BIOLOGICAL ACTIVITIES OF OLIVE OIL POLYPHENOLS

PROJECT INTERREG MED

"ARISTOIL"

Athens, October 2018

Interreg Mediterranean
EUROPEAN UNION
ARISTOIL

Project co-financed by the European Regional Development Fund

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One of the main objectives of Interreg MED project "Aristoil" is consumers' awareness on high quality olive oil benefits. This edition is the result of 3 Faculties of pharmacy efforts and includes the registration of researches published in scientific magazines. These researches prove that phenols in olive oil contribute to health claim of human body.

Olive oil, rich in phenols, is a valuable food with health claim protection.

According to the 432/2012 EU regulation, the daily consumption of 20gr of olive oil that contains at least 5mg tyrosol and hydroxytyrosol derivatives, is enough for blood lipids oxidative protection. This olive oil can be given the characterization of HEALTH CLAIM.

Dr. Nikolaos Krimnianiotis
Aristoil Project Coordinator

OLEOCANTHAL

1. Alzheimer's-associated Aβ oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal

This study of Pitt *et al.* has focused on oleocanthal (OC), as a compound capable of altering the assembly state of soluble oligomers of amyloid-β1-42 peptide (ADDL), which peptide is a neurotoxin that causes Alzheimer's disease (AD). OC increased the immunoreactivity of soluble Aβ species, indicating changes in oligomer structure. Analysis of oligomers in the presence of OC showed an upward shift in molecular weight and a ladder-like distribution of SDS-stable ADDL subspecies. In comparison with control ADDLs, oligomers formed in the presence of OC (Aβ-OC) showed equivalent co-localization at synapses but exhibited greater immunofluorescence as a result of increased antibody recognition. The enhanced signal at synapses was not due to increased synaptic binding, as direct detection of fluorescently-labeled ADDLs showed an overall reduction in ADDL signal in the presence of OC. Decreased binding to synapses was accompanied by significantly less synaptic deterioration assayed by drebrin loss. Additionally, treatment with OC improved antibody clearance of ADDLs. These results indicate oleocanthal is capable of altering the oligomerization state of ADDLs while protecting neurons from the synaptopathological effects of ADDLs and suggest OC as a lead compound for development in AD therapeutics.

Low dosages of oleocanthal prove to be protective against Alzheimer's disease

This study of Pitt et al indicates that oleocanthal is a substance capable of altering the assembly state of soluble oligomers of amyloid-β1-42 peptide (ADDL), which peptide is a neurotoxin that causes Alzheimer's disease (AD). The results show that Oleocanthal protects the neurons from the negative effects of Alzheimer's disease even at low doses and as a result in the future it could be used in a potential therapy.

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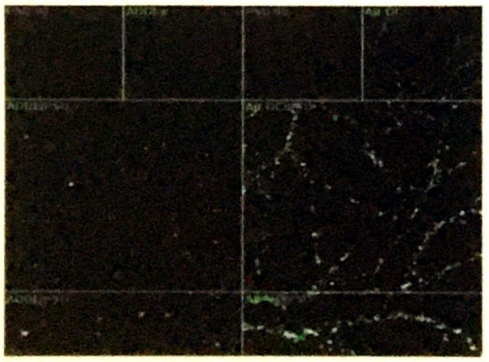


Figure. Specific binding of Aβ-OC to synapses shows greatly enhanced immunoreactivity compared to ADDLs PSD-95 (magenta) was used as a synaptic marker to determine if the pattern of binding was similar between ADDLs or Aβ-OC (both in green).

Pitt J et al., Toxicol Appl Pharmacol. 2009

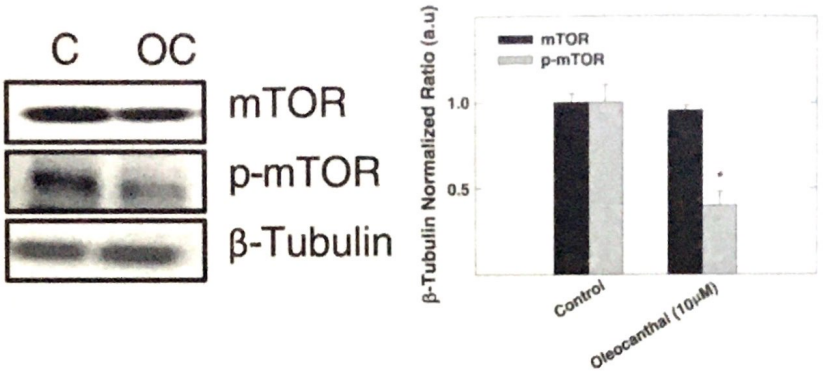
2. Olive-Oil-Derived Oleocanthal Enhances β- Amyloid Clearance as a Potential Neuroprotective Mechanism against Alzheimer's Disease: In Vitro and in Vivo Studies

The mechanism by which oleocanthal exerts its neuroprotective effect is still incompletely understood. Abuznait *et al.* with this study provide in vitro and in vivo evidence for the potential of oleocanthal to enhance Aβ clearance from the brain via up-regulation of P-glycoprotein (P-gp) and LDL lipoprotein receptor related protein-1 (LRP1), major Aβ transport proteins, at the blood-brain barrier (BBB). Results from in vitro and in vivo studies demonstrated similar and consistent pattern of oleocanthal in controlling Aβ levels. In cultured mice brain endothelial cells, oleocanthal treatment increased and LRP1 protein expression and activity. Studies showed that administration of oleocanthal to C57BL/6 wild-type mice resulted in Aβ clearance from the brain and increased the brain efflux index from 62.0 % for control mice to 79.9% for oleocanthal treated mice. Increased P-gp and LRP1 protein expression in the brain microvessels and inhibition studies confirmed the role of up-regulation of these proteins in enhancing Aβ clearance after oleocanthal treatment, which leads to Aβ degradation. In conclusion, these findings provide experimental support that potential reduced risk of AD associated with extra-virgin olive oil could be mediated by enhancement of Aβ clearance from the brain.

Oleocanthal promotes the removal of toxic proteins, called β-Amyloid related to Alzheimer's disease

In this study, Abuznait and his colleagues study the ability of oleocanthal to promote the removal of toxic proteins, called β-amyloids, related to Alzheimer's disease, from the brain. Administrating oleocanthal to mouse brain cells and in vivo to mice led to the increase of proteins that transport substances from and to the brain. The results showed that the increase of these protein levels led to Aβ clearance and brain function enhancement. In conclusion, these findings provide experimental support that potential reduced risk of AD associated with extra-virgin olive oil could be mediated by enhancement of Aβ clearance from the brain.

In cultured mice brain endothelial cells and in vivo in mice oleocanthal treatment helps to purify the brain from the toxic proteins that cause Alzheimer's disease



Effects of oleocanthal treatment on the levels of mTOR proteins in the human breast cancer cell line, MDA-MB-231.

Abuznait et al., ACS Chem. Neurosci. 2013

3. Modulation of tau protein fibrillization by oleocanthal

Oleocanthal is capable of altering the fibrillization of tau protein, which is one of the key factors at the basis of neurodegenerative diseases, and of covalently reacting with lysine amino groups of the tau fragment K18 in an unspecific fashion. In the present study, Monti *et al.* investigated the recognition process and the reaction profile between oleocanthal and the wild-type tau protein. As a result, oleocanthal has been found to interact with tau441, inducing stable conformational modifications of the protein secondary structure and also interfering with tau aggregation. These findings provide experimental support for the potential reduced risk of AD and related neurodegenerative diseases associated with olive oil consumption and may offer a new chemical scaffold for the development of AD modulating agents.

Monti et al., J. Nat. Prod. 2012

Oleocanthal modulates a selective protein associated with Alzheimer's disease

Oleocanthal reacts with tau protein, a brain protein related to Alzheimer's disease. Specifically, oleocanthal causes structure alterations, blocking the formation of fibers, which is an important factor in the beginning of neurodegenerative diseases, such as Alzheimer's.

Oleocanthal modifies a specific protein in the brain associated with the development of Alzheimer's disease

4. Inhibition of tau fibrillization by oleocanthal via reaction with the amino groups of tau

In Alzheimer's disease and related tauopathies, tau fibrillizes and aggregates into neurofibrillary tangles. Unpublished data of Li *et al.* indicate an inhibitory effect of oleocanthal on Aβ fibrillization, so I was reasoned that oleocanthal might inhibit tau fibrillization as well. Herein it is demonstrated that oleocanthal abrogates fibrillization of tau by locking tau into the naturally unfolded state. Using PHF6 peptide consisting of the amino acid residues VQIVYK, a hexapeptide within the third repeat of tau that is essential for fibrillization, it was shown that oleocanthal forms an adduct with the lysine via initial Schiff base formation. Structure and function studies demonstrate that the two aldehyde groups of oleocanthal are required for the inhibitory activity. These two aldehyde groups show certain specificity when titrated with free lysine and oleocanthal does not significantly affect the normal function of tau. These findings provide a potential scheme for the development of novel therapies for neurodegenerative tauopathies.

