Biochimie xxx (2010) 1–3

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

Which alkylglycerols from shark liver oil have anti-tumour activities?

Anne-Laure Deniau [a], Paul Mosset [b,c], Damien Le Bot [a], Alain B. Legrand [a,*]

[a] Laboratoire de Pharmacologie Moléculaire, Université de Rennes 1, France
[b] Ecole Nationale Supérieure de Chimie de Rennes, France
[c] Université européenne de Bretagne, France

ARTICLE INFO

Article history:
Received 18 September 2009
Accepted 14 December 2009
Available online xxx

Keywords:
Alkylglycerols
Shark liver oil
Anti-tumour activities
Bioactive lipids
Ether lipids

ABSTRACT

Alkylglycerols (alkyl-Gro) are ether lipids abundant in shark liver oil (SLO), and oral SLO or alkyl-Gro mix from this source have several *in vivo* biological activities including stimulation of haematopoiesis an immunological defences, or anti-tumour and anti-metastasis activities *in vivo*. Composition of natural alkyl-Gro mix contains several alkyl-Gro varying by chain length and unsaturation, and individual anti-tumour activity of each molecule present in natural mix remained unknown. We synthesized six prominent constituents of natural alkyl-Gro mix, namely 12:0, 14:0 16:0, 18:0, 16:1 n-7, and 18:1 n-9 alkyl-Gro. Using an *in vivo* model of grafted tumour in mice (3LL cells), we studied and compared the oral anti-tumour and anti-metastasis activities of each of these 6 alkyl-Gro. 16:1 and 18:1 alkyl-Gro showed strong activity in reducing lung metastasis number, while saturated alkyl-Gro had weaker (16:0) or no (12:0, 14:0, 18:0) effect. Spleen weights at day 20 after graft were also measured and showed tremendous variations depending on the treatment. Tumour graft resulted in a raise in spleen weight in control group, this raise was nearly abolished in 16:1 and 18:1 alkyl-Gro-treated mice, and was reduced in 14:0 and 16:0 alkyl-Gro-treated mice. Conversely, 18:0 alkyl-Gro-treated mice showed spleen weigh raise as compared with untreated grafted mice. These new data demonstrate a prominent role of unsaturation in the anti-tumour activities of alkyl-Gro.

1. Multiple biological activities of alkylglycerols

Natural 1-*O*-alkylglycerols (alkyl-Gro) are bioactive ether lipids present in body cells and fluids. They are found in milk, and are precursors of ether phospholipids which participate to structures and functions of membranes in certain cells such as white blood cells or macrophages. Alkyl-Gro are found as well in bone marrow lipids and in milk [1].

Marine sources of alkyl-Gro such as shark liver oil (SLO) contain high levels of these compounds as a mixture of few species varying by length and unsaturation of the alkyl chain [2] (Fig. 1).

Usual composition of alkyl chains in alkyl-Gro from SLO is found as follow: 12:0, 1–2%. 14:0, 1–3%; 16:0, 9–13%; 16:1 n-7, 11–13%; 18:0, 1–5%; 18:1 n-9, 54–68%; 18:1 n-7, 4–6%, and minor species (<1%).

Beneficial effects of SLO on health have been recognized in traditional medicine of northern counties involved in fishing, such as Japan, Norway or Iceland. In these countries the ancestral use of SLO was empirical as strengthening or wound heeling.

Experimental studies were performed during the late century, aiming to demonstrate whether alkyl-Gro from SLO, had biological properties and beneficial effects. Indeed several studies did observe interesting effects, such as haematopoiesis stimulation [3], lowering radiotherapy-induced injuries [4], or improving vaccination efficiency [5,6]. However, mechanisms for those multiple activities remained poorly understood.

Past few decades the family of bioactive lipids has grown tremendously. Lipids and phospholipids have prominent roles in cell-to-cell communications as well in intracellular signals. Membrane phospholipids are precursors of bioactive lipids either agonists of specific membrane receptors, or second messengers and transcription regulators involved in intracellular signallings. Interestingly lipids are the single biochemical class that allows variability in structure depending on the nutrition. Therefore one may modify by nutritional supply the lipid composition of cell membranes and as a result, the structures and functions of membrane–derived bioactive lipids.

