Available online at www.sciencedirect.com
SCIENCE DIRECT®
Prostaglandins, Leukotrienes and Essential Fatty Acids 71 (2004) 19–23
Prostaglandins Leukotrienes and Essential Fatty Acids
www.elsevier.com/locate/plefa

Natural 1-*O*-alkylglycerols reduce platelet-activating factor-induced release of [^{3}H]-serotonin in rabbit platelets

F. Pédrono, C. Cheminade, A.B. Legrand*

Laboratoire de Pharmacologie Moléculaire, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes I, 2 avenue du Pr Léon Bernard, 35043 Rennes Cedex, France

Received 3 July 2003; accepted 1 December 2003

Abstract

Natural 1-*O*-alkylglycerols have multiple biological activities with distinct mechanisms. In THP-1 monocytes, they amplify platelet-activating factor production. In endothelial cells, they participate in the production of 1-*O*-alkyl-2-acyl-*sn*-glycerol, a PKC inhibitor. Since PAF as well as PKC may interfere with platelet functions, we studied the effect of natural alkylglycerols purified from shark liver oil on [^{3}H]-serotonin release from rabbit platelets in vitro. [^{3}H]-alkylglycerols (1 μM) were consistently incorporated into platelet lipids and after a 2-h incubation, they were metabolised into phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, which represented 53.5±1.7%, 36.3±1.8%, 5.3±0.5% of metabolised [^{3}H]-alkylglycerols, respectively. Alkylglycerols (10 μM) had no effect on spontaneous [^{3}H]-serotonin release. However, alkylglycerols partially inhibited PAF-induced [^{3}H]-serotonin release while they did not modify thrombin-induced release. These data show that alkylglycerols inhibit partially and specifically PAF-induced platelet stimulation and suggest that this effect could result from interfering with PAF receptors.

Keywords: Alkylglycerols; Platelet activation; Platelet activating factor

1. Introduction

Blood platelets play a key role in haemostasis. Activation of platelets also participate in patho-physiological mechanisms involved in various diseases, such as thrombotic heart disease [1,2], hypertension [3–5], autoimmune diseases [6,7], diabetes [8], and might play an important role in metastasic growth of cancer cells [9–12]. Platelet activation is characterised by changes in cell morphology and by the release of both the contents of intracellular granules and neoformed lipidic mediators such as eicosanoids and platelet-activating factor (PAF).

1-*O*-alkylglycerols (alkyl-Gro) are naturally occurring ether-lipids, present in human or cow milk and in hematopoietic organs, such as bone marrow; they are particularly abundant in shark liver oil [13,14]. Alkyl-Gro have several biological activities at low concentrations, suggesting pharmacological mechanisms: They possess anti-tumour effect and prevent radiotherapy side effects [15–17], including leukopenia and thrombocytopenia [18] and they modulate the immune system [19–21]. Recently we have established that alkyl-Gro could also improve in vitro as well as in vivo gamete functions [22]. The mechanisms of these various effects are not clearly established; however, we have shown that alkyl-Gro could incorporate into membrane 1-*O*-alkyl-phospholipids of several cell types [22–24], allowing them to modify cell signalling pathways involving phospholipases. In monocytes and spermatozoa, alkyl-Gro increase the production of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF) [24] or of its precursor and metabolite lyso-PAF [22], while in endothelial cells, activation of phospholipases produce 1-*O*-alkyl-2-acyl-*sn*-glycerol [23], an analogue of diacylglycerol (DAG) which is an inhibitor of protein kinase C (PKC) [25–26]. PAF is a potent platelet activator [27–29] while several platelet activating mediators, including PAF or thromboxane A_2, act through receptors coupled to phospholipase C and induce PKC activation. Therefore,

*Corresponding author. Tel.: +33-2-23-23-48-75; fax: +33-2-23-23-49-75.
E-mail address: alain.legrand@univ-rennes1.fr (A.B. Legrand).

0952-3278/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2003.12.003

although 1-*O*-hexadecyl-*sn*-glycerol has no proper effect on platelet aggregation [30], one could expect that alkyl-Gro might modify platelet functions by interfering with lipidic signallings. In this paper, we have studied the effect of alkyl-Gro on platelet functions in vitro. We show that alkyl-Gro inhibit partially PAF-induced activation of rabbit platelets.

