# Monolaurin (glyceryl monolaurate) as a potential antitumor agent.

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Introduction

The current use of drugs to control or eradicate cancer has exposed a number of limitations. Chemotherapeutic approaches exploited thus far to eradicate malignant cells are limited by their toxicity, lack of cellular specificity and lowering our innate immune system. Therefore, new nontoxic compounds displaying specific killing of neo-plastic cells without major side effects need to be identified.

Hence, the Identification of new apoptosis-inducing or cytotoxic agents specifically targeting malignant cells while sparing their normal counterparts, is a crucial need. A group of chemicals that may fulfill this new paradigm are lipids. That lipids (fats) have effects above and beyond their caloric value has been elucidated in three volumes--The Pharmacological effects of Lipids American Oil Chemist Society. (1), (2), (3)

Monoglycerides, a special family of lipids consisting of a single saturated or unsaturated fatty acid moiety bound to a glycerol backbone, were discovered (circa 1966) to have wide spread biological activity (antimicrobial) without toxicity. (4) Little interest, however, has been shown in discovering other pharmacological properties of these lipids. Since a lack of toxicity and biological activity are remarkable to find in a chemical agent, the question of direct effects on normal cells and tumor cells is of great interest.

A study using Ehrlich ascites by Kato, et al, (5), (6) first reported an apparent antitumor activity for monoglycerides in vivo without damage to the animal host. Confirmation of the above observation was noted from lipids (fatty acids and monoglycerides) extracted from group A hemolytic streptococci. (7) These lipids were also examined for their antitumor effect against Ehrlich ascites carcinoma and found active.

These early studies gave us information primarily on the effects of monolaurin (glycerol monolaurate) on animal malignant cells (Ehrlich carcinoma). Two more recent reports (8), (9) showed monoglycerides (C12:0, C18:1) induced cell death in several human cell lines.

The objective of the current research report was to test monolaurin against five normal and five human cancer cell lines. The inhibition of growth by the monoglyceride was recorded.

Study Design

To determine whether other cell types would respond the same way the test facilities of KGK Synergize (London, ON NGA 4G5) were sponsored. Mal Evans, PhD. was the study director. The following cells were tested: CCD-34Sk (Skin-normal), SKMEL-5 (Skin-cancer); NL20 (lung-normal), A549 (lung-cancer; THLE-2 (liver-normal), Hep G2 (liver-cancer), RWPE-1 (prostate-normal), LNCaP (prostate-cancer), CCD-18Co (colon-normal), HT-29 (colon-cancer).

Cell Culture

Ten human cell lines were used. Five representing normal human cell lines and 5 representing various types of cancer were obtained from ATCC and cultured according to the supplier's instructions including media and sub-culturing recommendations. Briefly, cells were maintained in a humidified chamber at 37_C, 5% CO2. Cells were plated in three 96 -well plates (one plate for each of three time points; 24, 48 and 72 hours) at 1x[10.sup.10] cells/well (100 [micro]l of 1x[10.sup.5] cells/ml and incubated overnight to allow cells to adhere to the plate surface.

Treatment of Cells with Monolaurin Labeling with 3H-thymldlne and Harvesting of Plates

The test compound monolaurin was weighed and prepared immediately before use by dissolving in DMSO at a concentration of 10 mg/mL. A dilution of 1 mg/mL was prepared using complete media prepared for CCD-34SK cells and sterile filtered using a 0.2[micro]m syringe filter and syringe. Serial dilutions of 400 [micro]g/mL, 200 [micro]g/mL, 100 [micro]g/mL, 10 [micro]g/mL and 1 [micro]g/mL were aseptically prepared from the sterile filtered 1 mg/mL monolaurin solution using complete media. DMSO controls were prepared in a similar manner.

After the overnight incubation, cells were treated with 100 [micro]L of media, DMSO controls or test solutions of monolaurin. Each concentration was plated in triplicate wells for each time point.

The 24-hour plate was further incubated for approximately 6-8 hours at which time 0.5 [micro]Ci 3H-thymidine was added to each well in a volume of 50 [micro]L of complete media. The plate was further incubated for 16-18 hours in a humidified chamber at 37_C, 5% CO2 after which time the plate was harvested. The filtermat was prepared according to internal procedures using a melton scintillation wax. The filtermat was read using a mi-crobeta counter (Perkin Elmer) and 3H- was counted for 1 minute per well. The 48 and 72 hour plates were prepared in a similar manner with 3H--thymidine being added after 30-32 hours and 54 - 56 hours from initial cell seeding.

Resulting data (Table 1) is presented as the percent of inhibition of growth by monolaurin on normal and tumor cells. This was calculated using the DMSO controls corresponding to the appropriate monolaurin concentration.