We have demonstrated that alkyl-Gro can be incorporated into the phospholipids of several cell types such as THP1 monocytes [7] endothelial cells [8] or blood platelets [9]. Furthermore in THP1 and endothelial cells, resulting 1-O-alkylglycerophospholipids participated to the production of Platelet-activating Factor (PAF) [7] and 1-

* Corresponding author. Tel.: +33 2 23 23 48 75; fax: +33 2 23 23 48 76.
E-mail address: alain.legrand@univ-rennes1.fr (A.B. Legrand).

0300-9084/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2009.12.010

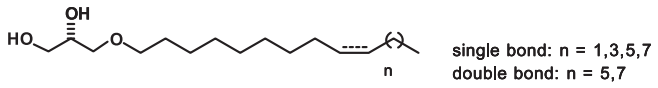

Fig. 1. General structure of natural alkyl-Gro from shark liver oil.

O-alkyl-2-acylglycerol [8], an analogue of diacylglycerol (DAG) with inhibiting effect on protein kinase C [10], respectively. Since PAF plays a key role in sperm functions an interesting hypothesis was that alkyl-Gro could modify sperm physiology, and actually we showed that alkyl-Gro improved boar sperm motility and fertility [11,12].

We have also demonstrated that oral alkyl-Gro from shark liver oil increased vaccination-induced raise in specific immunoglobulins in serum and colostrum of pregnant sows. Furthermore, alkyl-Gro and specific immunoglobulins were also increased in colostrum, and beneficial effects were observed on offspring haematopoiesis, immunological defences, and growth [13].

2. Anti-tumour activities of natural alkylglycerols

Using a model of solid tumour grafted in mice (Lewis lung carcinoma cells = LLC), we have evaluated the anti-tumour effects of oral SLO and of natural alkyl-Gro purified from the same source. We found that both treatments reduced significantly the growth of grafted tumours [14]. Furthermore, both treatments also reduced the number of pulmonary metastases. These data demonstrated that alkyl-Gro are active molecules accounting for anti-tumour activities of shark liver oil. The mechanisms by which alkyl-Gro exert these anti-tumour properties may be multiple. To evaluate anti-neoangiogenic activity we measured the density of an endothelial marker in tumours. After an alkyl-Gro oral treatment as short as 5 days, we established that density of von Willebrand factor in grafted tumours was decreased by 26% as compared to control group [14].

Anti-neoangiogenic activities might be one of the possible mechanisms for anti-tumour activities of alkyl-Gro [15]. Since basic Fibroblast Growth Factor (bFGF), is a major angiogenesis stimulator [16], we studied the effect of natural mix of alkyl-Gro on endothelial cell proliferation stimulated by bFGF and showed that alkyl-Gro reduced the bFGF stimulating effect [17].

3. Which 1-*O*-alkylglycerols from shark liver oil have anti-tumour activities?

Since most studies describing alkyl-Gro effects were obtained using a mix of several natural compounds, it was of interest to explore the activity of each single compound. We synthesized six of the major of natural alkyl-Gro mix, namely: 1) AKG 12:0 = 1-*O*-Dodecyl-*sn*-glycerol, 2) AKG 14:0 = 1-*O*-Tetradecyl-*sn*-glycerol, 3) AKG 16:0 = 1-*O*-Hexadecyl-*sn*-glycerol (chimyl alcohol), 4) AKG 18:0 = 1-*O*-Octadecyl-*sn*-glycérol (batyl alcohol), 5) AKG 16:1 = 1-*O*-(*Z*)-9′-Hexadecenyl-*sn*-glycerol, 6) AKG 18:1 = 1-*O*-(*Z*)-9′-Octadecenyl-*sn*-glycerol (selachyl alcohol).

The activities of each of these compounds were compared on the same *in vivo* model of solid tumour grafted in mice [14]. We found that AKG 16:1 and 18:1 were the most potent compounds on tumour growth and lung macrometastasis number, while other compounds (AKG 16:0 and 12:0) had weaker activities. We observed also that AKG 18:0 did not reduce but trended to increase tumour growth and metastasis number (Table 1).