2. Materials and methods

2.1. Reagents

[^{3}H]-serotonin (5-hydroxytryptamine binoxalate [1, 2, ^{3}H]) was obtained from Isotopchim (Ganagobie-Peyruis, France). Acetylsalicylic acid (Aspegic) was from Sanofi-Synthelabo (Paris, France). Gelatin type B, EDTA ((Ethylenedinitrilo) tetraacetic acid), Triton X100, PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), thrombin (from bovine plasma), formaldehyde and the adenosine diphosphate (ADP) scavenger complex phosphocreatine/creatine kinase (CP/CPK) were purchased from Sigma-Aldrich (La Verpillère, France). All solvents and silica gel 60 Å LK6 plates were purchased from Merck (Darmstadt, Germany).

2.2. Buffers

Buffer 1. Tyrode's gelatin without Ca^{2+}: 137 mM NaCl; 2.6 mM KCl; 12.1 mM $NaHCO_3$; 1.0 mM $MgCl_2$; 5.5 mM glucose; 4.2 mM HEPES; 0.25% (w/v) gelatin; pH 6.5 or 7.4; *Buffer 2*. Tyrode's gelatin without Ca^{2+} with EDTA: same as Buffer 1 but with 0.10 mM EDTA; *Buffer 3*. Tyrode's gelatin with Ca^{2+}: same as Buffer 1 but with 1.15 mM $CaCl_2$, pH 7.4; *Buffer 4*. Acid citrate dextrose (ACD): 71 mM citric acid, 85 mM sodium citrate, 111 mM dextrose, pH 4.4.

2.3. Alkylglycerols

Alkyl-Gro from liver oil of *Centrophorus squamosus* were a generous gift from Dr P. Allaume of the Centre Technique ID-MER (Lorient, France). The alkyl-Gro were prepared and purified from shark liver oil as described previously [24]. Alkyl-Gro species varied according to the alkyl-chain length, with composition as follows: 14:0 = 0.7%, 16:0 = 9.1%, 16:1n-7 = 12.5%, 18:1n-9 = 68.1%, 18:1n-7 = 4.8% and other minor species (<0.1%) = 4.8%. Tritiation of alkyl-Gro was performed by ^{3}H -labelling on the *sn*-3 position of glycerol as described elsewhere [24].

2.4. Platelet sampling

New Zealand white rabbit platelets were obtained as described previously [31]. Briefly, six volumes of blood were collected from the marginal vein into one volume of ACD and centrifuged at 375 *g* for 20 min. The platelet-rich plasma (PRP) was collected and centrifuged at 375 g for 20 min.

2.5. Incorporation of [^{3}H]-alkyl-Gro into rabbit platelet lipids

Platelets were suspended in buffer 1 (pH 7.4) (10^8 platelets/ml). [^{3}H]-alkyl-Gro (1 μM, 92.5 mCi/mmol) were added and the mixture was incubated at 37°C under 95% air + 5% CO_2. After indicated periods of time, 50×10^6 platelets were washed in buffer 1 (pH 7.4) and total lipids were extracted according to Bligh and Dyer's method [32]. Lipid extract was separated by thin layer chromatography (TLC) on silica gel plates using the following system chloroform:methanol:acetic acid (35:14:2.7, v/v) as mobile phase. Radioactive zones were visualised by a radiochromatogram scanner (Bioscan, Washington DC, USA). Phospholipid classes were identified by their retention factor (R_f), radioactive zones on the silica gel were scrapped off and the radioactivity was quantified in a liquid scintillation counter (Packard, USA). The silica containing non-polar lipids from the above TLC was scrapped off, extracted with ethyl acetate:0.1% acetic acid (1:0.025, v/v) and further analysed on silica gel TLC using the solvent system hexane:diethyl ether:acetic acid (40:10:0.2, v/v). The zones co-migrating with 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol (alkyl-DAG) or alkyl-Gro standards were scrapped off and the radioactivity was measured by liquid scintillation counting.