Table I

Growth Inhibition on Tissue Cells by Monolaurin

| TISSUE | TIME POINT | CONCENTRATION | PERCENT INHIBITION |
|---|---|---|---|
| CCD-34Sk (skin-normal) | 48 Hours | 100 [micro]g/ml | 1.80 [+ or -]: 7.7 |
| SKMEL-5 (skin-cancer) | | | 97.6 [+ or -]:0.2 |
| NL20 (lung-normal) | 48 Hours | 100 [micro]g/ml | 50.0[+ or -]1.2 |
| A549 (lung-cancer) | | | 95.2[+ or -]:0.6 |
| THLE-2 (liver-normal) | 24 Hours | 100 [micro]g/ml | 32.0[+ or -]14.1 |
| Hep G2 (liver-caner) | | | 87.2[+ or -]:1.4 |
| RWPE-1 (prostate-normal) | 72 Hours | 200 [micro]g/ml | 34.9[+ or -]:10.1 |

| | | | |
|---|---|---|---|
| LNCaP (prostate-cancer) | | | 98.1[+ or -]:0. 5 |
| CCD-18Co (colon-normal) | 72 Hours | 200 [micro]g/ml | 85.4[+ or -]:1.8 |
| HT-29 (color-cancer) | | | 92.5[+ or -]1.8 |

Results

The levels (50,100 and 200 [micro]g/ml) were selected based on the results of preliminary study. The data presented (Table 1) was taken at 24, 48 or 72 hours of exposure to monolaurin.

Skin

Melanoma is a skin cancer that begins in the melanocytes, the cells producing a pigment called melanin that colors the skin, hair, eyes and moles. It is, however, considered curable, if diagnosed early. In this study the effect of monolaurin was studied on the skin melanoma cell line SKMEL-5 and was compared to human fibroblast cell line, CCD-34SK. It was seen that after 48 hrs of incubation with concentrations of 100 [micro]g/ml cancer cells showed a statistically significant higher percent inhibition (97.8[+ or -]0.2%) as compared to normal cells (1.8[+ or -]7.7).

Lung

In its progression, lung cancer may lead to metastasis, invasion of adjacent tissue and infiltration beyond the lungs. In this study the effect of monolaurin was studied against the human lung cancer cell line A549 and was compared to the human normal lung cell line NL20. It was seen that there was a significantly higher (95.2 [+ or -] 0.6) percent reduction in proliferation of cancer lung cells after 48 hours of incubation in comparison to normal cells (50.0 [+ or -] 1.2) when treated with 100 [micro]g/ml of monolaurin.

Liver

Liver cancer is a disease in which liver cells become malignant (becoming abnormal and growing uncontrollably), forming a tumor. When cancer originates in the liver it is called primary liver cancer or HCC (hepatocellular carcinoma). The effect of LauricidinAE was studied on human liver cell line HepG2 and was compared to normal human liver cell line THLE-2. For liver cancer cells, 100 [micro]g/ml of monolaurin demonstrated a higher percentage (87.2[+ or -] 1.4%) of inhibition as compared to normal liver cells (32.0[+ or -] 14.1 %).

Prostate

Prostate cancer develops in the prostate and occurs when cells of the prostate mutate and begin to multiply out of control. In this report the effect of monolaurin was studied on the human prostate cancer cell line LNCaP and was compared to the normal human prostate cell line RWPE-1. For the prostate cells, there was a statistically significant difference in proliferation of cancer cells in comparison to normal when incubated with 200 [micro]g/ml monolaurin after 72 hours. Inhibition for cancer cells was 98.1[+ or -]0.5% while normal cells were inhibited 34, 9[+ or -]10.1%

Colon

Colorectal cancer or large bowel cancer includes cancerous growths in the colon, rectum and appendix. The effect of monolaurin was studied on the human colon cancer cell line HT-29 and was compared to the normal human colon cell line CCD-18Co. It was seen that there was a slightly greater percent

inhibition after 72 hours incubation in cancer cells (92.5 [+ or -] 1.8 when compared to normal cells (85.4 [+ or -]1.8)).

Discussion

Little interest has been shown in discovering other pharmacological properties of monoglycerides. Since a lack of toxicity with biological activity are remarkable, the question of effects on normal cells and tumor cells would be of great interest.

A study by Kato, et al, (5), (6) reported an apparent antitumor activity with monoglycerides in vivo without damage to the animal host. The antitumor study of nine different monoglycerides was assayed using Ehrlich ascites tumors in mice. While most monoglycerides exhibited some antitumor activity, monolaurin (C12)) treated mice showed complete cures after 30 days. Control mice survived only 15-17 days.

Confirmation of the above observation was noted from lipids (fatty acids and monoglycerides) extracted from group A hemolytic streptococci. (4) These lipids were also examined for their antitumor effect against Ehrlich ascites carcinoma and found active.

Prior studies only gave us information primarily on the effects of monolaurin on animal malignant cells. The isolation of two monoacylglycerides, 1-monolaurin and 1-monomyristin from Saw Palmetto was the first description showing moderate biological activities against renal (A-498) and pancreatic (PACA-2) human tumor cells and borderline cytotoxicity against human prostatic (PC-3) cells. (8)

A more recent report (9) showed that a monoglycerlde (C18:1) induces cell death in several human leukemic cell lines. Importantly, treatment of primary leukemic cells obtained from B-cell chronic lymphocytic leukemia (B-CLL), patients, resulted in rapid apoptosis. In striking contrast, resting or activated human peripheral blood mononuclear cells from healthy individuals were resistant to the same treatment. Therefore, these compounds could represent potential antileukemic food supplements or could allow for the design of novel therapeutic agents applied to leukemia.

