Anti-Tumor Activities of Analogues Derived from the Bioactive Compound of Zingiber zerumbet

1S.I.A. Wahab, 1A.B. Abdul, 1H.C. Yee1, 1,A.S. Alzahairi, 1M.M. Elhassan and 1M.M. Syam
1UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia
2Faculty of Medicine, Sana’a University, Sana’a, Yemen

Abstract: The aim of this study is to evaluate some derivatives of Zerumbone for their anti-tumor effects on human cervical cancer cell lines (HeLa). The MTT tetrazolium salt colorimetric assay was utilized to evaluate the cytotoxic effects of ZER, cisplatin and the derivatives were n-Butylbenzene (compound 5), 1,1’-(4-Chlorobutylidene) bis(4-fluorobenzene) (compound 6), alpha, alpha-Diphenyl-gamma-butyrolactone (compound 7) and (1,4’-Bipiperidine)-4’-carboxamide (compound 8). The results of this study showed that derivatives of ZER have shown lesser anti-tumor effects towards HeLa cancer cells compared to the principal compound (ZER).

Key words: Zerumbone, analogues, human cervical cancer cell line

INTRODUCTION

Zingiber zerumbet Smith locally known as lempoyang wild ginger belongs to Zingiberaceae family. It is native to South East Asia but has been widely cultivated plant in village gardens throughout the tropical and subtropical area around the world and has naturalized in some areas for its medicinal properties (Perry, 1980; Nharet Somchit and Nur Shukriah, 2003, Yu et al., 2008). Zingiber zerumbet is used in local traditional medicine as a cure for swelling, sores and loss of appetite. Besides that the juice of the boiled rhizomes has also been used as a medicine for worm infestation in children. The volatile oils of the rhizomes have been shown to contain zerumbone, humulene and campene (Hassan, 1991, Jarg et al., 2005).

Zerumbone (ZER), a monocyclic sesquiterpene from rhizomes of edible plant Zingiber zerumbet Smith. ZER has recently been found to suppress tumor promoter 12-0-tetradecanoylphosphol-13 acetate (TPA)-induced Epstein-Barr virus activation in a potent manner (Murakami et al., 2002). ZER is known to be a potent suppressant of cyclooxygenase (COX)-2 and inducible nitric oxide synthase expression (Murakami et al., 2003). ZER is a food phytochemical that has a distinct potential for use as an effective anticancer agent (Matthes et al., 1980), markedly suppresses free radical generation, proinflammatory protein production (Murakami et al., 2002), possibly by its apoptosis-inducing, antiproliferative influences (Kirana et al., 2003) and activates phase II drug metabolizing enzymes (Nakamura et al., 2004).

However, studies on the synthetic ZER derivatives for their cytotoxic ability in cervical cancer remains unresolved. Therefore, the objective of this study is to investigate the cytotoxic effects of several synthetic ZER derivatives compared to ZER and cisplatin in human cervical cancer cells.

Corresponding Author: Siddig Ibrahim Abdel Wahab. UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia
Tel: 0660126565990  Fax: 06601268972101

154
MATERIALS AND METHODS

ZER was extracted and was isolated from *Zingiber zerumbet* by Ms. Nirmala Devi Thailan, Department of Biomedical Science, Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM). Synthetic derivatives of ZER (Fig. 1), were a generous gift by Assoc. Prof. Dr. Muhd Nazrul Hakim Abdullah (IBS, UPM), were n-Butylbenzene (compound 5), 1,1'-4-Chlorobutylidene bis (4-fluorobenzene) (compound 6), alpha, alpha-Diphenyl-gamma-butyrolactone (compound 7) and (1,4'-Bipiperidine)-4'-carboxaminde (compound 8).

Cell Culture

HeLa, cervical cancer cells obtained from ATCC were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% penicillin-streptomycin and 1% amphotast. Flasks were incubated at 37°C in a humidified incubator with 5% CO₂, 95% air. Cultures were regularly examined using inverted microscope. Compounds were added to the cells in different concentrations and left for 72 h.

Fig. 1: Structure of several synthetic ZER derivatives
MTT (Microculture Tetrazolium) ASSAY

After incubated for 48 h, 20 μL MTT solution was added under dark condition, with gentle shaking. After 4 h, content in the wells was aspirated and 100 μL DMSO was added with gentle shaking. Finally, the wells were read using ELISA reader at 450 nm and Optical Density (OD) was recorded. The percentage of the cytotoxicity was calculated using the equation below:

\[
\text{Cell viability (\%)} = \left( \frac{\text{Average OD}_{\text{sample}}}{\text{Average OD}_{\text{control}}} \right) \times 100\%
\]

\[
\text{OD}_{\text{sample}} = \text{Optical Density of sample (treated cells)}
\]

\[
\text{OD}_{\text{control}} = \text{Optical Density of control (untreated cells)}
\]

A dose-response curve was drawn using Microsoft Excel, with cell viability versus ZER concentration. The Inhibition Concentration, IC₅₀, which is the drug concentration that inhibits 50% of HeLa cells growth, was determined from the graph. The experiment was conducted in triplicates.

Statistical Analysis

The values of IC₅₀ from each batch were recorded in table and were analyzed using One-way Analysis of Variance (ANOVA) to compare with Cisplatin, the positive control. The values of IC₅₀ from each batch were expressed as the mean ±SE of mean. DUNCAN test was conducted to compare the differences between groups mean value. The statistical analysis was done using SPSS (15.0) with p<0.05 as significant.