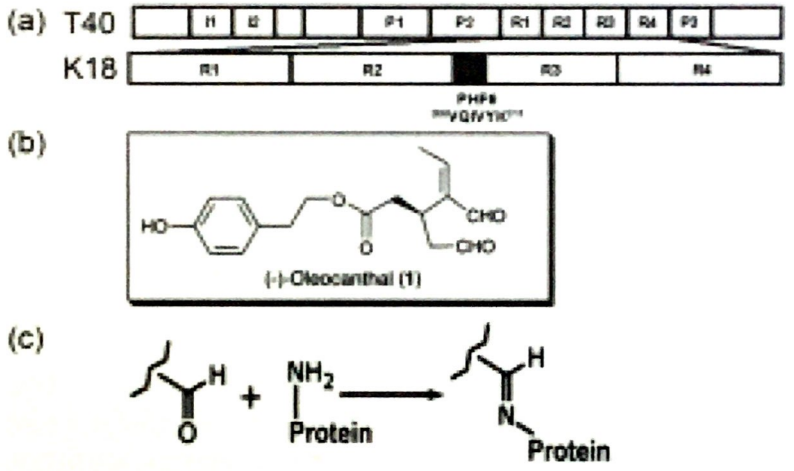


Figure: Human tau constructs, structure of oleocanthal and Schiff base reaction between oleocanthal and lysine side chain.

Li et al., J. Neurochem. 2009

Oleocanthal: a potential future treatment of Alzheimer's disease

Protein Tau alteration is one of the factors causing Alzheimer's disease. This particular study showed that oleocanthal can prevent this alteration, maintaining the original form of the protein. Further studies have shown that oleocanthal, due to its structure, leads to this obstruction without affecting the physiological function of Tau protein. These findings lead to a possible development of new treatments for diseases such as Alzheimer's.

Oleocanthal is a potential future treatment of Alzheimer's disease as it prevents the alteration of a specific protein associated with the development of the disease.

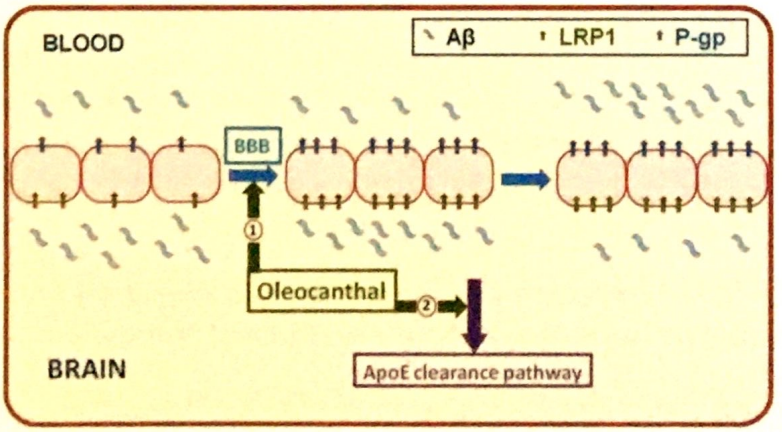
5. Oleocanthal Enhances Amyloid-β Clearance from the Brains of TgSwDI Mice and in Vitro across a Human Blood-Brain Barrier Model

In the current study, Hisham *et al.* investigated the effect of oleocanthal on pathological hallmarks of Alzheimer's disease in TgSwDI, an animal model of AD. Mice treatment for 4 weeks with oleocanthal significantly decreased amyloid load in the hippocampal parenchyma and microvessels. This reduction was associated with enhanced cerebral clearance of Aβ across the blood-brain barrier (BBB). Further mechanistic studies demonstrated oleocanthal to increase the expression of important amyloid clearance proteins at the BBB including Pglycoprotein and LRP1, and to activate the ApoE-dependent amyloid clearance pathway in the mice brains. The anti-inflammatory effect of oleocanthal in the brains of these mice was also obvious where it was able to reduce astrocytes activation and IL-1β levels. Finally, we Hisham *et al.* could recapitulate the observed protective effect of oleocanthal in an in vitro human-based model, which could argue against species difference in response to oleocanthal. In conclusion, findings from in vivo and in vitro studies provide further support for the protective effect of oleocanthal against the progression of AD.

A potential mechanism of action of oleocanthal against Anzheimer's disease

Protein Tau alteration is one of the factors causing Alzheimer's disease. This particular study showed that oleocanthal can prevent this alteration, maintaining the original form of the protein. Further studies have shown that oleocanthal, due to its structure, leads to this obstruction without affecting the physiological function of Tau protein. These findings lead to a possible development of new treatments for diseases such as Alzheimer's.

Oleocanthal acts against Alzheimer's disease, preventing the deposition of specific proteins called amyloid in the brain of mice.



Hisham et al., ACS Chem. Neurosci. 2015

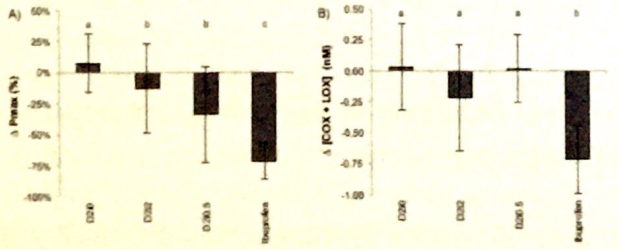
6. Oleocanthal-rich extra virgin olive oil demonstrates acute anti-platelet effects in healthy men in a randomized trial

The phenolic profiles of extra virgin olive oils (EVOOs) may influence their cardiovascular benefits. In a randomized crossover of acute EVOO intake on platelet function, participants (n = 9) consumed 40 mL of EVOO weekly. EVOOs were matched for total phenolic content and were either tyrosol-poor with 1:2 oleacein/oleocanthal (D2i0.5), or 2:1 oleacein/oleocanthal (D2i2), or predominantly tyrosol (D2i0). Ibuprofen provided a platelet inhibition control. Blood was collected pre- and 2 h post-EVOO intake. D2i0.5 and D2i2 reduced 1 mg/mL collagen-stimulated maximum platelet aggregation (Pmax), with effects best correlated to oleocanthal intake (R = 0.56, P = 0.002). Total phenolic intake was independently correlated to eicosanoid production inhibition, suggesting that cyclooxygenase blockade was not responsible for the Pmax inhibition. Five participants exhibited >25% ΔPmax declines with D2i0.5 and D2i2 intake and plasma metabolomic profiles discriminated subjects by oil responsivity. Platelet responses to acute EVOO intake are associated with oil phenolic composition and may be influenced by diet.

Olive oil rich in oleocanthal effects the cardiovascular system's function

Olive oil oleocanthal levels, can significantly affect the cardiovascular benefits from its consumption. In this study, 9 participants consumed 40ml of extra virgin olive oil (EVOO), with known polyphenol concentration, in order to evaluate the effects on blood platelets function, blood ingredients that are related to blood coagulation. The results were compared with ibuprofen action; a powerful anti-inflammatory agent. This study showed that EVOO samples rich in oleocanthal and oleacein caused a significant decrease in platelet aggregation levels (more than 25% reduction), that is the main cause for blood clotting, indicating that oleocanthal intake holds the bigger part. Finally, it seems that the effects of EVOO polyphenols is independent from their antioxidant action and turns out that the chemical profile of olive oil influences directly the cardiovascular system's function.

According to a randomized crossover of acute EVOO intake, olive oil rich in oleocanthal and oleacein helps to reduce thrombosis, as it causes a decrease in platelet aggregation



Karan Agrawal et al.., Journal of Functional Foods 36 (2017) 84–93

7. Phytochemistry: ibuprofen like activity in extra virgin olive oil.

Newly pressed extra virgin olive oil contains oleocanthal a compound whose pungency induces a strong stinging sensation in the throat, not unlike that caused by solutions of the nonsteroidal anti-inflammatory drug ibuprofen. In the study of Beauchamp *et al.*., 2005, this similar perception seems to be an indicator of a shared pharmacological activity, with oleocanthal acting as a natural anti-inflammatory compound that has a potency and profile strikingly similar to that of ibuprofen. Although structurally dissimilar, both these molecules inhibit the same cyclooxygenase enzymes in the prostaglandin biosynthesis pathway. Both enantiomers of oleocanthal, exhibited a dose-dependent inhibition of COX-1 and COX-2 activities, with no effect on lipoxygenase activity, much as observed with ibuprofen.

