Tocotrienols and Acute Myeloid Leukaemia

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INTRODUCTION

Tocotrienols are superior antioxidants that can combat free radicals in the human body, and have shown many biological functions such as having antioxidant and anti-inflammatory effects, maintaining fertility and regulating the immune system, associated with lowering tumour formation, having enhanced anti-cancer properties, as well as controlling tumour growth in certain types of cancer (Yam *et al.*, 2009; Abd Hafid *et al*., 2010; Inoue & Zhang, 2011; Wong *et al*., 2012; Abdul Hafid *et al.*, 2013). Some studies have shown that tocotrienols induce cell death in various cancers, such as breast, prostate, cervix and pancreas. Delta-tocotrienols (δ-T3) are believed to be more effective than other forms of tocotrienols in causing apoptosis or cell death in both oestrogen-nonresponsive and oestrogen-responsive breast cancer cells (Ahn *et al*., 2007; Inoue and Zhang, 2011; Wong *et al*., 2012). Since, tocotrienols as antioxidants have the ability in lowering oxidative stress, neutralise free radicals which functions as a chain-breaking antioxidant that prevents propagation of free radical reactions in all cell membranes (Devasagayam *et al*., 2004); it may be suggested as an alternative compound in treating and managing leukaemia diseases. There are only few reports on the effects of tocotrienols in leukaemic cells (Ahn *et al*., 2007; Al-Tonbary *et al*., 2008; Inoue and Zhang, 2011; Wong *et al*., 2012).

LEUKAEMIA

Leukaemia is a cancer caused by an overproduction of damaged white blood cells, and it is one of the top ten cancers affecting all races in the United States (Yamamoto and Goodman, 2008). It is the sixth most common cancer among Malaysians, being the seventh most common cancer in males and eighth in females (Zainal and Nor Saleha, 2011). Leukaemia is one of the most common childhood cancers, mostly under the age of 15 years, and most often occurring in older adults of over 55 years of age (Yamamoto and Goodman, 2008).

In leukaemia, abnormal blood cells are produced in the bone marrow, which involves the production of abnormal white blood cells, the cells that are responsible for fighting infection. Leukaemia affects white blood cells and can be classified by the type of white cells affected (myeloid or lymphatic) and by the mode of disease progression (acute or chronic). The common types of leukaemia are: acute lymphocytic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), chronic myelogenous leukaemia (CML) and acute myelogenous leukaemia (AML) (Christian *et al.,* 2017) (*Figure 1*). The American Cancer Society estimated that about 62 130 people were expected to receive a diagnosis of leukaemia in 2017, and around 24 500 deaths would likely be due to this disease (*Christian et al.,* 2017). Acute leukaemia develops quickly and worsens rapidly, but chronic leukaemia gets worse over time. Other subtypes include: hairy cell leukaemia, chronic myelomonocytic leukaemia (CMML), and juvenile myelomonocytic leukaemia (JMML) which occurs mostly in children and can be cured using haematopoietic stem cell transplantation (HSCT) (Niemeyer *et al*., 2005).

Treatment options for leukaemia depend on the type of leukaemia, the patient's age and overall state of health. Nevertheless, the main type of treatment is chemotherapy, and is tailored to the type of cancer which a patient has (Christian *et al*.,

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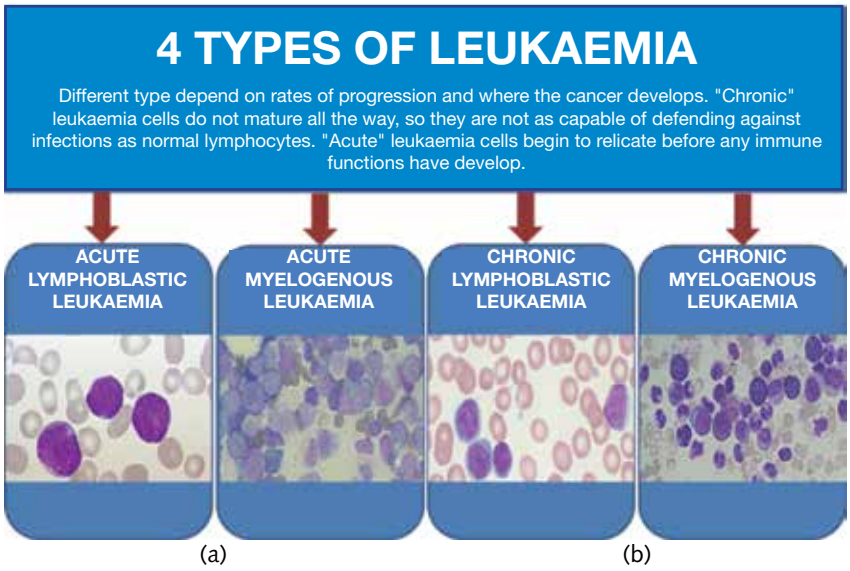


Note: Pictures (a) on acute lymphoblastic and acute myelogenous leukaemia adapted from Döhner *et al.,* (2015). Pictures (b) on figure of chronic lymphoblastic and chronic myelogenous leukaemia adapted from O'Brien and Gribben (2008).