2.6. [^{3}H]-serotonin release from platelets

Platelet activation was quantified by a bioassay of [^{3}H]-serotonin release. Platelets were labelled as previously described by Ardlie et al. [33]. Briefly the PRP was incubated at 37°C for 45 min with [^{3}H]-serotonin (1 μCi/ml, 27.5 Ci/mmol) and acetylsalicylic acid (0.1 mM) for inhibition of cyclooxygenases. The platelets were then centrifuged at 1400 *g* for 20 min and washed in buffer 1 (pH 6.5). Cells were incubated at 37°C for 2 h under 95% air + 5% CO_2 in the presence of indicated concentrations of alkyl-Gro or in vehicle (ethanol 0.2%). Platelets were centrifuged at 1400 *g* for 20 min and washed first in Buffer 2 and then in Buffer 1 (pH 6.5). The pellet was gently resuspended in Buffer 2 (1.25×10^9 platelets/ml). For stimulation, labelled platelets (50 μl) were added to Buffer 3 containing CP/CPK (1 mM/10 U/ml respectively), for inhibiting ADP activation, and indicated concentrations of thrombin or PAF, or vehicle for control. The platelet suspension was stirred at 37°C for 3 and 10 min, for thrombin- and PAF-stimulations, respectively. The reaction was stopped by addition of 9.25% formaldehyde (20 μl),

platelets were centrifuged at 2500 *g* for 15 min at 4°C, and supernatant was collected for measurement of released [^{3}H]-serotonin. A fraction of labelled platelets was lysed by 10% Triton X-100 (20 µl) and radioactivity was measured to establish total incorporated [^{3}H]-serotonin. Each data point was expressed as released [^{3}H]-serotonin/total incorporated [^{3}H]-serotonin × 100.

2.7. Calculation and statistics

All data are the mean of the indicated number of experiments performed in two or more repetitions. Significance of treatments was tested by ANOVA or by significance of correlation coefficient *r*, and individual differences were checked by Mann and Whitney non-parametric test.

3. Results

3.1. Incorporation of [^{3}H]-alkyl-Gro into platelet lipids

Incubation of platelets in the presence of [^{3}H]-alkyl-Gro (1 µM) resulted in a time-dependent incorporation of radioactive material into several phospholipid or neutral lipid classes. This incorporation was predominantly observed into 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkyl-PC) and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (alkyl-PE). After 4 h, this incorporation reached 44 ± 3.3, 29 ± 1.5 and 4.3 ± 0.4 pmol/10^8 platelets for alkyl-PC, alkyl-PE and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoinositol (alkyl-PI), which represents 53.5 ± 1.7%, 36.3 ± 1.8%, 5.3 ± 0.5% of metabolised radioactive material, respectively (Fig. 1). No significant amount of radioactivity was detected in 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoserine. We also observed incorporation of [^{3}H]-alkyl-Gro into alkyl-DAG. The sum of [^{3}H]-alkyl-Gro which were metabolised into lipids after 4-h-incubation represented 35 ± 3% of radioactive material associated to cells. Unmodified [^{3}H]-alkyl-Gro were also fast either bound or incorporated in platelets (Fig. 1).

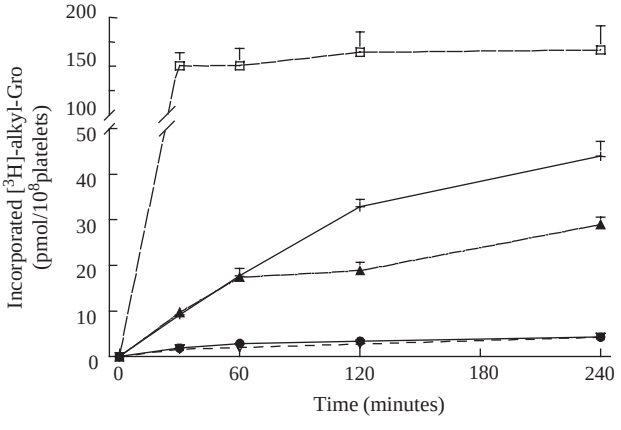

Fig. 1. Incorporation of [^{3}H]-alkyl-Gro into rabbit platelets. Platelets were incubated with [^{3}H]-alkyl-Gro (1 µM) for the indicated times. Lipids were then extracted and separated on TLC as described in Material and methods. Radioactivity of lipid classes was determined by liquid scintillation counting: alkyl-Gro (□), alkyl-DAG (▽), alkyl-PC (+), alkyl-PI (●), alkyl-PE (▲). Means ± SEM (n = 3, 3 repetitions).