Oleocanthal: a natural anti-inflammatory compound

Freshly extracted extra virgin olive oil contains oleocanthal, a substance responsible for a strong "pinch" feeling in the throat, similar to the effect of ibuprofen intake, a strong anti-inflammatory drug. According to this study, oleocanthal inhibits certain enzymes related to inflammation, showing the same action as ibuprofen, although structurally it shows many differences.

Oleocanthal acts as a natural anti-inflammatory compound that has a potency and profile strikingly similar to that of ibuprofen, although they are structurally dissimilar

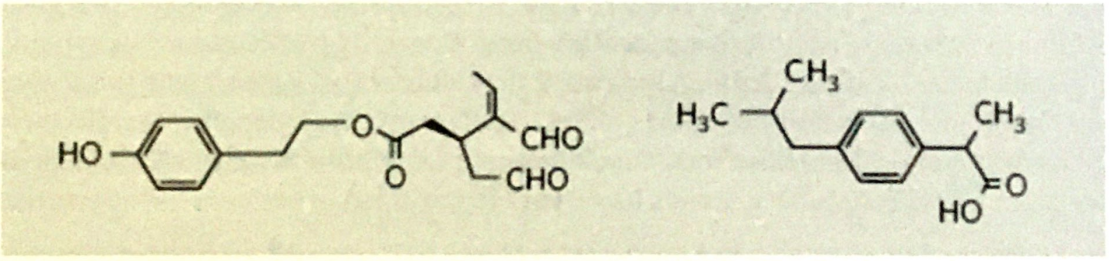


FIGURE. Structures of (-) oleocanthal (left) and the anti-inflammatory drug ibuprofen (right) (Nature 2005 Sep 1; 437(7055):456).

Beauchamp GK et al., Nature. 2005 Sep 1; 437(7055):456.

8. (-) Oleocanthal as a cMet inhibitor for the control of metastatic breast and prostate cancers

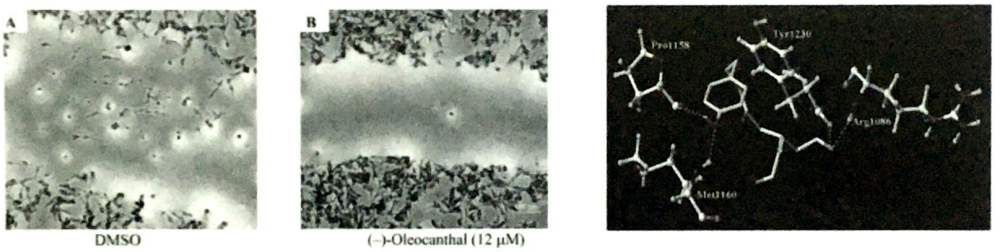
(-) Oleocanthal is a naturally occurring minor secoiridoid isolated from extra virgin olive oil, which showed potent anti-inflammatory activity. In the study of Enagar *et al.*, Computer Assisted Molecular Design (CAMD) identified oleocanthal as a potential virtual cMet inhibitor hit. In this study oleocanthal inhibited the

proliferation, migration, and invasion of the epithelial human breast and prostate cancer cell lines with an IC(50) of 4.47μM. Moreover, oleocanthal inhibited the phosphorylation of cMet kinase in vitro, with an IC (50) value of 4.8μM. These results show that oleocanthal and EVOO can have potential therapeutic use for the control of cMet-dependent malignancies.

The role of oleocanthal in breast and prostate cancer

Oleocanthal is a compound found in extra virgin olive oil showing a strong anti-inflammatory action. Research of Enagar and his colleagues showed that oleocanthal, at a cellular level, blocks the development and the metastatic action of breast or prostate cancer cells. Even in low dosage, oleocanthal and by extend extra virgin olive oil have a powerful healing role in breast and prostate cancer.

In this study oleocanthal inhibited the proliferation, migration, and invasion of the epithelial human breast and prostate cancer cell lines



Elnagar AY et al., Planta Med. 2011 Jul;77(10):10139.

9. (-)-Oleocanthal inhibits growth and metastasis by blocking activation of STAT3 in human hepatocellular carcinoma

In the present study was explored by Pei *et al.*, the anti-cancer capacity of oleocanthal in human hepatocellular carcinoma (HCC). Oleocanthal inhibited proliferation and cell cycle progression and induced apoptosis in HCC cells *in vitro* and suppressed tumor growth in an orthotopic HCC model. Oleocanthal also inhibited HCC cell migration and invasion *in vitro* and impeded HCC metastasis in an *in vivo* lung metastasis model. Oleocanthal acted by inhibiting epithelial-mesenchymal transition (EMT) through downregulation Twist, a protein which is a direct target of the transcription factor STAT3. Oleocanthal also reduced STAT3 nuclear translocation and DNA binding activity, ultimately downregulating its downstream effectors, including the cell cycle protein Cyclin D1, the anti-apoptotic proteins Bcl-2 and survivin, and the invasion-related protein MMP2. Overexpression of constitutively active STAT3 partly reversed the anticancer effects of oleocanthal, which inhibited STAT3 activation by decreasing the activities of JAK1 and JAK2 and increasing the activity of SHP-1. These data suggest that oleocanthal may be a promising candidate for HCC treatment.

The anticancer activity of oleocanthal

Pei and his colleagues studied the anticancer action of oleocanthal in human liver cancer cells. The results showed that oleocanthal reduced the proliferation of cancer cells, suspended tumor growth and at the same time caused the death of many cancer cells. Also, in an experiment that was held testing the antimetastatic action of the substance, oleocanthal blocked the metastasis on the lungs. These results, give hope for the use of this compound not only on a cellular level, but on the human organism.

Oleocanthal has strong anti-tumor properties in human liver cells, as it reduces tumor cell proliferation, inhibits tumor growth and simultaneously causes the death of many cancer cells.

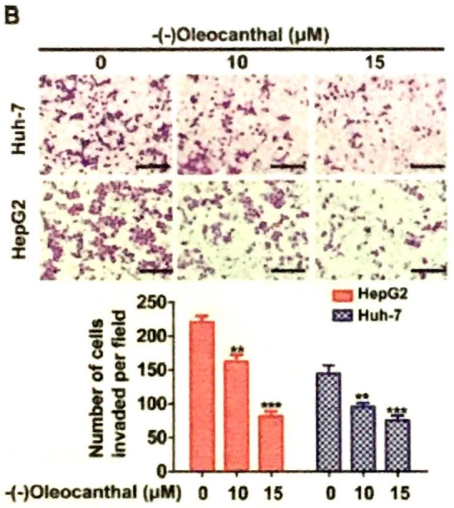


Figure: (-)-Oleocanthal inhibits migration and invasion abilities of HCC in vitro and in vivo. (B) Representative images of invasion assay for Huh-7 and HepG2 cells after the pre-treatment with increasing doses of (-)-oleocanthal for 24 h (top panel). The number of invaded cells was counted (bottom panel). Scale bar = 100 μm.

Tiemin Pei et al., Oncotarget, 2016, Vol. 7, No. 28, 43475-91

10. (-)-Oleocanthal rapidly and selectively induces cancer cell death via lysosomal membrane permeabilization

LeGendre *et al.* investigated the effect of oleocanthal (OC) on human cancer cell lines in culture and found that OC induced cell death in all cancer cells examined as rapidly as 30 minutes after treatment. OC treatment of non-transformed cells suppressed their proliferation but did not cause cell death. OC induced both primary necrotic and apoptotic cell death via induction of lysosomal membrane permeabilization (LMP). Here evidence are provided showing that OC promotes LMP by inhibiting acid sphingomyelinase (ASM) activity, which destabilizes the interaction between proteins required for lysosomal membrane stability. The data presented here indicate that cancer cells, which tend to have fragile lysosomal membranes compared to non-cancerous cells, are susceptible to cell death induced by

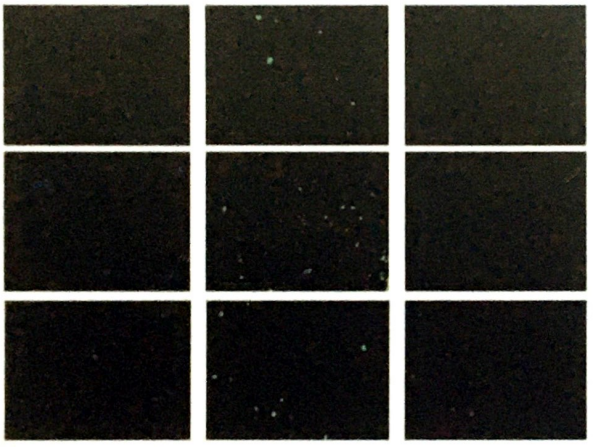
lysosomotropic agents. Therefore, targeting lysosomal membrane stability represents a novel approach for the induction of cancer-specific cell death.

