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Ginkgo biloba and its anti-inflammatory value as a medical tool

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ABSTRACT: Ginkgolides are mainly extracted from the leaves of *Ginkgo biloba* tree and exhibit, among other properties, an intense anti-inflammatory role. This characteristic is attributed to their anti-PAF (Platelet Activating Factor) properties as ginkgolides and especially BN 52021, are the most potent specific PAF inhibitors. PAF is a lipid mediator, present during the inflammatory processes and implicated that way in numerous pathophysiological situations. PAF is synthesized by a wide variety of organisms and cells exerting its biological functions by binding to its specific receptor. There is a wide range of PAF inhibitors that act variously. BN 52021 is a natural specific PAF inhibitor which acts competitively locking PAF receptor in its inactive state. Atherosclerosis and other pathological complications have as common a chronic or an acute inflammatory response and therefore the implication of PAF. Different extracts of *Ginkgo biloba* have been used either in animal models or in clinical trials giving promising results in the aforementioned situations, as a result of its antioxidant properties. As a conclusion, in this article it is proposed for the first time that the beneficial effects

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Ginkgo biloba και η αντιφλεγμονώδης δράση του ως κλινικό εργαλείο

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ΠΕΡΙΛΗΨΗ: Τα γκινκγολίδια, κυρίως προερχόμενα από τα φύλλα του δέντρου *Ginkgo biloba*, διαθέτουν ανάμεσα στις πολλές ιδιότητές τους, έντονο αντιφλεγμονώδη ρόλο. Αυτό το γεγονός προέρχεται από τις αντι-PAF (Platelet Activating Factor, Παράγοντας Ενεργοποίησης Αιμοπεταλίων) ιδιότητές τους αφού τα γκινκγολίδια, και ειδικά το BN 52021, είναι οι πιο ισχυροί ειδικοί αναστολείς του PAF. Ο Παράγοντας Ενεργοποίησης Αιμοπεταλίων αποτελεί έναν λιποειδικό κυτταρικό μεσολαβητή, εμπλεκόμενο στις φλεγμονώδεις διεργασίες και κατ' επέκταση σε πληθώρα παθοφυσιολογικών καταστάσεων. Ο PAF βρίσκεται σε διάφορους οργανισμούς και κύτταρα ασκώντας τις δράσεις του μέσω της δέσμευσής του στον ειδικό του υποδοχέα. Υπάρχουν πολλά διαφορετικά είδη αναστολέων του PAF οι οποίοι δρουν ποικιλοτρόπως. Το BN 52021 αποτελεί τον πιο δραστικό φυσικό, ειδικό αναστολέα του PAF που δρα ανταγωνιστικά, σταθεροποιώντας τον υποδοχέα του σε αδρανή κατάσταση. Η αθηροσκλήρωση, όπως και πολλές άλλες παθολογικές καταστάσεις, έχουν ως κοινό χαρακτηριστικό τη χρόνια ή την άμεση φλεγμονώδη απόκριση και συνεπώς την εμπλοκή του PAF. Διάφορα εκχυλίσματα του *Ginkgo biloba* έχουν δοκιμαστεί, είτε

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of *Ginkgo biloba* extracts may be mainly exerted by its anti-PAF properties. This suggestion should be further studied in order to shed light on the anti-inflammatory pharmaceutical use of *Ginkgo biloba*.

Key words: *Ginkgo biloba*, Platelet Activating Factor, atherosclerosis, anti-inflammatory properties.

σε πειραματόζωα είτε σε κλινικές μελέτες, με υποσχόμενα αποτελέσματα, τα οποία αποδίδονται στη βιβλιογραφία στις αντιοξειδωτικές του ιδιότητες. Συμπερασματικά, στο άρθρο αυτό προτείνεται για πρώτη φορά ότι τα ευεργετικά χαρακτηριστικά του *Ginkgo biloba* πιθανώς να προέρχονται κυρίως από τις αντι-PAF (αντιφλεγμονώδεις) ιδιότητές τους. Η υπόθεση αυτή πρέπει να μελετηθεί εκτενέστερα με σκοπό να διαφωτιστεί η αντι-φλεγμονώδης φαρμακευτική δράση του *Ginkgo biloba*.