3.2. Effects of alkyl-Gro on [^{3}H]-serotonin release

When platelets were incubated in the presence [^{3}H]-serotonin, we observed its uptake into the cells. The platelet content in [^{3}H]-serotonin was not modified when platelets were further incubated in the presence of up to 20 µM of alkyl-Gro for 2 h (data not shown).

3.2.1. PAF-stimulated platelets

Incubation of platelets with increasing concentrations of PAF resulted in a concentration-dependent release of [^{3}H]-serotonin (Fig. 2). When platelets were first incubated for 2 h with alkyl-Gro (10 µM) before PAF stimulation, we observed a partial and significant inhibition of the stimulating effect of PAF (Fig. 2). To assess that this inhibition was concentration-dependent, platelets were stimulated by PAF (120 pM) after 2-h-incubation in the presence of increasing concentrations of alkyl-Gro. This resulted in a significant concentration-dependent drop in PAF stimulating effect on [^{3}H]-serotonin release (r = 0.741, P < 0.01) (Fig. 3).

3.2.2. Thrombin-stimulated platelets

Platelet stimulation by increasing concentrations of thrombin resulted in a concentration-dependent raise in [^{3}H]-serotonin release. When platelets had been first

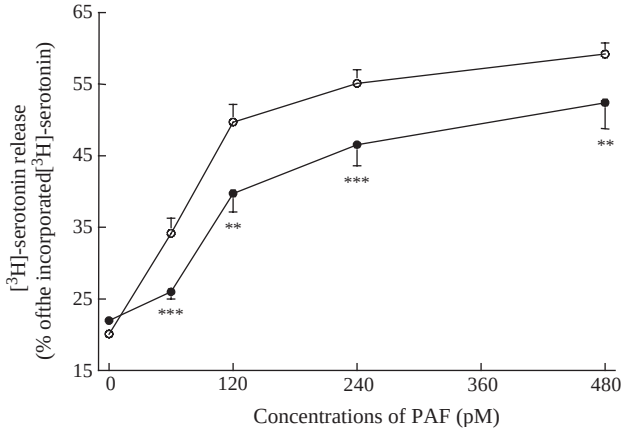

Fig. 2. [^{3}H]-serotonin release from rabbit platelets treated with alkyl-Gro and stimulated with different concentrations of PAF. Platelets were labelled with [^{3}H]-serotonin, incubated for 2 h with alkyl-Gro (10 µM) (●) or vehicle (○) and then stimulated with indicated concentrations of PAF for 10 min as described in Materials and methods. Mean ± SEM (n = 7, 2 repetitions). Significance of differences between alkyl-Gro and control: P < 0.001 (three-way ANOVA) and individual differences tested by Mann and Whitney's test: $^{**}P$ < 0.01 and $^{***}P$ < 0.001.

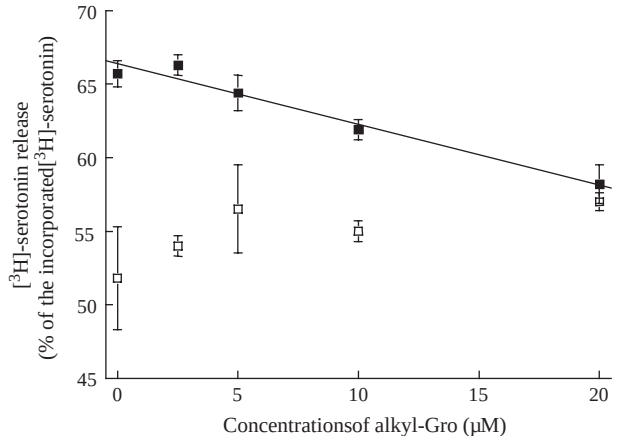

Fig. 3. [^{3}H]-serotonin release from rabbit platelets treated with different concentrations of alkyl-Gro and stimulated with PAF. Platelets were labelled with [^{3}H]-serotonin, incubated with indicated concentrations of alkyl-Gro for 2 h and then stimulated for 10 min with PAF (120 pM) (■) or vehicle (□) as described in Materials and methods. Mean ± SEM ($n = 4$, 2 repetitions). Correlation between [^{3}H]-serotonin release and alkyl-Gro concentration in the presence of PAF: $r = 0.741$, $P < 0.01$.

