualified, Gibco, Invitrogen TM USA) and penicillin-streptomycin 1 % (v/v) (Gibco, Invitrogen Corporation, Grand Island, NY, 14072, USA). These cell lines were kindly provided by the Department of...
Drugs

5-Flourourasill from Ebeew (vial 10 mg/5 ml) purchased from P.T. Ferron Par Pharmaceutical (Cikarang, Indonesia) was diluted directly in culture medium.

Cytotoxic Assay-MTT Method

HeLa, Widr and T47D cells (5x10^3cells/well) were transferred into 96-well plate and incubated for 24 hours (70-80% confluent). Cells were treated by EAA, 5-FU, and their combination, then incubated for 24 hour. At the end of the treatment incubation, MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] 0.5 mg/ml were added to each well followed by 4 hours incubation in 37°C chamber. Viable cells react with MTT to form purple formazan crystals. After 4 hours, stopper sodium dodesil sulphate (SDS) 10% in 0.1 N HCl solution were added to dissolve the formazan crystals. Following overnight incubation (with protection from light exposure), the cells were shaken for 10 minutes before being read by ELISA reader at λ 595 nm. The obtained absorbance of each well converted to percentage of viable cells:

\[
\text{% viable cells} = \frac{\text{Treated cells abs} - \text{Medium control abs}}{\text{Cell control abs} - \text{Medium control abs}} \times 100\%
\]

Combination index (CI) for determining synergism additivity or antagonism

The combined effects of EAA and 5-FU were subjected to median effect analysis with the mutually nonexclusive model as previously described [1]. The combination index (CI) for determining synergism and antagonism between the substances was calculated using SPSS 17.0; SPSS Inc.). CI < 1, CI = 1, and CI > 1 indicate synergism, additivity, and antagonism respectively. The results by ATP assay were analyzed for CI determination.

RESULTS AND DISCUSSION

Component identification of EAA Fraction by TLC fingerprint

Chromatographic fingerprinting is a powerful technology for authentication of natural products. The application of chromatographic fingerprinting in component identification of natural products continues to expand. TLC – Densitometry fingerprint printing of EAA fraction for quality control is shown in Fig. 1. The 3 main compounds of EAA fraction found in this study, with major compound is andrographolide.

Cytotoxic Assay

To explore the effects of EAA fraction on human cancer cell lines and normal cells in vitro, the cytotoxicity of EAA fraction at 10 – 100 µg/ml for 24 h was assessed by MTT assays in a panel of human cancer cell lines namely HeLa, Widr and T47D. Fig. 2 shows that growth was strongly inhibited in all cancer cells with IC_{50} of EAA are 18.486 µg/ml in HeLa cells, 13.467 µg/ml in Widr cells and 21.618 µg/ml in T47D cells. Therefore, EAA showed strong and broad-spectrum anticancer activity. But IC_{50} of 5-FU in different human cancer cells lines are 71.50 µg/ml in HeLa cells, 38.05 µg/ml in Widr cells and 29.7 µg/ml in T47D cells. A previous study reported chemotherapy drug induced cell membrane peroxidation leads to membrane leakage and increased transport EAA into cells [42].

The present study explored the effect of EAA fraction alone and in combination with 5-FU on cytotoxicity of HeLa, Widr and T47D cells. Single treatment of EAA fraction showed potent cytotoxic effect, but 5-FU did not show potent cytotoxic to HeLa and Widr cells, while combination with 5-FU increased cytotoxic effect of 5-FU. These results are interesting to be evaluated. Combination of EAA and 5-FU probably increased EAA and 5-FU intracellular concentrations. Previous study reported chemotherapy drug induced cell membrane peroxidation leads to membrane leakage and increased transport EAA into cells [42].

The present of TLC-Densitometry fingerprinting of EAA fraction that shown 3 main compounds of EAA fraction found in this study, with major compound is andrographolide, with have strong anticancer effect in HeLa, Widr and T47D.

This result showed that combination of EAA fraction and 5-FU strongly synergistic in inhibited the growth of cancer cells, this is similar with Yang et al. has reported [43]. Yang et al. [43], found that andrographolide, a natural diterpenoid lactone with potent anti-inflammatory activity, could significantly enhance the 5-FU anticancer activity against HCC cell line SMMC-7721. Our results indicated that: (a) andrographolide inhibits the growth of SMMC-7721 cells and potentiates the cytotoxic effect of 5-FU in HCC cell line (SMMC-7721); (b) inhibition of cell growth and induction of apoptosis were synergistic or additive but not antagonistic; (c) Bax played a key role in andrographolide /5-FU-related apoptosis; (d) mitochondrial membrane potential significantly disappeared after combination treatment; (e) andrographolide / 5-FU-induced SMMC-7721 apoptosis through a caspase-8-dependent mitochondrial pathway; (f) p53 may also play a role in the apoptosis induced by the combination.
Combination of 5-FU and EAA fraction produced synergistic effects on human cancer cells

In order to investigate the anticancer activity of a cocktail containing EAA fraction and 5-FU, cytotoxic studies were performed in human cancer cell lines (HeLa, WiDr and T47D). As shown in Fig 4 the viability levels of all cell lines decreased.

The present study, showed the potency of Andrographis paniculata Nees to be developed as a co-chemotherapeutic agent for 5-FU. The use of 5-FU together with EAA is expected to increase the activity and reduce the side effect of 5-FU. However, the molecular mechanism of cytotoxic effect by this combination need to explored in detail.
CONCLUSION

The research showed that combination of EAA and 5-FU increased the effect of 5-FU against various human cancer cell line (HeLa, WiDr, and T47D). Based on this result, EAA is potential to be developed as a co-chemotherapeutic agent for 5-FU in cervical, colon and breast cancer therapy.

ACKNOWLEDGMENTS

This study was supported by the Penelitian Hibah Kompetensi 2013, Dijen Dikti, Ministry of Education, Indonesia.

REFERENCES