Λέξεις ευρετηρίου: *Ginkgo biloba*, παράγοντας ενεργοποίησης αιμοπεταλίων, αθηροσκλήρωση, αντι-φλεγμονώδεις ιδιότητες.

1. Introduction

Herbal products are known for their medicinal properties since the ancient years and they are still used individually or as complementary therapies to deal with various pathological conditions. St John's wort (depression), garlic (cardiovascular risk factors), echinacea (immunostimulation), cranberries (urinary infection), mistletoe (cancer), peppermint (irritable bowel syndrome), saw palmetto (prostate hyperplasia) and valerian root (insomnia) are some of the most known herbal products with medicinal use.[1]

1.1. Ginkgo biloba

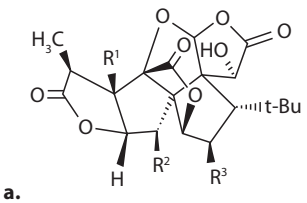
An outstanding position among herbal products holds the *Ginkgo biloba* extract (*Gb*E) which belongs to the living fossils as its species date back to the Permian period. In contrast to other plants and animals of that period, *Ginkgoaceae* tree manage to survive along the centuries evolving to *Ginkgo biloba*.[2] This plant comes from China with a therapeutic history of 5000 years when it was mainly used as an asthma, heart disease and bronchitis remedy. Its medicinal properties may have been known since then but it was not until 1983 that it was found out that the origin of its biological activity is coming from its leaves and roots.[3,4]

1.2. Ginkgo biloba's secondary metabolites

The main secondary metabolites of *Ginkgo biloba* are terpenes, flavonoids and organic acids but carbohydrates, long chain hydrocarbons and lipids, inorganic salts or complexes and other miscellaneous organic compounds have also been reported. From all of them, the ones that have mostly concerned the international bibliography because of their biological activity are: ginkgolides, bilobalide, polyphenols, kaempferol, quercetin, ginkgetin and isoginkgetin.[5] The most commonly used preparation of *Ginkgo biloba* is called EGb761 and contains 24% flavonoid glycosides (mainly quercetin, kaempferol and isorhamnetin), 6% terpene lactones (ginkgolides and bilobalide) and no more than 5 ppm ginkgolic acids.[6]

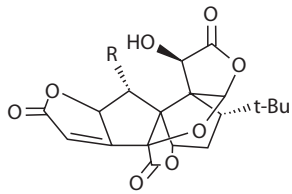
Flavonoids and polyphenols provide *Ginkgo biloba* extracts with antioxidant properties mostly by reducing free radical formation and scavenging free radicals but also by promoting the expression of antioxidant proteins which, in turn, increase endogenous antioxidant metabolites such as glutathione.[7–9] While flavonoids and polyphenols can be obtained from many other plants, ginkgolides and bilobalide, only occur in *Ginkgo biloba* extracts.[10]

1.2.1. Ginkgolides and bilobalide. Although ginkgolides were first isolated by Furukawa in 1932, their structure was resolved by other scientists as Nakanishi et al, Maruyama et al and Weinges et al who characterized and named them as BN 52020, BN 52021, BN 52022, BN 52024 and BN 52023 (also termed as A, B, C, J and M).[11] Later Wang et al proposed two new trace ginkgolides, namely K and L,[4,5,12] and recently two more structures of ginkgolides, named as P and Q, have been isolated from the leaves of *Ginkgo biloba*.[13] Ginkgolides are diterpenes with a structure of twenty carbon cage molecules[4] and bilobalide is closely related to them as a sesquiterpene differing actually by the absence of tetrahydrofuran ring[5] (fiture 1).

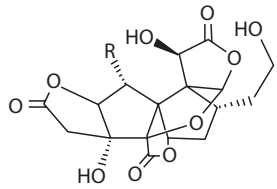


a.