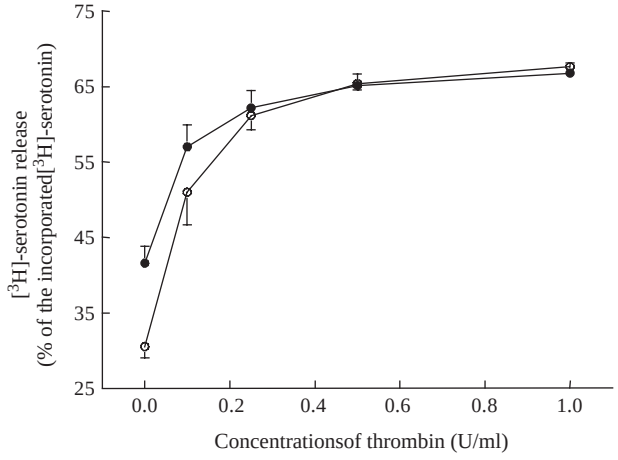

Fig. 4. [^{3}H]-serotonin release from rabbit platelets treated with alkyl-Gro and stimulated with different concentrations of thrombin. Platelets were labelled with [^{3}H]-serotonin, incubated for 2 h with alkyl-Gro (10 μM) (●) or vehicle (○) and then stimulated with indicated concentrations of thrombin for 3 min as described in Materials and methods. Mean ± SEM ($n = 7$, 2 repetitions). No significance between alkyl-Gro and control (three-way ANOVA).

incubated for 2 h in the presence of 10 μM alkyl-Gro, we observed, in contrast with data obtained with PAF, that thrombin-induced [^{3}H]-serotonin release was not modified (Fig. 4).

4. Discussion

Naturally occurring alkyl-Gro have potent biological activities on various cells or systems [34]. They are incorporated into phospholipids of cultured transformed monocyte-like cell line THP1 [24], endothelial cells [23] or, in a lesser extent, spermatozoa [22]. Therefore, mechanisms of their effects may include interfering with the production of lipidic second messengers [23] or mediators [22,24]. Effects of alkyl-Gro on platelets are poorly documented; Le Blanc [30] found a weak effect of 1-*O*-hexadecyl-*sn*-glycerol on human platelet aggregation. Since platelet phospholipids have a key role as precursors of potent bioactive lipids such as DAG, PAF or eicosanoids, we investigated the in vitro effect of alkyl-Gro on rabbit blood platelets. We first observed that [^{3}H]-alkyl-Gro were highly incorporated into platelet phospholipids, predominantly into the PAF precursor alkyl-PC, and to a lesser extent into alkyl-PE. Alkyl-PC also might be hydrolysed by phospholipases C or D and provide 1-*O*-alkyl analogues of DAG. Such DAG analogues with an ether bond at 1-*sn* position have no ability to activate PKC, and inhibit the stimulating effect of DAG on PKC [25,26]. Since PKC activation is a ubiquitous signalling for secretion, it was of interest to explore the effects of alkyl-Gro on [^{3}H]-serotonin release by platelets.

Alkyl-Gro had no significant effect on spontaneous [^{3}H]-serotonin release. Alkyl-Gro also decreased the magnitude of the PAF-induced [^{3}H]serotonin release, while they had no such effect when thrombin was used as [^{3}H]-serotonin release activator.

Our data show that alkyl-Gro display contrasted effects on [^{3}H]-serotonin release depending on the stimulus. Since alkyl-Gro had no inhibiting effects on thrombin-triggered [^{3}H]-serotonin release, their inhibiting effect observed with PAF-stimulated platelet is not likely to result from influencing common pathway such as alteration of PKC activation. Either alkyl-Gro are not likely to stimulate the PAF production, because this would result in an increased [^{3}H]-serotonin release under stimulation with low concentrations of PAF. The selective inhibition of alkyl-Gro on PAF stimulating effect could result from an antagonist effect of alkyl-Gro on PAF receptor.