We also observed important variations in spleen weights at the end of the experiment (day 20). In non-grafted mice, natural mix of alkyl-Gro had no effect on spleen weights. Tumour graft induced a strong increase in spleen weights of the control group. Alkyl-Gro treatments resulted in a reduction of spleen weights in most groups, with stronger effects observed in AKG 16:1 and 18:1 groups in which spleen weights decreased to levels similar of those in non-grafted groups. By contrast, spleen weight in AKG 18:0 group was significantly increased as compared with olive group (Fig. 2).

4. Cytotoxic and cytostatic effects on endothelial cells

On Human umbilical vein endothelial cells (HUVEC), MTT test [18] revealed absence of cytotoxicity after 72 h at concentrations lower than 20 μM for any synthetic alkyl-Gro. We studied the effect of 72 h incubation with synthetic alkyl-Gro on [^{3}H]-thymidine incorporation into HUVEC, and we observed that AKG 18:1 and 16:1 again had the strongest effects at concentrations devoid of cytotoxic effects (Table 2).

5. Discussion

The anti-tumour and anti-metastasis activities of natural alkyl-Gro from SLO are essentially supported by the unsaturated compounds with 16 and 18 carbon alkyl chain. These activities are associated with a strong reduction of tumour-induced increase in spleen weight. These two compounds represent mainly 70–80% of total alkyl-Gro from SLO, explaining activity of natural mix of alkyl-Gro. However, the natural mix contains compounds without or with weak activities, and a minor compound, AKG 18:0 which has remarkable opposite effects on spleen weight. Therefore the use of the two most active compounds could be of interest for improving anti-tumour and anti-metastasis efficiency of alkyl-Gro. The splenomegaly observed after 3LL cell graft reflects spleen invasion by immature myeloid cells, proeminently neutrophil granulocytes [19,20], and is associated with a decrease in blood B and T lymphocytes [19], both events impairing efficient immune responses to tumour cells. Spleen size reduction observed in grafted mice treated with AKG 18:1 and 16:1 has poorly understood mechanism, it could however contribute to restore immune defences against tumour cells.

Table 1
Effect of alkyl-Gro on tumour volume and lung metastasis number after Lung Lewis Carcinoma (LLC) cell grafting in mice. LLC cells were grafted in the leg of the mice which were treated orally (25 mg/day) with alkyl-Gro or with olive oil (control) for 20 days. Tumour volume was followed every other day. At day 20, lungs were collected and lung metastases were counted. (2 separate experiments, 10 mice per group) Significance of the difference between groups was assessed by one-way ANOVA and the rank test of Mann and Whitney was used to compare groups. $^{**}p < 0.01$ and $^{***}p < 0.001$.

Alkyl-Gro	None	12:0	14:0	16:0	18:0	16:1	18:1	Natural mix
Tumour volume (percent of control)								
Mean	100	81.15***	85.72	95.48	103.05	26.92***	50.5**	78.09***
± SEM	2.13	3.27	6.73	8.62	8.93	3.31	2.37	2.32
Lung metastases (percent of control)								
Mean	100	87.26	81.89	64.98**	104.4	9.07***	25.14***	37.84***
± SEM	9.45	16.28	17.31	15.42	20.21	3.53	5.46	7.01

Please cite this article in press as: A.-L. Deniau, et al., Which alkylglycerols from shark liver oil have anti-tumour activities?, Biochimie (2010), doi:10.1016/j.biochi.2009.12.010