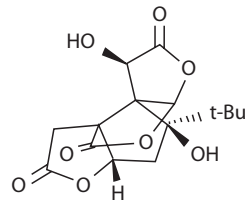
ginkgolide	R_1	R_2	R_3
A	H	H	OH
B	OH	H	OH
C	OH	OH	OH
J	H	OH	OH
M	OH	OH	H



ginkgolide	R
P	H
Q	OH



ginkgolide	R
K	OH
L	H



b.

Fiture 1. Structure of (a) ginkgolides and (b) bilobalide.

The main characteristic action of ginkgolides is their antagonistic properties against Platelet Activating Factor (PAF).[4] Until this discovery it was believed that from all the secondary metabolites, only the ginkgolides display this anti-PAF action. Nevertheless, bilobalide has also been reported to interact with PAF receptor by down-regulating its expression.[14]

2. Platelet activating factor

Platelet activating factor belongs to the class of glyceryl ether phospholipids and is characterized as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine.[15] PAF is one of the most potent mediators of inflammation and a significant signaling molecule of the immune system. Particularly, PAF stands as the main representative of a ubiquitous, potent and unique class of lipid cellular mediators while it has also been considered as a hormone.[16,17] The name of PAF underlies a greater family of molecules which includes a large number of PAF-structurally related phospholipids as well as PAF-like activity molecules.[18]

Apart from mammals, many other kinds of organisms,[19] can synthesize PAF by a great variety of cells.[17] PAF exerts its biological functions by binding to a specific transmembrane G-protein coupled receptor (PAFR) located on the plasma membrane of a wide variety of cells.[20] The interaction between PAF and PAFR initiates a cascade of signaling pathways, translating the given message to a cell response.[21]

PAF holds an essential role in numerous biological functions, either physiological (reproduction, hemostasis, neurotransmission) or pathological (inflammation, cardiovascular diseases, allergy).[18] Depending on the needs of the organism, PAF can be synthesized either by the *de novo* or the remodeling pathway.[22,23] Studies have shown that during inflammatory conditions the *de novo* pathway is activated during the chronic pathological conditions while the remodeling pathway is activated during acute inflammatory situations, as a direct response of the organism.[24] When PAF levels are elevated, its actions are moderated by intracellular PAF-specific acetylhydrolase and its circulating isoform Lipoprotein-associated phospholipase A_2 ($LpPLA_2$),[25] which has been reported to have a controversial role, acting either protectively or harmfully.[26]

2.1. Platelet activating factor inhibition by ginkgolides

Ginkgolides belong to the natural specific inhibitors and antagonize the binding to PAF's membrane receptor by a competitive way. It is important to notice that these molecules act by a high specificity as they do not interact with any other known receptors like these of adenosine, thromboxane, leukotriene or histamine. Studies around the inhibitory effect of ginkgolides have revealed that the less polar the ginkgolide is, the better PAF antagonist gets. According to these studies, among all ginkgolides structures, BN 52021 (B) which has two hydroxyl groups on C1 and C3, is the one with the most potent inhibitory effect and the best specific PAF-inhibitor known until now.[4]

The mechanisms by which PAFR is activated upon binding to an endogenous agonist, PAF, and its inactivation by an exogenous antagonist, *Ginkgo biloba*, where clarified according to the 3D models of human PAFR that were constructed. Based on these molecular dynamics simulations, it was shown that the binding of PAF to PAFR leads to its activated state,[27] while, the binding of *Ginkgo biloba* locks PAFR in its inactive state.[28]

3. *Ginkgo biloba* and atherosclerosis

Atherosclerosis is a chronic inflammatory disease of blood vessels characterized by slow thickening of arterial walls. In 2003 a new theory came up called "*The PAF Implicated Atherosclerosis Theory*", which was a new approach to this scientific issue and managed to unify the so far proposed theories about inflammation, oxidation and response-to-retention. The above new theory proposes an integrated biochemical mechanism and reveals the pivotal role of the inflammatory mediator Platelet Activating Factor (PAF) for the initiation of atheromatosis.[29] According to that, compounds which exert strong inhibitory activities against PAF, such as *Gb*E, can prevent the initiation of atheromatosis. In addition, the antioxidants, also found in *Gb*E, play a significant role in preventing –partly– LDL oxidation and therefore PAF production.[4]

*Gb*Es and mainly EGb761 have been studied for their possible beneficial effect on atherosclerosis. The exact mechanism by which *Gb*Es are implicated in the disease of atherosclerosis is not clear yet.