Thus, the multiple biological activities of alkyl-Gro could stem from several and distinct mechanisms. Amplification of PAF production by THP1 cells can be explained mainly by increasing PAF precursors in these cells [24]. This mechanism might account for the immunostimulating properties of alkyl-Gro since PAF is a potent mediator involved in macrophage-triggered immunological responses [19]. Another and totally different mechanism is the production of DAG analogues with inhibiting effects on PKC. We have observed such an effect in endothelial cells [23]. A similar mechanism is suggested in the inhibition by alkyl-Gro of phorbol ester-induced arachidonic acid release [35]. Our present data suggest that alkyl-Gro may also alter PAF-induced responses, possibly by antagonist action on PAF-receptors.

The in vivo consequences of alkyl-Gro effect on platelet functions have not yet been explored. Since shark liver oil is used as a nutritional complement and contains two lipidic compounds with known or potential activities on platelets, namely n-3 unsaturated fatty acids and alkyl-Gro, further exploration of shark liver oil consumption on platelet functions in vivo would deserve further attention and exploration.

Acknowledgements

This study was supported by "Région Bretagne" and "Ligue Nationale contre le Cancer".

References

[1] M. Vejar, G. Fragasso, D. Hackett, D.P. Lipkin, A. Maseri, G.V. Born, G. Giabattoni, C. Patrono, Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina, Thromb. Haemost. 63 (1990) 163–168.
[2] V.K. Puri, M. Verma, A.K. Saxema, K. Shanker, Platelet serotonergic mechanisms in ischaemic heart disease, Thromb. Res. 57 (1990) 445–451.
[3] S. Nityanand, B.L. Tekwani, M. Chandra, K. Shanker, B.N. Singh, Kinetics of serotonin in platelets in essential hypertension, Life Sci. 46 (1990) 367–372.
[4] P. Guicheney, M.A. Devynck, J.F. Cloix, M.G. Pernollet, M.L. Grichois, P. Meyer, Platelet 5-HT content and uptake in essential hypertension: role of endogenous digitalis-like factors and plasma cholesterol, J. Hypertens. 6 (1988) 873–879.
[5] P. Hervé, L. Drouet, C. Dosquet, J.M. Launay, B. Rain, G. Simonneau, J. Caen, P. Duroux, Primary pulmonary hypertension in a patient with a familiar platelet storage pool disease: role of serotonin, Am. J. Med. 89 (1990) 117–120.
[6] J. Zeller, E. Weissbarth, B. Baruth, H. Mielke, H. Deicher, Serotonin content of platelets in inflammatory rheumatic diseases. Correlation with clinical activity, Arthritis Rheum. 26 (1983) 532–540.
[7] M.L. Biondi, B. Marasini, E. Bianchi, A. Agostoni, Plasma free and intraplatelet serotonin in patients with Raynaud's phenomenon, Int. J. Cardiol. 19 (1988) 335–339.
[8] M.A. Barradas, D.S. Gill, V.A. Fonseca, D.P. Mikhailidis, P. Dandona, Intraplatelet serotonin in patients with diabetes mellitus and peripheral vascular disease, Eur. J. Clin. Invest. 18 (1988) 399–404.
[9] I. Helland, B. Klementsen, L. Jorgensen, Addition of both platelets and thrombin in combination accelerates tumor cells to adhere to endothelial cells in vitro, In vitro Cell Dev. Biol. Anim. 33 (1997) 182–186.
[10] B. Klementsen, L. Jorgensen, Mechanisms involved in the early interaction between HeLa cells, platelets and endothelial cells in vitro under the influence of thrombin. Effects of acetylsalicylic acid and Na-salicylate, APMIS 105 (1997) 391–401.
[11] H.M. Pinedo, H.M. Verheul, R.J. D'Amoto, J. Folkman, Involvement of platelets in tumour angiogenesis? Lancet 352 (1998) 1775–1777.
[12] S. Karpatkin, E. Pearlstein, C. Ambrogio, B.S. Coller, Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo, J. Clin. Invest. 81 (1988) 1012–1019.