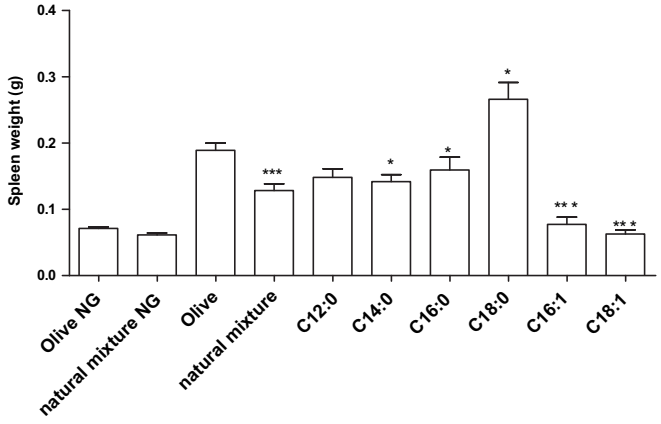

Fig. 2. Effects of alkyl-Gro on spleen weight (g) in mice grafted with Lewis lung carcinoma cells (LLC). Alkyl-Gro were administered orally (25 mg/day) by incorporation in the food for 20 days. At day 0, mice receive intramuscular implantation of LLC (10^6 cells/100 μl). Control groups received olive oil (25 mg/day). At day 20, spleens were collected and weighed. Values are mean ± SEM. (2 separate experiments, 10 mice per group). NG = non-grafted mice. Significance of the difference between treated and untreated groups in LLC grafted mice was assessed by the rank test of Mann and Whitney, $^{*}p < 0.05$ and $^{***}p < 0.001$.

Table 2
Inhibiting effect of alkyl-Gro on [^{3}H]-thymidine incorporation into HUVEC. HUVEC were incubated for 72 h without or with increasing concentrations (1–50 μM) of alkyl-Gro. At $t = 64$ h, [^{3}H]-thymidine was added and at $t = 72$ h incubation was stopped by methanol. Labelled DNA was precipitated using 10 percent perchloric acid (v/v), dissolved in 0.5 N NaOH, and incorporated radioactivity was quantified by liquid scintillation counting. Concentrations inhibiting 50 percent of [^{3}H]-thymidine incorporation into control cells (IC 50) were established graphically.

Alkyl-Gro	18:1	16:1	12:0	16:0	14:0	18:0
IC 50 (μM)	9.2	9.6	13.8	15	27	–

Alkyl-Gro have only weak in vitro cytotoxic effects on 3LL cells [21] and cytotoxicity is not likely to be the major mechanism of alkyl-Gro anti-tumour activity,

Mechanisms of anti-tumour effects also could be related to anti-angiogenic activities, since both unsaturated alkyl-Gro reduced endothelial proliferation which is involved in neo-angiogenesis.

Several biochemical mechanisms could explain the multiple activities of alkyl-Gro. Their role in increasing PAF precursor and amplifying PAF production could explain several effects related to PAF activities such as immuno-stimulation or immuno-modulation. Another interesting track is the interaction of 1-*O*-alkyl-2-acylglycerol with protein kinase C. This compound which is produced following cell stimulation after alkyl-Gro incorporation in membrane phospholipids [8], is an analogue of DAG which inhibits PKC [10]; 1-*O*-alkyl-2-acylglycerol binds to, but do not activates PKC-ε and compete with DAG; this could result in cell growth arrest [22] as observed in endothelial cells [17].

Finally the relation between unsaturation and anti-tumour activities of alkyl-Gro remains uncertain and deserves to be studied in the future.

Statistics

Data are presented as mean ± SEM. Significance of the difference between treated and control groups was assessed by the indicated statistical test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Patents

WO 2006/024742, USA 11/ 453/309, FR n°0953443.

Acknowledgements

Ligue Nationale Contre le Cancer, Région Bretagne, Société Polaris, Pleuven (56), France.