Oleocanthal promotes only the death of cancer cells and not of normal cells

LeGendre and his colleagues studied the effects of oleocanthal in human cancer cells. Their results showed that oleocanthal causes the death of cancer cells within 30 minutes, whithout affecting the normal cells. This happens because oleocanthal acts on a certain cell mechanism that cancer cells are much more sensitive than normal cells, leading to their death while normal cells are not damaged.

Oleocanthal induces cell death in all cancer cells examined as rapidly as 30 minutes after treatment, with a selective mechanism of action.

In the presence of serum, 10 mM Oleocanthal induced a maximum 10% inhibition of ASM activity



LeGendre et al., Molecular & Cellular Oncology 2014

11. Cytotoxic Activity of Oleocanthal Isolated from Virgin Olive Oil on Human Melanoma Cells

Oleocanthal's potential anticancer activity has already been reported but only limited evidence has been provided in cutaneous malignant melanoma. The present study of Fogli S *et al.* is aimed at investigating the selective in vitro antiproliferative activity of oleocanthal against human malignant melanoma cells. Cell viability experiments demonstrated that oleocanthal had a remarkable and selective activity for human melanoma cells versus normal dermal fibroblasts with IC50s in the low micromolar range of concentrations. Such an effect was paralleled by a significant inhibition of ERK1/2 and AKT protein phosphorylation and downregulation of the gene Bcl2 expression. These findings may suggest that extra virgin olive oil phenolic extract enriched in oleocanthal deserves further investigation in skin cancer.

Fogli S, Nutr Cancer. 2016 Jul;68(5):8737

Oleocanthal's anticancer activity in cutaneous malignant melanoma

The aim of this study is to investigate the anticancer activity of oleocanthal against cutaneous malignant melanoma. Oleocanthal had a remarkable and selective activity for human melanoma cells versus normal skin cells, even at low dosages.

Oleocanthal has a selective in vitro antiproliferative activity against human malignant melanoma cells, even at low doses.

12. In Cell Interactome of Oleocanthal, an Extra Virgin Olive Oil Bioactive Component.

Cassiano C *et al.* revealed in their research via chemical proteomics that heatshock proteins, HSP70 and HSP90, as main oleocanthal interactors in living systems. These two proteins are involved in cancer development and, thus, our findings could have important outcomes for a deep evaluation of the biopharmacological significance of oleocanthal.

Cassiano C et al., Nat Prod Commun. 2015 Jun;10(6):10136.

The interaction of oleocanthal with mechanisms associated with cancer

Cassiano and his colleagues proved that two proteins (HSP70 and HSP90) are the main molecules with which oleocanthal interacts in living systems. These two proteins are involved in the development of cancer and therefore the results of this study may have significant benefits for the pharmacological action of oleocanthal against cancer.

The interaction of oleocanthal with mechanisms associated with cancer is indicative of its anti-cancer activity

13. Olive Oil-derived Oleocanthal as Potent Inhibitor of Mammalian Target of Rapamycin: Biological Evaluation and Molecular Modeling Studies

Mammalian target of rapamycin (mTOR) is a protein that integrates signals from energy homeostasis, metabolism, stress response, and cell cycle, with reported role in cancer and Alzheimer's disease development. This function encouraged the team of Mohammad A. Khanfar *et al.* to examine the possibility that oleocanthal inhibits mTOR. Subsequent experimental validation indicated that oleocanthal indeed inhibited the enzymatic activity of mTOR with an IC50 value of 708 nM. Oleocanthal inhibits the growth of several breast cancer cell lines at low micromolar

concentration in a dose-dependent manner. Oleocanthal treatment caused a marked downregulation of phosphorylated mTOR in metastatic breast cancer cell line (MDA-MB-231). These results strongly indicate that mTOR inhibition is at least one of the factors of the reported anticancer and neuroprotective properties of oleocanthal.

Khanfar MA et al. Phytother Res. 2015 November ; 29(11): 1776–1782

The effect of oleocanthal on breast cancer

The aim of this study is to investigate the effect of oleocanthal treatment on specific breast cancer cells, even at low dosages. The results have shown that oleocanthal reduces the impact of a protein, that plays an important role in the development of cancer cells and of breast cancer as well.

Oleocanthal inhibits the enzymatic activity of a protein that plays an important role in the development of cancer cells, in a dose-dependent manner.

14. Olive Phenolics as c-Met Inhibitors: (-)-Oleocanthal Attenuates Cell Proliferation, Invasiveness, and Tumor Growth in Breast Cancer Models

Dysregulation of the Hepatocyte growth factor (HGF)/c-Met signaling axis upregulates diverse tumor cell functions, including cell proliferation, survival, scattering and motility, epithelial-to-mesenchymal transition (EMT), angiogenesis, invasion, and metastasis. The aim of this study was to characterize the intracellular mechanisms involved in mediating the anticancer effects of (-)-oleocanthal treatment and the potential involvement of c-Met receptor signaling components in breast cancer. Results showed that (-)-oleocanthal inhibits the growth of human breast cancer cell lines MDA-MB-231, MCF-7 and BT-474 while similar treatment doses were found to have no effect on normal human MCF10A cell growth. In addition, (-)-oleocanthal treatment caused a dose-dependent inhibition of HGF-induced cell migration, invasion and G1/S cell cycle progression in breast cancer cell lines. Moreover, (-)-oleocanthal treatment effects were found to be mediated via inhibition of HGF-induced c-Met activation and its downstream mitogenic signaling pathways. This growth inhibitory effect is associated with blockade of EMT and reduction in cellular motility. Further results from in vivo studies showed that (-)-oleocanthal treatment suppressed tumor cell growth in an orthotopic model of breast cancer in athymic nude mice. Collectively, the findings of this study suggest that (-)-oleocanthal is a promising dietary supplement lead with potential for therapeutic use to control malignancies with aberrant c-Met activity.

Intracellular mechanisms of oleocanthal treatment against breast cancer: Oleocanthal reduces tumor proliferation and tumor growth

The aim of this study was to characterize the intracellular mechanisms involved in mediating the anticancer effects of oleocanthal treatment. The results showed that the oleocanthal inhibits the growth of cancer cells, without any effect on normal cells. In particular, oleocanthal modulates the activity of specific proteins associated with the growth, proliferation and migration of cancer cells. In further experiments on mice with cancer of their skin showed remarkable inhibition of tumor growth. In conclusion, the findings of this study suggest that oleocanthal is a promising dietary supplement with potential for therapeutic use to control malignancies.

Oleocanthal has a strong anti-cancer effect, by affecting the activity of specific proteins associated with the proliferation and migration of cancer cells, without showing any effect on normal cells.

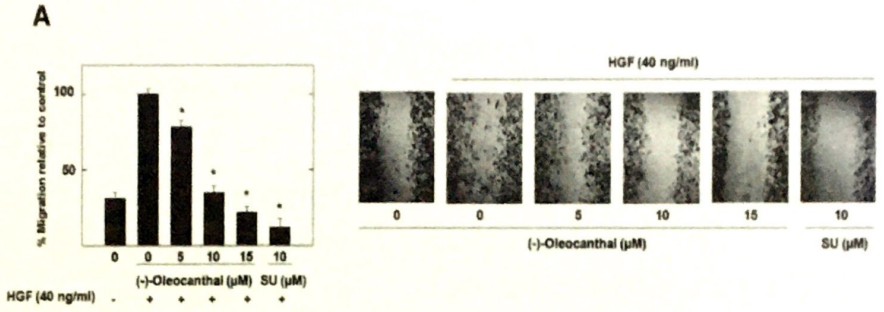


Figure (-)-Oleocanthal treatment caused a dose-dependent suppression of HGF-induced mammary tumor cell migration and invasion and Brk/paxillin/Rac1 pathway signaling.

Mohamed et al.., PLoS ONE 2014

15. Effect of Oleocanthal and Its Derivatives on Inflammatory Response Induced by Lipopolysaccharide in a Murine Chondrocyte Cell Line

In joint diseases, cartilage homeostasis is disrupted by mechanisms that are driven by combinations of biologic factors. Osteoarthritis progression is characterized by increased nitric oxide (NO) production, which has been associated with cartilage degradation. Oleocanthal displays antiinflammatory drug activity similar to that of ibuprofen, a drug widely used in the therapeutic management of joint inflammatory diseases. In this study Iacono *et al.* evaluated the effect of oleocanthal and its derivatives on the modulation of NO production in chondrocytes. Oleocanthal and its derivatives decreased lipopolysaccharide-induced NOS2 synthesis in chondrocytes without significantly affecting cell viability at lower concentrations. Among the derivatives that were examined, derivative 231 was the most interesting, since its inhibitory effect on NOS2 was devoid of cytotoxicity even at higher concentrations. This class of molecules shows potential as a therapeutic weapon for the treatment of inflammatory degenerative joint diseases.