Figure 1. Leukaemia and types of leukaemia.

2017), such as radiation therapy. If treatment starts early, the chance of remission is greatly improved. Acute lymphocytic leukaemia (ALL) is a curable disease with an expected long-term survival rate of at least 70% after treated with modern therapeutic regimens. A common therapy for ALL begins with induction of chemotherapy with combination of drugs and less intensive course of chemotherapy used to reduce the risk of the disease recurring after treatment has completed. In CLL, the prognosis is poor, and ibrutinib is approved for the treatment of patients who have received at least one prior therapy (Christian *et al.,* 2017). For CML, the prognosis also very poor, and treatment may include radiation therapy, chemotherapy or stem cell transplant and immunotherapy. The commonly used chemotherapy drugs are gleevec (imatinib), sprycel (dasatinib) and tasigna (nilotinib). The survival rate for acute myeloid leukaemia (AML) is very slim; thus, there is a strong demand for the development of more effective treatment therapies such as combination therapies, radiation, stem cell and immunotherapy.

Few studies have reported the use of tocotrienols or single isomers of tocotrienol in combating leukaemic cells. A study conducted by Wong *et al*. (2012) showed that delta- and gamma-tocotrienols induced apoptosis cell death in human T lymphoblastic leukaemic cell line, CEM-SS. Furthermore, a clinical study conducted by Al-Tonbary *et al*. (2009) showed a significant increase serum Glu.Px in acute lymphoblastic leukaemia (ALL) patient who received vitamin E and N-acetyl cystine (NAC) after phase II of therapy in comparison with those who received chemotherapy alone without any supplementation. In the study conducted by Al-Tonbary *et al*. (2009), it was concluded that vitamin E and NAC supplementation significantly decreased the level of free radicals resulting from oxidation as evidenced by the increased level of GLu.Px and the lowered level of malondialdehyde (MDA)awith Acute lymphoblastic leukaemia (ALL) patient who took the supplementation. So far, no study has been reported the effects of combination therapy using palm vitamin E and acute myeloid leukaemia (AML) drug.

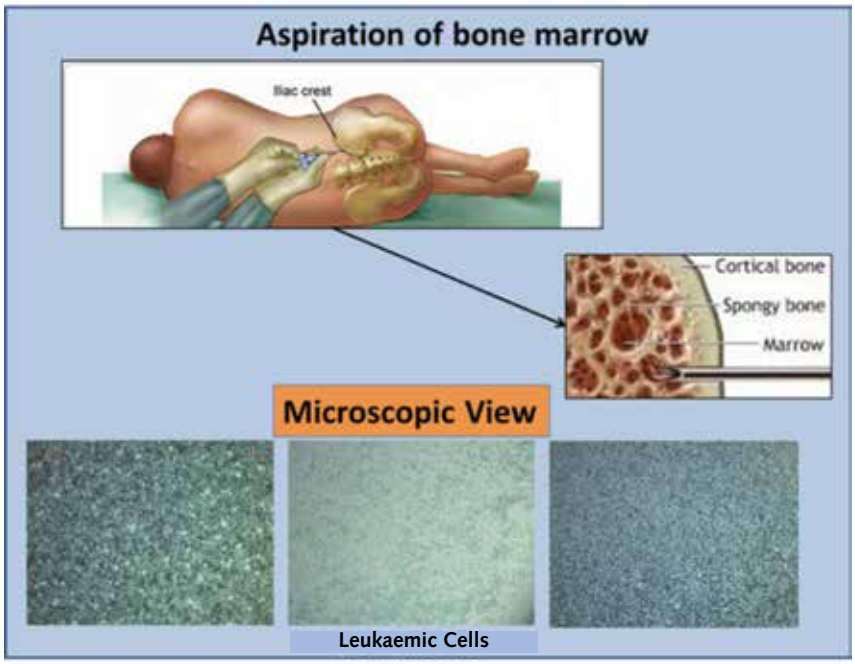
ACUTE MYELOID LEUKAEMIA

Acute myeloid leukaemia is also known by other names, such as acute myelogenous leukaemia (AML), acute granulocytic leukaemia and acute non-lymphocytic leukaemia. It is a fast-growing cancer of in blood and bone marrow. In AML, the bone marrow makes many cancerous cells called leukaemic blasts (Dohner *et al*., 2009). Normal blasts develop into white blood cells that fight infection (Dohner *et al*., 2009; Betz and Hess, 2010). Leukaemic blasts do not develop properly and cannot fight infections.