References

[1] B. Hallgren, S.O. Larsson, The glyceryl ethers in man and cow. J. Lipid Res. 3 (1962) 39–43.
[2] C.G. Bordier, N. Sellier, A.P. Foucault, F. Le Goffic, Purification and characterization of deep sea shark Centrophorus squamosus liver oil 1-*O*-alkylglycerol ether lipids. Lipids 31 (1996) 521–528.
[3] J.W. Linman, M.J. Long, D.R. Korst, F.H. Bethell, Studies on the stimulation of hemopoiesis by batyl alcohol. J. Lab. Clin. Med. 54 (1959) 335–343.
[4] A. Brohult, J. Brohult, S. Brohult, I. Joelsson, Effect of alkoxyglycerols on the frequency of injuries following radiation therapy for carcinoma of the uterine cervix. Acta Obst. Gynecol. Scand. 56 (1977) 441–448.
[5] B.Z. Ngwenya, D.M. Foster, Enhancement of antibody production by lysophosphatidylcholine and alkylglycerol. Proc. Soc. Exp. Biol. Med. 196 (1991) 69–75.
[6] A. Brohult, J. Brohult, S. Brohult, Effect of irradiation and alkoxyglycerol treatment on the formation of antibodies after salmonella vaccination. Experientia 28 (1972) 954–955.
[7] A. Hichami, V. Duroudier, V. Leblais, et al., Modulation of platelet-activating-factor production by incorporation of naturally occurring 1-*O*-alkylglycerols in phospholipids of human leukemic monocyte-like cells THP-1. Eur. J. Biochem. 250 (1997) 242–248.
[8] K. Marigny, F. Pédrono, C. Martin–Chouly, H. Youmine, B. Saïag, A.B. Legrand, Modulation of endothelial permeability by 1-O-alkylglycerols. Acta Physiol. Scand. 176 (2002) 263–268.
[9] F. Pédrono, C. Cheminade, A.B. Legrand, Natural 1-*O*-alkylglycerols reduce platelet-activating factor-induced release of [^{3}H]-serotonin in rabbit platelets. Prostag. Leukot. Ess. Fatty Acids 71 (2004) 19–23.
[10] F. Heymans, C. Da Silva, N. Marrec, J.-J. Godfroid, M. Castagna, Alkyl analogs of diacylglycerols as activators of protein kinase C. FEBS Lett. 218 (1987) 35–40.
[11] C. Cheminade, V. Gautier, A. Hichami, P. Allaume, D. Le Lannou, A.B. Legrand, 1- *O*-alkylglycerols improve boar sperm motility and fertility. Biol. Reprod. 66 (2002) 421–428.
[12] R. Mitre, C. Cheminade, P. Allaume, P. Legrand, A.B. Legrand, Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm. Theriogenology 62 (2004) 1557–1566.
[13] R. Mitre, M. Etienne, S. Martinais, et al., Humoral defence improvement and haematopoiesis stimulation in sows and offspring by oral supply of shark liver oil to mothers during gestation and lactation. Br. J. Nutr. 94 (2005) 753–762.
[14] F. Pédrono, B. Martin, C. Deluc, et al., Natural alkylglycerols restrain growth and metastasis of grafted tumour in mice. Nutr. Cancer 48 (2004) 64–69.
[15] E. Skopinska-Rozewska, M. Krotkiewski, E. Sommer, et al., Inhibitory effect of shark liver oil on cutaneous angiogenesis induced in Balb/c mice by syngeneic sarcoma L-1, human urinary bladder and human kidney tumour cells. Oncol. Rep. 6 (1999) 1341–1343.
[16] M. Presta, P. Dell'Era, S. Mitola, E. Moroni, R. Ronca, M. Rusnati, Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 16 (2005) 159–178.
[17] F. Pédrono, B. Saïag, J.-P. Moulinoux, A.B. Legrand, 1-O-alkylglycerols reduce the stimulating effects of bFGF on endothelial cell proliferation in vitro. Cancer Lett. 251 (2007) 317–322.
[18] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65 (1983) 55–63.
[19] J.-K. Lee, T.C. Back, K.L. Komschlies, F.W. Ruscetti, H.A. Young, R.H. Wiltrout, Hematopoietic switch from lymphoid to granulocytic development in 3LL tumor-bearing mice. In Vivo 15 (2001) 255–264.
[20] M. Shin-Ya, O. Mazda, C. Tsuchihara, H. Hirai, J. Imanishi, M. Takeuchi, Interleukin-2 abolishes myeloid cell accumulation induced by Lewis lung carcinoma. J. Interferon Cytokine Res. 23 (2003) 631–638.
[21] F. Pédrono, Effets anti-angiogénique et anti-tumoral d'alkylglycérols naturels: etude in vitro et in vivo. PhD thesis, University of Rennes 1, France, 2 Décembre 2002, pp. 129–131.
[22] K.L. Houck, T.E. Fox, L. Sandirasegarane, M. Kester, Ether-linked diglycerides inhibit vascular smooth muscle cell growth via decreased MAPK and PI3K/Akt signalling. Am J Physiol 295 (2008) H1657–H1668.

Please cite this article in press as: A.-L. Deniau, et al., Which alkylglycerols from shark liver oil have anti-tumour activities?, Biochimie (2010), doi:10.1016/j.biochi.2009.12.010