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Therapeutic Effect of Avocatin B in Avocados Toward Acute Myeloid Leukemia

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ABSTRACT

Acute myeloid leukemia is the abnormal division of immature myeloid precursor cells in the bone marrow affecting mostly older people. Till date, there are no potent treatments due to the fact that it cannot target leukemic stem cells which are responsible for relapse and initiation of leukemia. Current treatments can also pose side effects that might to be too harsh for older people. Avocatin B is a lipid derived from avocado that can be a novel potent drug to replace or go along with the current therapies. Avocatin B is able to induce leukemic cell death and their stem cells while leaving normal cells unharmed. Their mechanisms of actions include inhibiting fatty acid oxidation which causes reactive oxygen species mediated apoptosis and lipotoxicity. Their selectivity towards leukemic cells over normal cells are due to the fact that they are able to target leukemic mitochondria which are different from the normal cells' mitochondria in terms of their metabolic pathways.

Keywords: Acute myeloid leukemia, Avocatin B, Leukemic Stem Cells, Fatty acid oxidation

ABSTRAK

Leukemia myeloid akut merupakan sel precursor myeloid dalam sumsum tulang dewasa yang abnormal. Hingga saat ini, belum ada terapi yang tepat untuk leukemia myeloid akut. Avocatin B adalah lipid yang berasal dari alpukat, dapat menjadi obat baru atau digabungkan dengan terapi saat ini. Avocatin B mampu menginduksi kematian sel leukemia dan sel induknya. Mekanisme Avocation B meliputi penghambatan oksidasi asam lemak yang menyebabkan oksigen reaktif memediasi apoptosis dan lipotoksisitas. Selektivitas avocatin B terhadap sel-sel leukemia karena dapat menarget mitokondria penderita leukemia yang berbeda jalur metabolisme dari mitokondria sel-sel normal. **Dias Rima Sutiono, Anthony Ryan Pantoni. Efek Terapetik Avocatin B dari Alpukat untuk Leukemia Myeloid Akut.**

Kata kunci : Leukemia mieloid akut, Avocatin B, Sel Punca Leukemik, Oksidasi asam Lemak

INTRODUCTION

Acute myeloid leukemia (AML) is the abnormal division of immature myeloid precursor cells in the bone marrow which fails to fully differentiate. These underdifferentiated cells known as leukemic blast cells have deteriorated function and prevent the formation of normal functioning cells. AML is commonly caused by genetic alteration usually of the FLT3 or NPM1 gene[1]. The term acute indicates that the disease can spread and turn severe rapidly. According to American Cancer Society, there are estimated to be 19,950 cases of AML with 10,430 deaths in United States alone at 2016, most of them adults[2]. In fact, 80% AML cases occur in adult compared to 20% in kids. People of increasing age are more susceptible[1]. Elder people at ages 65 and above are ten times more likely to develop AML compared to those at their 30's and faces 90% death rate after two years of diagnosis[1].

In most cases, AML is treated using either one or combination of chemotherapy, radiation and stem cell transplantation. Chemotherapy itself involves two steps which are induction therapy and post-remission therapy. The induction therapy involves the elimination of leukemic cells as much as possible. The post remission therapy is the elimination of any remaining AML cells that can possibly cause relapse. These therapies together are capable of eliminating most of the AML cells. However, they post two main problems which are their harmful side effects and inability to target leukemic stem cells. These therapies can cause hair loss, mouth ulcers, vomiting and in the long term might cause heart and liver damage and development of other cancer. These side effects can be extremely fatal to older people especially those with other medical complications, causing only 30% of elder people to receive treatment [1,3]. Moreover, similar to hematopoiesis, cancer begins with stem cells which are capable of self-renewing, causing disease and being drug resistant. The current therapy lacks the ability to target these cells, which increases the chance of relapse[3].