[13] B. Hallgren, S. Larsson, The glyceryl ethers in the liver oils of elasmobranch fish, J. Lipid Res. 3 (1962) 31–38.
[14] B. Hallgren, S. Larsson, The glyceryl ethers in man and cow, J. Lipid Res. 3 (1962) 39–43.
[15] 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 Obstet. Gynecol. Scand. 56 (1977) 441–448.
[16] A. Brohult, J. Brohult, S. Brohult, Regression of tumor growth after administration of alkoxyglycerols, Acta Obstet. Gynecol. Scand. 57 (1978) 79–83.
[17] A. Brohult, J. Brohult, S. Brohult, I. Joelsson, Reduced mortality in cancer patients after administration of alkoxyglycerols, Acta Obstet. Gynecol. Scand. 65 (1986) 779–785.
[18] J.W. Linman, Hemopoietic effects of glyceryl ethers. III. Inactivity of selachyl alcohol, Proc. Soc. Exp. Biol. Med. 104 (1960) 703–706.
[19] S. Homma, N. Yamamoto, Activation process of macrophages after in vivo treatment of mouse lymphocytes with dodecylglycerol, Clin. Exp. Immunol. 79 (1990) 307–313.
[20] B.Z. Ngwenya, D.M. Foster, Enhancement of antibody production by lysophosphatidylcholine and alkylglycerol, Proc. Soc. Exp. Biol. Med. 196 (1991) 69–75.
[21] S.Y. Oh, L.S. Jadhav, Effects of dietary alkylglycerols in lactating rats on immune responses in pups, Pediatr. Res. 36 (1994) 300–305.
[22] 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.
[23] K. Marigny, F. Pédrono, C.A.E. Martin-Chouly, H. Youmine, B. Saïag, A.B. Legrand, Modulation of endothelial permeability by 1-O-alkylglycerols, Acta Physiol. Scand. 176 (2002) 263–268.
[24] A. Hichami, V. Duroudier, V. Leblais, L. Vernhet, F. Le Goffic, E. Ninio, A. Legrand, Modulation of platelet-activating-factor production by incorporation of naturally occurring 1-O-alkylglycerols in phospholipids of human leukemic monocyte-like THP-1 cells, Eur. J. Biochem. 250 (1997) 242–248.
[25] F. Heymans, C. Da Silva, N. Marrec, J.J. Godfroid, M. Castagna, Alkyl analogs of diacylglycerol as activators of protein kinase C, FEBS Lett. 218 (1987) 35–40.
[26] L.W. Daniel, G.W. Small, J.D. Schmitt, C.J. Marasco, K. Ishaq, C. Piantadosi, Alkyl-linked diglycerides inhibit protein kinase C activation by diacylglycerols, Biochem. Biophys. Res. Commun. 151 (1988) 291–297.
[27] P. Braquet, L. Touqui, T.Y. Shen, B.B. Vargaftig, Perspectives in platelet-activating factor research, Pharmacol. Rev. 39 (1987) 97–145.
[28] M. Chignard, J.P. Le Couedic, M. Tencé, B.B. Vargaftig, J. Benveniste, The role of platelet-activating factor in platelet aggregation, Nature 279 (1979) 799–800.
[29] W. Chao, M.S. Olson, Platelet-activating factor: receptors and signal transduction, Biochem. J. 292 (1993) 617–629.
[30] K. Le Blanc, J. Samuelsson, J. Brohult, A. Berg, J. Palmblad, 1-O-hexadecyl-2-methoxy-glycero-3-phosphatidylcholine—a methoxy ether lipid inhibiting platelet activating factor-induced platelet aggregation and neutrophil oxydative metabolism, Biochem. Pharmacol. 49 (1995) 1577–1582.
[31] M.J. Bossant, E. Ninio, D. Delautier, J. Benveniste, Bioassay of paf-acether by rabbit platelet aggregation, Methods Enzymol. 187 (1990) 125–130.
[32] E.G. Bligh, W.J. Dyer, Arapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[33] N.G. Ardlie, M.A. Packham, J.F. Mustard, Adenosine diphosphate-induced platelet aggregation in suspensions of washed rabbit platelets, Br. J. Haematol. 19 (1970) 7–17.
[34] P.T. Pugliese, K. Jordan, H. Cederberg, J. Brohult, Some biological actions of alkylglycerols from shark liver oil, J. Altern. Complement Med. 4 (1998) 87–99.
[35] M. Robinson, R. Burdine, T.R. Warne, Inhibition of phorbol ester-stimulated arachidonic acid release by alkylglycerols, Biochim. Biophys. Acta 1254 (1995) 361–367.