As far as the antioxidant activity of EGb761 is concerned, it has been reported to scavenge various free radicals,[30–34] which play primary role in the pathogenesis of atherosclerosis, considering *Ginkgo biloba* as a potential anti-atherogenic factor. Other studies support the protective activity of *Gb*Es against atherosclerosis by their ability to decrease the levels of the highly atherogenic lipoprotein (a).[35,36] *Gb*Es can also inhibit vascular endothelial growth factor,[37] P-selectin mediated leukocyte adhesion and inflammation,[38] while by increasing endothelial NO production[39] exhibit vasculoprotective effects.[40]

Chen et al proposed potential clinical use of *Gb*Es in atherosclerosis taking into consideration their *in vitro* protection against mechanisms of atherogenesis such as the modulation of redox sensitive transcription pathways and reduction of the endothelial adhesion molecule expression, in human aortic endothelial cells.[41]

Ou et al noticed the dual role of *Gb*E, as antioxidant and anti-inflammatory, on the amelioration of the damaged condition in endothelial cells, by decreasing the ox-LDL induced ROS generation and impairment of antioxidant enzymes (SOD, catalase and glutathione peroxidase), the expression of adhesion molecules (ICAM, VCAM, and E-selectin), and the adhesiveness between human cells.[42]

As atherosclerosis is an inflammatory pathological condition, the levels of cytokines play an important role. When *Gb*E was administered to atherosclerotic rats it inhibited the expression of the pro-inflammatory cytokines IL-1β and TNF-α in the brain, which are known to be induced as secondary effect of the atherosclerotic procedure in the brain. *Gb*E was also found to up-regulate the expression of the anti-inflammatory cytokine IL-10 in the brain confirming the anti-atherosclerotic actions of *Gb*E through its anti-inflammatory actions.[43]

Apart from the effect of *Gb*E alone, its combination with an anti-atherosclerotic therapy has also been studied in mice. In particular, the co-treatment of cilostazol with *Gb*E decreased superoxide generation, macrophage infiltration and expression of pro-inflammatory molecules such as VCAM-1 and MCP-1. Cilostazol and *Gb*E exert synergistic anti-atherosclerotic effects[44] without prolonging bleeding or coagulation times.[45]

Tsai et al investigated how EGb761 ameliorates the formation of foam cells in human cell lines. They proposed a novel mechanism underlining the crucial role of haem oxygenase-1 in the EGb761-mediated anti-atherogenic property in macrophages, which reduces lipid accumulation in foam cells via a decrease in cholesterol uptake and an increase in cholesterol efflux which is regulated by EGb761 via transcriptional down regulation of SR-A expression and post-transcriptional up-regulation of ABCA1 expression[46] proposing a new approach at the anti-atherogenic action of EGb761.

Last but not least, the study of Rodriquez et al reveals the reduction of atherosclerotic nanoplaque formation and size of eight patients after a two-month therapy with *Gb*E. Furthermore, superoxide dismutase activity was up-regulated and ox-LDL, LDL and lipoprotein (a) concentrations were decreased in the patients' blood. These results set a new perspective at the treatment of atherosclerosis as well as at the regression of the atherosclerotic plaques.[47]

3.1. Other PAF implicated pathological conditions

*Gb*Es have been also used in a wide variety of other, apart from atherosclerosis, pathological conditions with promising results. The following table (table 1) displays indicatively some of these conditions where PAF is also implicated.