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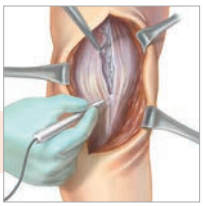
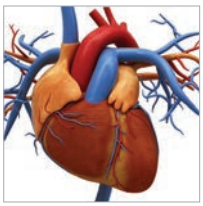




Avocatin B, a lipid isolated from avocado, is a potent candidate as a novel treatment and possible cure for acute myeloid leukemia. Based on functional stem cell assay, avocatin B can eliminate both leukemic stem cell and leukemic blast cells without harming the normal cells. This is done by targeting leukemic mitochondria which are different to that of normal cells in terms of their metabolism in which they depend on fatty acid oxidation (FAO) for survival. Due to the fact that it does not harm any normal cells, Avocation B is suspected to have harmless to no side effects which is suitable for elder patients[4].

We would like to emphasize on what are leukemic stem cells, metabolism changes in the mitochondria of leukemic cells and the mechanism of action of Avocatin B towards leukemic cells which subsequently leads to their death.

Leukemic Stem Cells

The major disadvantage of the current treatment is their inability to target the leukemic stem cells. These stem cells or also commonly known as tumor initiating cells make up 0.1-1% of leukemic blast cells and have the ability to self-renew, cause the occurrence and be resistant to drugs [3,5]. Leukemic stem cells possess 5 main properties that allowed them to counter current therapy. This include: they contain efflux proteins to pump out medications, they contain more anti-apoptotic proteins such as Bcl-2, they have more cell cycle restriction points and therefore improved DNA repair mechanisms, they exist mostly in dormant state. Therefore, they can avoid treatments such as chemotherapy that targets rapidly dividing cells, and they might not have specific antigens to certain drugs and immune systems[5]. Aside from these characteristics, leukemic stem cells and normal leukemic cells also differ in their mitochondria metabolism from normal cells, allowing it to be a potent target for Avocatin B.

Mitochondrial Alteration 1: Glycolysis Dependent

Leukemic cells have different mitochondrial metabolism due to increased uncoupling proteins (UCP). Due to this UCP, leukemic cells do not follow the normal respiratory cascade. Glycolysis step was uncoupled from the Krebs Cycle, avoiding the oxidation of pyruvate. As a result, instead of using glucose products, the mitochondria depend on fatty acid for Krebs Cycle. Moreover, the uncoupling protein also causes the reduction in proton gradient causing the mitochondria to depend on glycolysis for ATP production instead of oxidative phosphorylation. The glycolysis gives advantage to leukemic cells which include: It limits the probability of forming another pathway that can interrupt biosynthesis, it allows them to survive conditions where oxygen is limited, it increases formation of Bcl-2 which are anti-apoptotic agents and down regulate Bax and Bak proteins which are pro-apoptotic proteins, and it allows mitochondria to depend on fatty acid oxidation for survival[3].

Mitochondrial Alteration 2: Fatty acid oxidation for survival

Energy depending tissues such as heart, muscle and liver require fatty acid oxidation (FAO) for their energy. This is due to the fact that fatty acid oxidation yields twice ATP compared to carbohydrates. On the other hand, leukemic cells depend on fatty acid oxidation mostly for survival, not ATP requirement. In fact, too much ATP from FAO can be toxic to the leukemic cells. Therefore, they have the increased uncoupling proteins to down regulate the proton pump [6].

The process of fatty acid oxidation involves several processes which yields NADH, FADH and Acetyl-CoA. Acetyl CoA is converted to form citrates which are essential for formation of cell membrane, cellular proliferation and down regulation of proapoptotic proteins (Bax and BAK). Citrates are also then processed to form NADPH. The presence of NADPH increases the concentration of reduced glutathione (GSH) which is essential antioxidant that can neutralize the free radical's reactive oxygen species (ROS) [6,7].

Despite the advantages, depending on FAO requires them to perform a rate limiting step beforehand. Fatty acids or fatty Acyl-CoA are unable to pass through the mitochondria. Therefore, they require the help of carnitine. Prior to FAO, the acyl group must be transferred to the carnitine by the carnitine palmitoyltransferase (CPT1) forming the acylcarnitine. In the mitochondria, these acylcarnitine are then converted back to Acyl-Coa by CPT2 to be broken down via FAO[7]. Avocatin B utilizes this process to eradicate leukemic cells.