Iacono et al., ARTHRITIS & RHEUMATISM 2010

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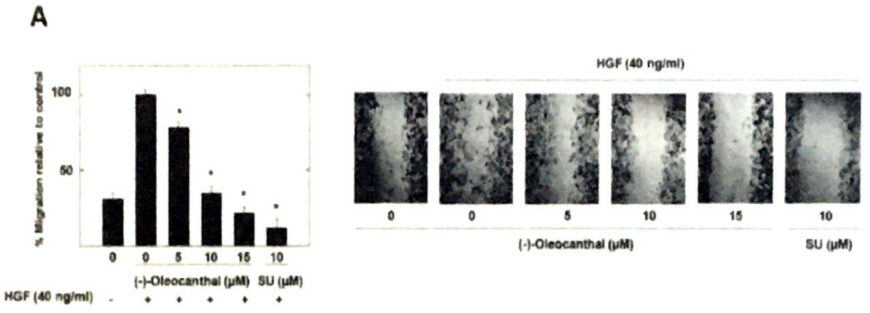

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15. Effect of Oleocanthal and Its Derivatives on Inflammatory Response Induced by Lipopolysaccharide in a Murine Chondrocyte Cell Line

In joint diseases, cartilage homeostasis is disrupted by mechanisms that are driven by combinations of biologic factors. Osteoarthritis progression is characterized by increased nitric oxide (NO) production, which has been associated with cartilage degradation. Oleocanthal displays antiinflammatory drug activity similar to that of ibuprofen, a drug widely used in the therapeutic management of joint inflammatory diseases. In this study Iacono *et al.* evaluated the effect of oleocanthal and its derivatives on the modulation of NO production in chondrocytes. Oleocanthal and its derivatives decreased lipopolysaccharide-induced NOS2 synthesis in chondrocytes without significantly affecting cell viability at lower concentrations. Among the derivatives that were examined, derivative 231 was the most interesting, since its inhibitory effect on NOS2 was devoid of cytotoxicity even at higher concentrations. This class of molecules shows potential as a therapeutic weapon for the treatment of inflammatory degenerative joint diseases.

Iacono et al., ARTHRITIS & RHEUMATISM 2010

Oleocanthal and its derivates on the treatment of inflammatory degenerative joint diseases

Oleocanthal and its derivatives show potential as a therapeutic weapon for the treatment of inflammatory degenerative joint diseases, such as osteoarthritis. Osteoarthritis is characterized by increased production of nitric oxide (NO) associated with cartilage damage. Oleocanthal and its derivatives reduce the synthesis of NO and inhibit the progression of the disease.

Oleocanthal shows potential as a therapeutic weapon for the treatment of inflammatory degenerative joint diseases, such as osteoarthritis, by reducing the synthesis of nitrogen monoxide, which is associated with cartilage damage.

16. Oleocanthal exerts antitumor effects on human liver and colon cancer cells through ROS generation.

Oleocanthal (OC) shows an anti-inflammatory and anticancer activity, which guided Cusimano *et al.* to study the anticancer effects of OC in hepatocellular (HCC) and colorectal carcinoma (CRC). Several cell lines were used at the study that were treated with OC and estimated the cell viability and apoptosis. OC was more effective comparing with other anti-inflammatory agents like ibuprofen, indomethacin and nimesulide, and induced cell growth inhibition. Moreover, experiments with OC showed inhibition of colony formation and apoptosis induction. Finally, OC showed no toxic effect on normal hepatocytes. All this lead to the conclusion, that OC is a potent agent against in HCC and CRC. These findings provide a strong support of the potential use of extra virgin olive oil as chemotherapeutic.

Anticancer effects of oleocanthal in hepatocellular (HCC) and colorectal carcinoma (CRC)

Oleocanthal (OC) shows an anti-inflammatory and anticancer activity in liver and colon cancer cells. OC inhibits the growth of cancer without affecting normal cells, providing a strong support of the potential use of extra virgin olive oil as chemotherapeutic.

Oleocanthal (OC) shows a remarkable anti-inflammatory and anticancer activity in liver and colon cancer cells without affecting normal cells

Cusimano A, Balasus D, Azzolina A, Augello G, Emma MR, Di Sano C, Gramignoli R, Strom SC, McCubrey JA, Montalto G, Cervello M
Int J Oncol. 2017 Aug;51(2):533-544

17. The olive oil phenolic oleocanthal modulates estrogen receptor expression in luminal breast cancer in vitro and synergizes with tamoxifen treatment

The goal of this study was to explore the effect of oleocanthal treatment on growth of luminal breast cancer cells and to examine the effect of combination of oleocanthal with tamoxifen. Results showed that oleocanthal inhibited growth of various human breast cancer cells in mitogen-free media with IC_{50} values of 32.7 to 80.93μM. Similarly, oleocanthal suppressed growth of these cells in 17β-estradiol-supplemented media with IC_{50} values of 22.28 to 83.91μM. Combined oleocanthal and tamoxifen treatments resulted in a synergistic growth inhibition of the cells with combination index values of 0.65 to 0.53 for each cell line. Studies indicated high degree of overlapping for the binding of oleocanthal and 17β-estradiol to estrogen receptors, while oleocanthal and tamoxifen have distinguished binding modes. Treatment with 5mg/kg or 10mg/kg (-)-oleocanthal resulted in 97% inhibition of tumor growth in mice. (-)-Oleocanthal treatment reduced total levels of estrogen receptors in cells both in vitro and in vivo. Collectively, (-)-oleocanthal showed a potential beneficial effect in suppressing growth of hormone-dependent breast cancer and improving sensitivity to tamoxifen treatment. These findings provide rational for evaluating the effect of (-)-oleocanthal in combination with endocrine treatments in luminal breast cancer.

Oleocanthal treatment on growth of luminal breast cancer cells and the effect of combination of oleocanthal with tamoxifen

In the present study, oleocanthal seems to prevent the development of breast cancer, but also combined oleocanthal and tamoxifen treatments resulted in a synergistic growth inhibition of the cells. In particular, oleocanthal treatment reduces the total level of estrogen receptors, concerning experiments both on cells and animals, with 97% inhibition of tumor growth in mice. In conclusion, oleocanthal improves the sensitivity to tamoxifen treatment, so it can be used in combination with endocrine therapy for better breast cancer results.

Oleocanthal showed a potential beneficial effect in suppressing growth of hormone-dependent breast cancer and improving sensitivity to tamoxifen treatment in mice.

Ayoub NM[1], Siddique AB[2], Ebrahim HY[2], Mohyeldin MM[2], El Sayed KA[2].
Eur J Pharmacol. 2017 Sep 5;810:100-111. doi: 10.1016/j.ejphar.2017.06.019. Epub 2017 Jun 15.

18. Oleocanthal ameliorates amyloid-β oligomers' toxicity on astrocytes and neuronal cells: In vitro studies.

In the current study, Batarseh *et al.* investigated oleocanthal effect on modulating Aβ oligomers (Aβo) pathological events in neurons and astrocytes. The findings

demonstrated oleocanthal prevented Aβo-induced synaptic proteins, SNAP-25 and PSD-95, down-regulation in neurons, and attenuated Aβo-induced inflammation, glutamine transporter (GLT1) and glucose transporter (GLUT1) down-regulation in astrocytes. The inflammation that was induced by Aβo was characterized by interleukin-6 (IL-6) increase and glial fibrillary acidic protein (GFAP) upregulation that were reduced by oleocanthal. In conclusion, this study comes to add more to support the role of oleocanthal against AD pathology.

Neuroscience. 2017 Jun 3;352:204-215. doi: 10.1016/j.neuroscience.2017.03.059. Epub 2017 Apr 7.
Batarseh YS[1], Mohamed LA[1], Al Rihani SB[1], Mousa YM[1], Siddique AB[1], El Sayed KA[1], Kaddoumi A[2].

Oleocanthal reduces the toxicity of Aβ oligomers in Alzheimer's disease.

Oleocanthal reduces the toxicity of Aβ oligomers associated with the pathogenesis of Alzheimer's disease. The findings showed that oleocanthal prevents the deregulation of specific proteins in the neurons and attenuates the inflammation that is generated. In conclusion, oleocanthal seems to play a very important role in the treatment of the disease.