In AML, there are too many of a specific type of white blood cells called myeloblasts (Betz and Hess, 2010). These cells crowd out the healthy blood cells, making it hard for the blood to do its work of warding off infections. These leukaemic blasts grow quickly and crowd out the bone marrow, preventing it from making the normal red blood cells, white blood cells and platelets that the body needs (Betz and Hess, 2010; Cook and Pardee, 2013).

Nearly 15 000 people in the United States are diagnosed with AML each year (Howlader *et al*., 2013) (*Figure 2*). AML can affect people of any age, but it is the most common type of leukaemia among adults. AML is more common in men than in women, but is rare in individuals under the age of 40 (Dohner *et al.*, 2009; Betz and Hess, 2010).

The cause of AML is unknown. Possible risk factors include smoking, blood disorders, previous chemotherapy treatment including the use of etoposide and drugs known as alkylating agents, exposure to certain chemicals and harmful substances, radiation, and a weakened immune system due to



Note: Picture on iliac crest adapted from Nursingcrib.com and picture on bone marrow adapted from Nursingcrib.com.

Figure 2. Aspiration of bone marrow.

Figure 3. Possible treatments for AML disease.

an organ transplant. Problems with genes may also play a role in the development of AML (Koeffler and Golde, 1980; Betz and Hess, 2010; Cook and Pardee, 2013).

Normally, myeloid and other blood cells are produced in the bone marrow (the spongy area in the middle of bones) in a carefully controlled fashion. In someone with AML, the blood cell production process is abnormal and large numbers of immature myeloid cells are produced and may be released into the blood stream (Chakraverty, 2012). Sometimes, the number of white blood cells in the circulation system is abnormally high because of over-production of malignant blood cells. The over-production of myeloid cells prevents the bone marrow from producing other important blood cells, including red blood cells, other types of white blood cells and platelets. This results in a variety of body-wide symptoms, including anaemia, bleeding, and an increased risk of infection. As AML is a heterogeneous malignant disease in which disease progression at the level of $CD34^+$ cells has a major impact on resistance to chemotherapy and relapse, the inability to undergo apoptosis is a crucial mechanism of the multi-drug resistance in AML patients (Chakraverty, 2012; Wiernik *et al*., 2013).

Factors that clearly need to be taken into account when considering treatment options such as age, health status and certain gene or chromosome changes which are more likely to benefit from more intensive treatment. There are a few possible treatments for AML, such as chemotherapy and in combination with drugs, targeted therapy, non-chemo drugs, surgery, radiation and stem cell therapy (*Figure 3*). The chemo drugs that often used for AML are cytarabine or daunorubicin, or a combination of two types of drugs. Patients undergoing treatment for AML receive multi-agent chemotherapy. Unfortunately, many of the agents (cytosine arabinoside, doxorubicin, cyclophosphamide, and methotrexate) are associated with free radical production that may affect the antioxidant status during therapy (Ahn *et al*., 2007). During the initial intensive period for multi-agent chemotherapy induction, common difficulties experienced by patients include hepatic and haematological complications. Usually these complications can cause discontinuation of the therapy, prolong the duration of stay in hospital, and may affect the overall prognosis and outcome of the disease. For targeted therapy, midostaurin (Rydapt) is a tablet drug that can be taken orally, which is used to block FLt3 and other proteins in cancer cells. Radiation and surgery treatments are the type of typical treatments

for all types of cancers, including AML, that have a located or isolated tumour, for example granulocytic sarcoma. Stem cell treatment will usually be done using a transplant of blood or bone marrow from a donor who closely matches the patient. Non-chemo drugs or natural compounds, such as ATRA, will be used for induction treatment in AML. ATRA is a form of vitamin A, currently used in leukaemia patients. Other possible treatments, such as tocotrienols or single isomers of tocotrienol, e.g. gamma-tocotrienol, are reported to have an effect in inhibiting AML (American Cancer Society). Gamma-tocotrienol has been reported to activate the p53 gene (tumour suppressor gene) and inhibit NFK-b and Ras signalling to lower down the disease (https://www.lls.org/content/nf-kb-inhibition-in-aml).