4. Conclusion

All the above indicate that the use of *Ginkgo biloba* extracts ameliorate various pathological situations. Most of the studies have until now underlined the antioxidant

Table 1. Results of Ginkgo biloba use on various pathological conditions where PAF is implicated.

Pathological condition (PAF implicated)	***Ginkgo biloba* Extracts**	**Results**
Cognitive disorders[48] (dementia, Alzheimer, Parkinson's disease, brain injury, tinnitus) and also disorders like depression, anxiety, memory loss	EGb761	Beneficial for patients on *Ginkgo biloba* Extracts compared to those on placebo according to the ADAS-cog (Alzheimer's Disease Assessment Scale cognitive) and the GERRI (Geriatric Evaluation by Relative's Rating Instrument)[49]
	*Gb*E	Improvement on several scores of patients with cerebral insufficiency on *Ginkgo biloba* compared to patients on placebo[50]
		Neuro-protective and cognitive enhancing role in several clinical trials for treating a variety of medical, neurologic and physiologic symptoms[51]
HIV infection[52]	Ginkgolic acid	Inhibits HIV protease activity[53]
	Ginkgobilobin	Suppresses the activity of HIV reverse transcriptase[54]
	EGb761	Acts against Tat protein induced neurotoxicity[55]
	Bilobols and alkylsalicylic acids	Bilobols can stand as a new class of protease inhibitors and alkylsalicylic acids as a new inhibitory group against both against protease and RNase H reverse transcriptase[56]
Ischemia[57]	Bilobalide	Protects against neuronal death in global brain ischemia and in glutamate induced excitotoxicity[58,59]
Tissue injuries[60]	Flavonoids	Stimulate the proliferative activity and increased production of collagen and extracellular fibronectin of human skin fibroblast *in vitro*[61]
Cancer[62]	Ginkgetin or isoginkgetin	Inhibits lymphocyte proliferation[63] and the proliferation of human ovarian carcinoma cells OVCAR-3 via the induction of apoptosis in a dose dependent manner[64]
	Kaempferol	Inhibits DNA synthesis and growth of human breast adenocarcinoma cell line MCF-7 cells[65] and also pancreatic cancer cell growth while it induces cell apoptosis *in vitro*.[66] Kaempferol provides antiproliferative effects in different systems based on its striking inhibition of diverse cellular events associated with tumor pathogenesis[67] and inhibits the activity of several enzymes involved in cell growth and signal transduction pathway including cAMP-phosphodiesterase and tyrosine kinase[68] cdc25 phosphatase,[67] DNA topoisomerase II, topoisomerase I catalyzed DNA relegation,[69] proline directed protein kinase fatty acid in human prostate carcinoma cells, and myosin light chain kinase[70]
Airway diseases as asthma and bronchitis[71]	*Gb*Es	In combination with anti-asthmatic drugs *Ginkgo biloba* extracts reduce eosinophil and lymphocyte numbers in BALF compared to the asthmatic subgroups treated only with drugs[72] and also provides improvement of clinical symptoms and pulmonary functions when was orally administrated to asthmatic patients[73]
Allergy[74]	Flavonoids	Present a pro-apoptotic effect on eosinophils[75]
	BN 52021	Inhibits human eosinophilc hemotaxis *in vitro*[76]
	Secondary metabolites of *Ginkgo biloba*	Reduce eosinophil recruitment and/or eosinophilia, along with the production of chemokines and TH2 cytokines involved in allergic and parasitic disorders[77]

role of *Ginkgo biloba* as the principal one for its beneficial actions. However, all the aforementioned conditions have as common the implication of PAF either in their pathogenesis or in their progression.[21] This fact drives to the conclusion, proposed for the first time, that the beneficial effects of *Ginkgo biloba* may be mainly exerted by its anti-PAF/anti-inflammatory action and secondary by its antioxidant properties. Studies around this point of view have to be conducted in order to shed light on the anti-inflammatory pharmaceutical use of *Ginkgo biloba*.

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