Oleocanthal significantly reduces the toxicity of Aβ oligomers associated with Alzheimer's disease and it can be a potential future treatment for the disease

19. Oleocanthal exerts anti-melanoma activities and inhibits STAT3 signaling pathway.

In this study Gu Y *et al.* explored the effects of oleocanthal (OC) on the three processes in melanoma and investigated underlying mechanisms. In vitro, OC suppressed proliferation, migration, invasion, in melanoma and human umbilical vascular endothelial cells, and additionally induced apoptosis and suppressed the tube formation, respectively. In vivo studies showed potent activity in suppressing tumor growth. Furthermore, OC suppressed proliferation and angiogenesis. In addition, OC was found to inhibit metastasis of melanoma in a lung metastasis model. Mechanistically, OC significantly suppressed phosphorylation of the protein signal transducer and activator of transcription 3 (STAT3), and, moreover, decreased and inhibited STAT3 nuclear localization and transcriptional activity, respectively. OC also downregulated STAT3 target genes, including Mcl-1, Bcl-xL, MMP-2, MMP-9, VEGF, which are involved in apoptosis, invasion and angiogenesis of melanoma. These results support further investigation of OC as a potential anti-melanoma drug.

The beneficial effect of oleocanthal on melanoma

In this study Gu Y et. al explored the effects of oleocanthal (OC) on melanoma and investigated the mechanisms. Experiments conducted in cells showed a suppression

of tumor growth, migration and penetration of melanoma. Furthermore, animal treatment with oleocanthal led to significant inhibition of tumor growth, through various biochemical pathways. These results support future studies of OC as a drug against melanoma.

Oleocanthal is a potential anti-melanoma drug, as it showed potent activity in suppressing tumor growth and inhibiting metastasis of melanoma, by affecting specific proteins that are important for tumor growth and its metastasis.

Oncol Rep. 2017 Jan;37(1):483-491. doi: 10.3892/or.2016.5270. Epub 2016 Nov 23.
Gu Y[1], Wang J[1], Peng L[1].

Effect of High-Oleocanthal and oleacein olive oil in patients with chronic lymphocytic leukemia

Andrea Paola Rojas Gil,[1] Iannis Kondonis,[2] Anastasios Ioannidis,[1] Tzortzis Nomikos,[3] Eleni Melliou,[4] Prokopios Magiatis[4]

[1]*Nursing Department, University of Peloponnese,* [2]*General Hospital of Lakonia Hematology Department,* [3]*Harokopio University,* [4]*Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Greece*

Oleocanthal and oleacein are major secoiridoid-phenolic ingredients of olive oil with well-known activity against cancer cells *in vitro* and *in vivo*. The concentration of oleocanthal and oleacein in olive oil is dependent on several factors related with the variety, the harvest season and the parameters of the production procedure (e.g. malaxation time and temperature). Oleocanthal, has important health promoting anti–cancerous properties, since it can inhibit *in vitro* the growth and promote the apoptosis of several cancer cells lines. Despite the known *in vivo* activity there are no data about their anticancer activity in humans.

After screening of >3000 olive oil samples we selected an extra virgin olive oil with high oleocanthal and oleacein concentration of 416 and 284 mg/Kg respectively (Lianolia Corfu; The Governor). As a control oil we selected one with high phenolic content (500 mg/Kg) represented mainly by free tyrosol and hydroxytyrosol and with low concentration of oleocanthal 82 mg/Kg and oleacein 33 mg/Kg. The two oils were administered daily at a dose of 40 ml for a three months period to two groups of 10 patients with CLL that were not following a treatment.

At the end of the intervention period the number of white blood cells in the study group was reduced in 8/10 patients. In 5/10 patients the reduction was statistical significant ($p<0.05$) and in 2/10 patients the reduction was >50%. In contrast the control group didn't show any change. A statistically significant reduction of the antiapoptotic protein survivin was observed in all patients of the intervention group. The levels for survivin significantly decreased at 1.5 months and remained decreased at 3 months in the intervention group of 5 CLL patients, that also significantly increased the apoptotic markers ccK18 and ApoFas1 at 3 months, in comparison to their control group. The opposite effect was observed in the control group. Interestingly, the lipid profile, the blood sugar as well as the hepatic function in both groups didn't show any significant change.

For the first time we were able to show that oleocanthal and oleacein are promising dietary agents for the stability and maybe improvement of CLL since they can increase apoptosis and decrease cell survival. Further and largest studies are necessary to clarify the exact role and the ability of such interventions to increase the expectation life.

Phytochemical Society of Europe conference, Naples 4-7 September 2018

Olive oil and Alzheimer's disease: The first clinical study in Greece

Magda Tsolaki, MD, PhD, Neurologist-Psychiatrist, Theologos

Professor of Medical School, Director of the Neurology Clinic

Aristotle University of Thessaloniki,

President of the Pan-Hellenic Society of Alzheimer's Disease

In 2015 it was estimated that 46.8 million people suffered from dementia worldwide. It is estimated that the number of patients will almost double every 20 years, with a projection to reach 74.7 million in 2030 and 131.5 million in 2050. AD is a global health problem as the population ages. There is no effective treatment that can protect against the onset or worsening of the Disease. Some claim that the lack of effective treatment is due to the delay in the diagnosis of Disease. Until the diagnosis of AD, the neurodegenerative lesions have progressed so that very few neurons can be rescued by medication. The research community created the term "Mild cognitive dysfunction" in an effort to detect individuals before the dementia stage that could benefit from potential therapies that they had not previously achieved. The last drug for AD was adopted in 2001. All the medicines we use today deal with the symptoms and not the disease. Emergency drugs are needed to modify the disease.

Hippocrates in the 5th century BC he said, "Let your medicine be your food and your food become your medicine." Based on Hippocratic Medicine we started the first clinical study with the administration of unripe olive oil to patients with Mild cognitive Disfunction. We gave olive oil from unripe olives from Chalkidiki with high oleocanthal content to 50 patients, extra virgin olive oil to 50 patients and a third group of 50 patients was only following a Mediterranean diet similarly to the first two groups.

Our first results after a year showed statistically significant differences in favor of the oil from unripe olives in most of the neuropsychological tests used in the study. The study continues for a second year. This study is being carried out for the first time worldwide. The study is performed by the Alzheimer Society and YANNI'S OLIVE GROVE, which supplies the extra virgin olive oil to patients with mild cognitive disfunction and will be completed in two years

OLEACEIN

1. Effects of olive oil polyphenols on erythrocyte oxidative damage.

In this work, Pavla-Martins *et al.*. studied the capacity of oleacein to protect red blood cells (RBCs) from oxidative injury. The in vitro oxidative stress of RBCs was induced by the water-soluble radical initiator 2,2'azobis (2amidinopropane) dihydrochloride and changes were evaluated either by optical microscopy or by the amount of hemolysis. Oleacein was shown to significantly protect RBCs from oxidative damage in a dose-dependent manner. Oleacein had the most powerful effect at 20mM, within the other polyphenols. Even at 3mM, oleacein still had an important protective activity. For the first time it was demonstrated that oleacine may play a noteworthy protective role against ROS-induced oxidative injury in human cells since lower doses of this compound were needed to protect RBCs in vitro from oxidative mediated hemolysis.

Paiva-Martins F et al.., Mol Nutr Food Res. 2009

Oleacein protects red blood cells (RBCs) from oxidative injury

In this work, Pavla-Martins *et al.*. studied the capacity of oleacein to protect red blood cells (RBCs) from oxidative injury. Oleacein was shown to significantly protect RBCs from oxidative damage in a dose-dependent manner. Even at low dosages exhibited a remarkable protective role for RBCs.

Oleacein protects red blood cells from oxidative mediated hemolysis, even at low dosages.

2. Oleacein. Translation from Mediterranean Diet to Potential Antiatherosclerotic Drug

Oleacein, due to its abundance, in olive oil, it may play a special role in decreasing the progression of atherosclerosis. Some bioactivities of oleacein, such as antioxidant, anti-inflammatory, anti-proliferative and antimicrobial, were documented. There is also evidence of the bioavailability of oleacein in humans as well. However, due to the lack of clinical data, further studies are needed to provide information about the usefulness of this compound in antiatherosclerotic therapy.

Marek Naruszewicz et al, Current Pharmaceutical Design, 2015

Oleacein: a potential Antiatherosclerotic Drug

Oleacein may play a special role in decreasing the progression of atherosclerosis, the most important cause of stroke or heart attack. Some bioactivities of oleacein have

been already documented, further studies are needed about the usefulness of this compound in antiatherosclerotic therapy.