Many studies have shown that the anti-oxidant properties of palm tocotrienols play a protective role against oxidative stress-induced DNA damage, and neutralise free radical formation which can induce cell death in various cancers and other types of leukaemic cells (Ahn *et al*., 2007; Al-Tonbary *et al*., 2009; Inoue and Zhang, 2011; Wong *et al*., 2012; Lee *et al*., 2017). Hence, therapy with tocotrienols or in combination with drugs might be effective in inhibiting acute myeloid leukaemia. The purpose of using palm vitamin E (especially tocotrienols) with leukaemic drugs in our study is to improve the management of side effects by lowering the oxidative stress and free radical generation during the maintenance chemotherapy, and may also improve survival outcome including a disease-free status and overall survival.

SUPPRESSION OF ACUTE MYELOID LEUKAEMIA (AML) WITH TOCOTRIENOL SUPPLEMENTATION

In our recent study, we examined the effects of single isomers such as gamma (γ)- and delta (δ)- tocotrienols (T3), and the mixed fraction called tocotrienol-rich fraction (TRF), to produce synergistic effects in the treatment of acute myeloid leukaemia. A leukaemic drug, cytarabine, was used as the commercial drug for comparison. In our preliminary data, we were able to show that the tocotrienol treatments on different acute myeloid leukaemia (AML) cell lines were able to suppress the leukaemic cells at the dose of 20 ug ml^{-1}. TRF treatment at 20 ug ml^{-1} on all the AML cell lines tested showed that HL-60 and Kasumi-6 cell lines seemed to be more sensitive and died faster compared with THP-1 and Wehi-3 cell lines.

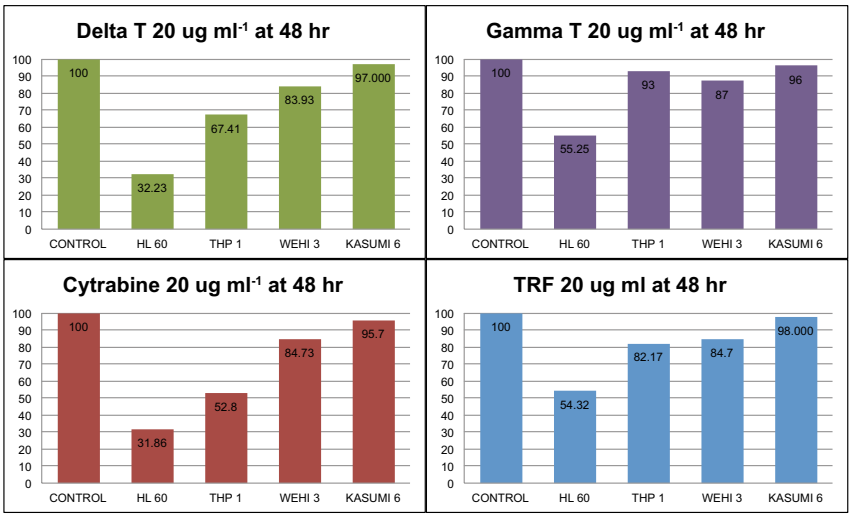


Figure 4. Effects of delta-T3, gamma-T3, TRF and cytarabine at a concentration of 20 ug ml^{-1} on different acute myeloid leukaemia (AML) cell lines. Cells were plated down at 5 x 10^5 and treated for 48 hr (unpublished data).

The effects of single isomers (delta- and gamma-T3), tocotrienol rich-fraction (TRF) and the commercial drug cytarabine were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide MTT assay to look at cell proliferation and cytotoxicity in response to the treatments on the cells themselves, and the results are shown in *Figure 4*. According to the results, delta-T3 and cytarabine showed the same pattern towards inhibition of AML cells when tested at 20 ug ml^{-1} for 48 hr. Gamma-T3 and TRF at the same concentration seemed to exhibit a similar pattern according to each treatment tested. We found that HL-60 cells treated with gamma-T3 were inhibited by almost 70% (IC70), and it was observed that TRF inhibited 50% (IC50) of the cells. As we can see from the figure, THP-1 cell lines exhibited an almost comparable inhibitory action (IC50) for delta-T3 and cytarabine. For Wehi-3 and Kasumi-6 cell lines, a different pattern was observed. Gamma-T3 and TRF treatments appeared to be less sensitive than treatments with cytarabine and delta-T3. In conclusion, the results show that different cell lines exhibited different patterns of inhibition and in a concentration and time dependent manner. Treatments tested in our study such as delta-T3, gamma-T3 and TRF have shown the ability of tocotrienols to inhibit the proliferation of AML cell lines, especially HL-60, THP-1, Wehi-3 and Kasumi-6.

Therefore, elucidation and further verification of the efficacy of tocotrienols and single isomers in treating acute myeloid leukaemia are needed. Combination therapy between tocotrienols or single isomers together with leukaemic drugs was one of our intentions to be tested in this study.

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