RESULTS

IC₅₀ obtained from MTT assay was used to determine the viability of HeLa cells. The higher IC₅₀ value, the more viable cells remaining after treatment, which indicates the lesser effective the compound was, for cytotoxicity. The synthetic analogues of ZER were screened at concentration ranged from 10 to 100 μM. Based on the Cytotoxicity Screening Index from the National Cancer Institute Chemotherapeutic Standard (Geran et al., 1972), the IC₅₀ value less than 18 μM is considered very significant whilst between 18 and 137.6 μM is considered significant. The finding (Fig. 2) indicated that IC₅₀ of the analogues fell between 18 and 137.6 μM, which is considered significant.

From Fig. 2, the IC₅₀ for the analogues obtained were 70.0±2.5 μM in n-Butylbenzene, 79.4±1.1 μM 1, 1'-(4-Chlorobutylidene) bis(4-fluorobenzene), 87.6±4.6 μM in alpha,alpha-Diphenyl-gamma-butyrolactone and 80.0±7.8 μM in (1,4'-Bipiperidine)-1'-carboxamidine. The finding indicated significant different (p<0.05) in IC₅₀ among analogues. In comparison within the analogues as seen in the Fig. 2, n-Butylbenzene shown significant lower value of IC₅₀ while alpha, alpha-Diphenyl-gamma-butyrolactone shown significant higher value of IC₅₀. Among the screened synthetic compounds, n-Butylbenzene considered most cytotoxic compound as its lowest IC₅₀.

Parent compound, ZER and commercial drug, cisplatin were previously established their IC₅₀ which were 20.7±0.9 and 5.3±1.3 μM. This study found that the analogues were incompetent to neither ZER nor cisplatin for cytotoxicity in HeLa cells as their IC₅₀ were higher (Fig. 2). Thus, ZER was the most active natural compound, comparing to its synthetic analogues and comparable to cisplatin as well.
DISCUSSION

Natural products possess a pedigree to justify quality and appreciation in drug discovery and development (Nielsen, 2002; Bogevik et al., 2008). Currently, there is rapid increase in application of natural products in combinatorial chemistry and vice versa (Kochn, 2008). The therapeutic areas of oncology are emerging (Saif, 2008). Natural products or intermediates have served as building blocks or scaffolds in the synthesis of complex natural products, bioactive analogues or designed hybrid molecules (Ortholam and Ganesan, 2004). Finally, structural motifs from the biologically active parent molecule have been identified and have served for design of natural product mimicry, which improves the knowledge and research in this area (Erlt et al., 2008).

ZER is a crystalline sesquiterpene derived from the wild ginger, Z. zerumbet. This bioactive component has its unique structure, with a cross-conjugated ketone in an 11-membered ring, as well as remarkable biological activity (Kitayama et al., 2003). Antiproliferative activity of Z. zerumbet is mainly modulated by the ZER component which is the main cytotoxic compound that constitutes about 37% of the whole Z. zerumbet content (Murakami et al., 2002). Terpenoids, including mono-, sesqui-, di- and triterpenoids, are biosynthesized by tandem reactions of the phosphorylated isoprene unit consisting of five carbons and are ubiquitously found in the plant kingdom. Some of these dietary terpenoids have anti-carcinogenic activities in a variety of rodent experiments and clinical trial results also demonstrated that their potential of treating cancers without major toxicity (Damodaran and Dev, 1968; Crowell, 1999; Rabi and Gupta, 2008). The further understanding of their biological and physiological mechanisms may lead to the identification of more effective compounds in this category for the prevention and treatment of targeted cancer types.

Studies demonstrated that ZER inhibited the proliferation of human colonic adenocarcinoma cell lines in a dose-dependent manner, while the growth of normal human dermal and colon fibroblasts was less affected. Intriguingly, a-humulene (HUM), a structural analogue lacking only the carbonyl group in ZER, was virtually inactive in all experiments conducted, indicating that the a, b-unsaturated carbonyl group in ZER may play some pivotal roles in interactions with unidentified target molecule(s) (Murakami et al., 2002). Thus, second-generation chemicals may be synthesized to mimic naturally occurring compounds, ZER but with greater specificity and less toxicity.
The MTT tetrazolium salt colorimetric assay previously described by Mosmann (1983); to measure cytotoxicity and cell proliferation. The level of MTT cleavage by viable cells of various origins was found to be directly proportional to the number of cells (Gerlier and Thomasset, 1986).

The synthetic analogues of ZER screened in this study, were n-Butylbenzene, 1, 1’-(4-Chlorobutylidene) bis(4-fluorobenzene), alpha,alpha-Diphenyl-gamma-butyrolactone and (1,4’-Bipiperidene)-4’-carboxamide. The synthetic analogues were shown in the result, to have cytotoxic effect towards cervical cancer cells. Yet, these analogues were not as effective as ZER and Cisplatin, if compare to their MTT findings established previously in the laboratory. Since IC_{50} of the analogues were high, we suggest that it may be used for anti-proliferative studies in order to investigate it preventive potential in cancer development. Nevertheless, the screening stage of this study concluded that ZER remains the most active cytotoxic natural compound on cervical cancer cells. A future chemical synthesis is required to produce more analogues, however, this approach must be followed by preserving the most functions biological active groups in ZER.

ACKNOWLEDGMENTS

The authors wish to thank gratefully Universiti Putra Malaysia (RUGS 91143) and the Malaysian National Council for Cancer for financial support and The Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia for providing analogues of zerumbone.

REFERENCES