Oleacein plays a special role in decreasing the progression of atherosclerosis, the most important cause of stroke or heart attack.

3. Oleacein enhances anti-inflammatory activity of human macrophages by increasing CD163 receptor expression.

Filipek *et al.*. examined whether oleacein could increase CD163 and IL10 receptor expression as well as intracellular secretion of protein heme oxygenase 1 (HO1) in human macrophages. Effect of oleacein (10 and 20 µmol/l) or oleacein together with complexes of haemoglobin (Hb) and haptoglobin 11 (Hp11) or haptoglobin 22 (Hp22) on expression of IL10 and CD163 receptors was determined by Flow Cytometry. HO1 intracellular secretion in macrophages was investigated by enzyme-linked immunosorbent assay (ELISA). Oleacein together with complexes HbHp11 or HbHp22 stimulated the expression of CD163 (30-100 fold), IL10 (170-300 fold) and HO1 secretion (60-130 fold) after 5 days of co-incubation. Our results suggested that oleacein enhances anti-inflammatory activity of complexes haemoglobin with haptoglobin 11 and 22 and could play a potential role in the prevention of inflammatory disease related to atherosclerosis.

The anti-inflammatory effect of oleacein and mechanisms of action

Filipek *et al.* examined the ability of oleacein to increase the production of specific anti-inflammatory proteins. In particular, oleacein is either associated with specific blood components and induces the production of some anti-inflammatory proteins or inflammatory-related proteins. Thus it seems that oleacein may play an important role in the prevention of inflammatory disease related to atherosclerosis.

Oleacein enhances anti-inflammatory activity of specific complexes and could play a potential role in the prevention of inflammatory disease related to atherosclerosis.

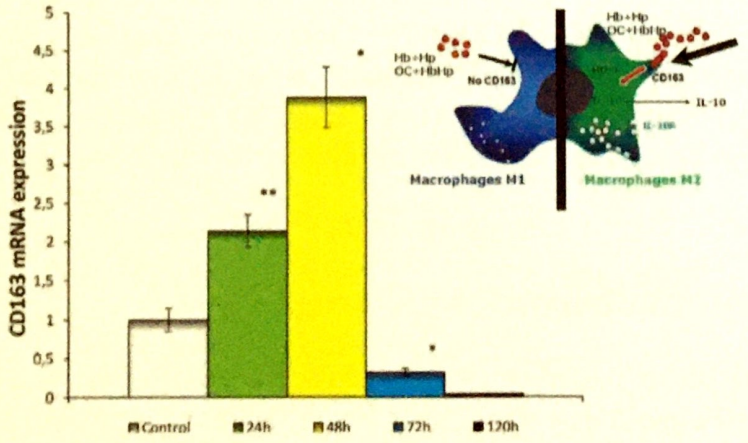

Fig. Influence of oleacein together with complexes of haemoglobin and haptoglobin on increases CD163 mRNA transcription. Quantification of CD163 mRNA expression by real-time RT-PCR in human monocytes/macrophage cells. mRNA levels are shown as arbitrary units normalized to GAPDH expression.Data from 24 experiments ±SEM. Statistical significance *P<0.05,**P<0.005 compared to control.

Filipek A et al., Phytomedicine. 2015

4. Oleuropein and oleacein may restore biological functions of endothelial progenitor cells impaired by angiotensin II via activation of Nrf2/heme oxygenase1 pathway.

Oleacein was examined if is able to protect Endothelial progenitor cells EPCs against impairment of their functions due to angiotensin-induced cell senescence. CD31(+)/VEGFR2(+) cells were cultured with angiotensin in presence or absence of increasing concentrations (from 1.0 to 10.0 μM) of oleacein. As compared to angiotensin II-treated cells, EPCs exposed to oleacein prior to angiotensin II showed a significant increase of proliferation and telomerase activity, and a decrease in the percentage of senescent cells and intracellular ROS formation. Oleacein restored migration, adhesion and tube formation of EPCs diminished by angiotensin II in a concentration-dependent manner. This effect was related to NFE2-related factor 2 (Nrf2) transcription factor activation and the increase of heme oxygenase1 (HO1) expression.

Oleacein protects cells from aging

Oleacein was examined if it is able to protect cells from aging and hence from their death. Endothelial progenitor cells exposed to oleacein showed a significant increase of proliferation in a dose-dependent manner.

Oleacein protects cells from aging and death in a dose-dependent manner

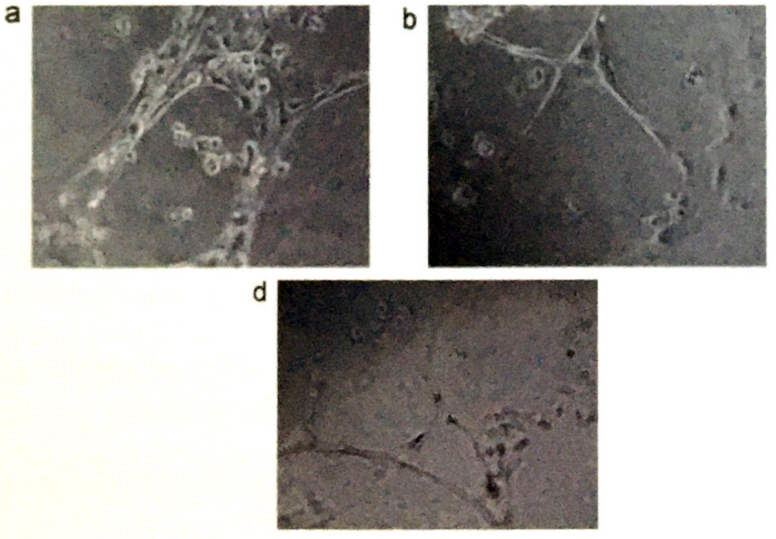

Fig. Effects of tested compounds on the angiogenesis in vitro. Representative micrographs are presented: (a) control–untreated cells; (b) angiotensin II-treated cells (1 M) (d) cells treated with oleacein (10 M) and angiotensin.

Parzonko A , Czerwińska ME, Kiss AK, Naruszewicz M. Phytomedicine. 2013

5. One-step semisynthesis of oleacein and the determination as a 5-lipoxygenase inhibitor.

5-lipoxygenase is a direct target for oleacein with an inhibitory potential (IC50: 2 μM) more potent than oleocanthal and oleuropein. This enzyme catalyzes the initial steps in the biosynthesis of pro-inflammatory leukotrienes. This investigation presented here an alternative solution to isolation or total synthesis for the procurement of oleacein, thus facilitating the further development as a potential anti-inflammatory agent.

Vougogiannopoulou K et al., J Nat Prod. 2014

6. Oleacein may inhibit destabilization of carotid plaques from hypertensive patients. Impact on high mobility group protein-1.

The aim this study was to investigate a potential role of oleacein in attenuation of carotid plaque destabilization ex vivo. Oleacein at the concentrations of 10 and 20 μM significantly ($P < 0.001$) decreased secretion of HMGB1 (up 90%), MMP-9 (up to 80%) proteins, MMP-9/NGAL complex (up to 80%) and TF protein (more than 90%) from the treated plaque, as compared to control. At the same time IL-10 and HO-1 release increased by more than 80% ($P < 0.001$).

Those results indicate that oleacein possess ability to attenuate the destabilization of carotid plaque and could be potentially useful in the reduction of ischemic stroke risk.

Oleacein reduces the ischemic stroke risk.

Oleacein has an effect against the destabilization of carotid plaques, by affecting the production of specific proteins associated with atherosclerosis. Ex vivo experiments showed that oleacein has the potential to attenuate the destabilization of carotid plaques and may be useful in reducing the risk of ischemic stroke.

Oleacein possess ability to attenuate the destabilization of carotid plaque and could be potentially useful in the reduction of ischemic stroke risk.

Phytomedicine. 2017 Aug 15;32:68-73. doi: 10.1016/j.phymed.2017.06.004. Epub 2017 Jun 13.
Filipek A1, Czerwińska ME1, Kiss AK1, Polański JA2, Naruszewicz M3.

LIGSTROSIDE AGLYCONE

1. Olive secoiridoids and semisynthetic bioisostere analogues for the control of metastatic breast cancer.

In the study of Busnena *et al.*, ligstroside aglycone showed the best antimigratory activity against the highly metastatic human breast cancer cell line MDAMB231. Generally, tyrosol esters showed better activities versus carbamate analogues. Tyrosol esters with a phenolic acid containing hydrogen bond donor and/or acceptor groups at the para-position have better anticancer and c-MET protein inhibitory activities. Olive oil secoiridoids, like ligstroside aglycon, are excellent scaffolds for the design of novel c-MET inhibitors.