Avocatin B

The potential efficacy and safety of Avocatin B was tested against several cell type: Leukemia cells, TEX leukemia cells with stem cell properties, primary human samples from AML patients with more than 80% of AML cells and peripheral blood stem cells (PBSC). High throughput screening of natural health product library showed that Avocatin B, a lipid from seed and peels of unripe avocado, is the most potent bioactive compound to eradicate AML [4].

Safety

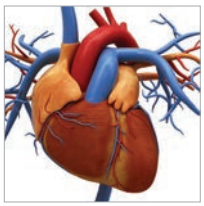
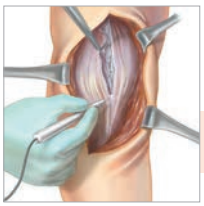
Three umol/L of Avocatin B on the AML patient's cells and normal cells proved that it was able to reduce AML growth by more than 70% while leaving the normal cells unaffected. Moreover, they also showed that Avocatin B had EC50 of 3.9 2.5 umol/L on AML cells while have no effect on PBSC up to 20 umol/L. These experiments proved that Avocatin B can be a potent novel drug to treat AML without harming the normal cells [4].

Mechanism of Action

Avocatin B induces cellular death by using two methods which are fatty acid oxidation inhibition and lipotoxicity of the mitochondria. Avocatin B can only be activated and exert its effect inside the mitochondria with the help of CPT1 enzyme. Experiment showed that in the presence of CPT1 inhibitor, etomoxir, the action of Avocatin B was vastly reduced. This was repeated using RNA interference to reduce the activity of CPT1 and showed the same result. Moreover, Avocatin B showed no effect in mitochondria that have been treated with ethidium bromide which made them lose their function. Due to this fact, it is believed that Avocatin B selective killing ability is by targeting the altered mitochondria of leukemic cells[4].

Fatty acid oxidation inhibition resulted leukemic cell death via the formation of AIF or cytochrome c. Although experiment performed by Lee and colleagues showed elevation in both of these enzymes, they also revealed that the cause might be independent from caspase enzymes due to the fact that caspase enzyme inhibition did not affect Avocatin B. Therefore, they proposed that AIF is a more probable cause [4].

Fatty acid oxidation inhibition by Avocatin B

is due to their odd-numbered carbon chain which is seventeen. The difference is that in even-numbered carbon chain fatty acid, their break down yields two acetyl CoA. On the other hand, breaking down of Avocatin B produces one acetyl CoA and one propionyl CoA in which their breakdown requires an extra ATP. Therefore, performing this pathway is metabolically slower and more energy depleting. Due to the lack of fatty acid byproducts, NADPH level is decreased together with the subsequent decrease in GSH which is essential to protect leukemic cells from ROS. Once the GSH lowers and protection was lesser, the leukemic cells become more susceptible to ROS mediated apoptosis[4].

On the other hand, Avocatin B can also cause lipotoxicity due to the accumulation of fatty acid in the mitochondria. Reactive oxygen species can convert excess fatty acids into lipid peroxides that can cause damage to the mitochondrial DNA, lipid and proteins [4].

Another advantage of using Avocatin B is that it has high partition coefficient which means that it can be targeted into the bone marrow where they will exert the effect towards the acute myeloid leukemia cells [4].

Limitations regarding Safety and Future Recommendations

Although experiments proved that Avocatin B can induce leukemic cell death without harming the normal cells, safety test specifically towards muscle, liver and kidney cells is essential. This is due to the fact Avocatin B depends heavily on fatty acid oxidation while those cells also depend on fatty acid oxidation for energy. If the action of Avocatin B affects those cells, it might pose a great threat towards our body. In fact, impaired fatty acid oxidation mechanism has been proven to cause hypoketonic hypoglycemia and cardiomyopathy[3].

CONCLUSION

Current drug therapy is unable to eradicate acute leukemic cells due to the fact that it cannot target the leukemic stem cells. Avocatin B is a potent alternative medication that can induce leukemic cells death including the stem cells while not affecting the normal cells. This is done by fatty acid oxidation pathway that causes ROS mediated apoptotic cell death and lipotoxicity. However, additional safety measures must be tested for the activity of Avocatin B towards muscles, liver and kidneys.

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