Monoglycerides were indeed able to induce cell death in human leukemic cell lines, albeit with different potencies. Following a 3-hour or 24-hour stimulation, neither the glycerol back bone nor the fatty acid alone were active. In contrast to leukemic cells, epithelial cells from mammary, prostate and endometrial tumors were quite resistant to monoglyceridc treatment even after 48-hour incubation. Hence, these results indicate that monoglycerides efficiently kill some human leukemic cell lines while being relatively less effective against the adenocarcinomas tested.

The potent activity of monoglycerides toward leukemic cell lines prompted examination whether these compounds would be as efficient in primary leukemic cells, such as leukemic B cells from patients suffering from B-CLL. Notably, monoglycerides triggered dose -dependent death in B cells from B-CLL patients, whereas PBMCs (peripheral blood mononuclear cells) from healthy individuals treated with the same concentrations remained unaffected, even after a 24-hour incubation .

Although unsaturated C16:l and saturated C16:0 monoglycerides were not as potent as C18:1 monoglyceride, they nevertheless can kill B-CLL cells while sparing normal PBMCs under the same doses and conditions. Together, these findings illustrate that primary leukemic B-CLL cells undergo apoptosis upon exposure to monoglycerides.

The cytotoxic activity of monoglycerides toward selected populations of normal PBMCs was also evaluated. As previously shown, the total population of PBMCs remained resistant to monoglyceride treatment. Among normal PBMCs, T-helper cells, T-cytotoxic cells, conventional (CD5-) and B-Ia (CD5+) B cells, natural killer cells, and monocytes remained unaffected by monoglycerides. Similarly, activated T and B cells, obtained from healthy donors, were refractory to monoglyceride treatment.

Therefore, the data shows that under the conditions tested, monoglycerides can have a direct cytotoxic effect in vitro on leukemic cells while sparing normal resting and activated human PBMCs. However, because only major leukocyte subsets were tested, the possibility remains that other blood cells, especially in the bone marrow, could be susceptible to monoglycerides. Nevertheless, the mechanism of monoglyceride action is uncertain and remains to be investigated.

Conclusion

Overall results showed that monolaurin has the capacity to decrease the proliferation of animal cancer cells both in vitro and in vivo and human tumor cells in vitro in comparison to normal organ specific tissues. Clinical cancer studies are needed to determine the usefulness of using monoglycerides as therapeutic agents.

Altogether, these studies demonstrate that monoglycerides represent a novel family of apoptotic compounds that appear to specifically kill cancer rather than normal cells. Clearly, further studies are still required before considering monoglycerides as potential therapeutic agents. For instance, elucidation of the mechanism involved in monoglyceride induced death would shed some light on a molecular basis for the relative selectivity towards cancer cells displayed by these compounds.

References

(1) Kabara, j.j. ed.--The Pharmacological effects of Lipids 1 Champaign IL American Oil Chemist Society, 1978

(2) Kabaraj.j. ed.--The Pharmacological effects of Lipids 1I Champaign IL American Oil Chemist Society, 1985

(3) Kabara,j.j. ed.--The Pharmacological effects of Lipids 1 Champaign IL American Oil Chemist Society, 1989

(4) Kabara JJ, Swieczkowski DM, Conley AJ, Truant JPFatty acids and derivatives as antimicrobial agents Antimicrob Agents Chemother. 1972 Jul;2 (I):23-8.

(5) Kato A, Ando K, Kodama K, Tamura G, Alima K. Identification and chemical properties of antitumor active monoglycerides from fungal mycelia. Studies on antiviral and antitumor antibiotics. J Antibiot (Tokyo)._1969 Feb;22(2):77-82.

(6) Akiko Kato, Kunio Ando, Seikichi Suzuki, Gakuto Tamura, Kei Arima Antitumor Activity of Monoglycerides and other Esters of fatty acids J Antibiot (Tokyo). 1969 Feb;22(2):83-4

(7) Kiaoshi S, Kitaji Effect of streptococcal lipids on Ehrlich ascite tumor cells. Jpn J Pharmacol. 1981 Apr;31(2):201-9

(8) Hiroko Shimada, Varro E. Tyler, and Jerry L. McLaughlin* Biologically Active Acylglycerides from the Berries of Saw-Palmetto (Serenoa repens) J. Nat. Prod., 1997, 60 (4), pp 417-418

(9) Philippoussis F, Arguin C, Mateo V, Steff AM, Hugo P Monoglycerides induce apoptosis in human leukemic cells while sparing nonnal peripheral blood mononuclear cells. Blood 2003 Jan l;101(l):292-4. Epub 2002 Aug 1

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