Busnena BA, Bioorg Med Chem. 2013

Ligstroside aglycone against metastatic breast cancer

In the study of Busnena *et al.*, ligstroside aglycone showed the best antimigratory activity in experiments performed in highly metastatic human breast cancer cells. Due to its particular chemical structure, it inhibits the activity of a specific protein, the c-MET protein, associated with the development of malignant tumor.

Ligstroside aglycone showed the best antimigratory activity against the highly metastatic human breast cancer cells.

2. tabAnti-HER2 (*erb*B-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO)

Menendez *et al.* in their study explored the ability of ligstrocide aglycone to modulate HER2 tyrosine kinase receptor-induced in vitro transformed phenotype in human breast epithelial cells. Using MCF10A normal breast epithelial cells it was further determined the relationship between chemical structure of ligstrocide aglycone and its inhibitory activities on the tyrosine kinase activity of the HER2 oncoprotein. When compared with untreated cells, MCF10A/HER2 cells, treated with ligstroside aglycone, grew less dense, were significantly bigger in volume and showed a profound reorganization of cell-cell contacts with the appearance of multiple extrusions. Ligstroside aglycone was one of the most active inhibitors of HER2 expression in MCF10A/HER2 cells, with a reduction 68%, and IC50 10μM. HER2 overexpression further promoted an exacerbated sensitivity to the apoptotic effects of ligstroside aglycone. These findings molecularly support epidemiological evidence revealing that lingstrocide aglycone anti-breast cancer effects primarily affect the occurrence of breast tumors overexpressing the type I receptor tyrosine kinase HER2

but further suggest that its stereochemistry might provide an excellent and safe platform for the design of new HER2 targeted anti-breast cancer drugs.

The effect of Ligstroside aglycone on breast cancer

Menendez *et al.* in their study explored the ability of ligstroside aglycone to modulate HER2 tyrosine kinase receptor-induced, which is present in large amounts in breast cancer cells and causes their uncontrolled proliferation. Ligstroside aglycone was one of the most active inhibitors of HER2 expression in cells, with a reduction 63%, even in very small doses and eventually the cancer cells were led to programmed cell death. These findings provide an excellent and safe platform for the design of new anti-breast cancer drugs

Ligstroside aglycone inhibits the growth of breast cancer. It causes a reduction in the number of HER2 tyrosine kinase receptors, which are presented in large amounts in breast cancer cells and lead to their uncontrolled proliferation.

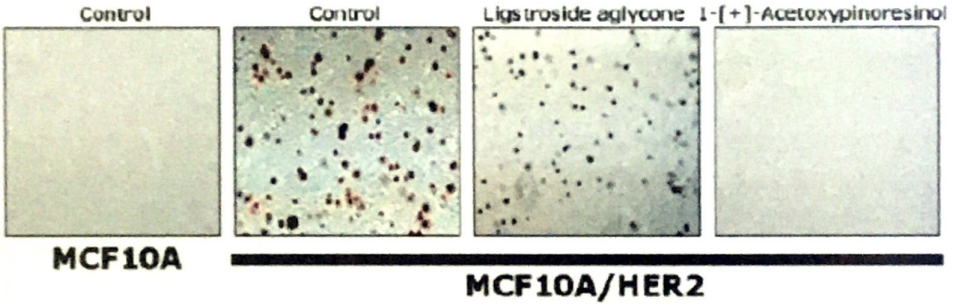

Figure. Effects of the EVOO polyphenols on the transforming ability of HER2. MCF10A/HER2 and MCF10A/pBABE matched control cells (10,000 per well) were seeded in 35-mm multi-well plates in culture medium containing 0.35% low-melting agarose over a 0.7% agarose basal layer and incubated for 14 days at 37°C in a humidified 95% O2 5% CO2 atmosphere in DMEM/F12 medium supplemented with 10% horse serum + 20 ng/ml EGF in the absence or presence of 50 μM ligstroside aglycone and 50 μM 1-(+)-acetoxypinoresinol. Colonies were then stained with p-iodonitrotetrazolium violet (1 mg/ml stock diluted 1:500) for 18 h. Colonies >50 μm in diameter were counted (see representative microphotographs).

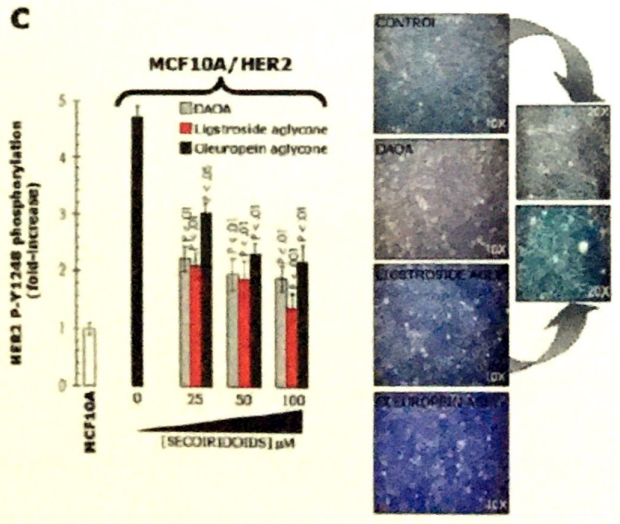

Figure. Effects of EVOO secoiridoids (c) on the activation status of HER2 tyrosine kinase. Overnight serum-starved MCF10A/HER2 cells were cultured in DMEM/F12 medium- 0.1% horse serum in the absence or presence of increasing concentrations of EVOO phenolics for 6 and 24 h. Assessment of the active/inactive status of the HER2 tyrosine kinase receptor was performed by semi-quantitatively determining the degree of degree of phosphorylation of the 1248 tyrosine residue (Tyr1248) of HER2 by using the FACE ErbB-2 (Y1248) kit as described in 'Materials and methods'. The total HER2 antibody supplied in the FACE ErbB-2 kit allows determining HER2 phosphorylation relative to the total HER2 protein found in the cells. Data were plotted after correction for cell number (performed through use of crystal violet staining) and the measurement of phosphor-HER2 (Y1248) in untreated HER2-negative MCF10A cells was arbitrarily designed as 1.0-fold. Data are the mean (columns) and 95% confidence intervals (bars) of three independent experiments performed in duplicate. One-factor ANOVA was used to analyze differences in the relative levels of phosphor-HER2 (Y1248) in MCF10A/HER2 cells following 6 h treatment with EVOO phenolics. Statistically significant differences (one-factor ANOVA analysis) between experimental conditions and unsupplemented control cells are labeled. All statistical tests were two-sided. N.S, Not statistically significant. Figure also shows the impact of exogenous supplementation with EVOO phenolics on cell morphology of MCF10A/HER2 cells as assessed by phase contrast microscopic analysis.

Javier A Menendez et al., BMC Cancer 2008

OLEUROPEIN AGLYCONE

1. The Polyphenol Oleuropein Aglycone Protects TgCRND8 Mice against Aß Plaque Pathology

In their research, Grossi *et al.* used the double transgenic TgCRND8 mice, which overexpressing the Swedish and Indiana mutations in the human amyloid precursor protein, to examine in vivo the effects of 8 weeks dietary supplementation of oleuropein aglycone at the dose of 50 mg/kg. The dietary supplementation of oleuropein aglycone strongly improves the cognitive performance of young/ middle-aged TgCRND8 mice. Immunofluorescence analysis of cerebral tissue in these mice showed remarkably reduced ß-amyloid levels and plaque deposits. Moreover, microglia migration to the plaques for phagocytosis and a remarkable reduction of the astrocyte reaction were evident. Finally, oleuropein aglycone-fed mice brain displayed an astonishingly intense autophagic reaction, as shown by the increase of autophagic markers expression and of lysosomal activity. Data obtained with cultured cells confirmed the latter evidence, suggesting mTOR regulation by oleuropein aglycone. These results support, and provide mechanistic insights into, the beneficial effects against Alzheimer-associated neurodegeneration of oleuropein aglycone.

Experiments in mice show the beneficial effect of oleuropein aglycon on the progression of Alzheimer's disease

In their research, Grossi *et al.* used special mice, which produce a special mutant human protein, which is associated with Alzheimer's disease. The dietary supplementation of oleuropein aglycone for 8 weeks at the dose of 50 mg/kg showed a remarkable improvement of the cognitive performance of mice. The effect of oleuropein aglycone on the stabilization of disease progression was also examined by biochemical analysis of mouse brains, showing significant results. These results support the beneficial role of oleuropein aglycone against Alzheimer's disease.

The dietary supplementation of oleuropein aglycone strongly improves the cognitive performance of young/ middle-aged mice, fact that indicates the beneficial effect of oleuropein aglycone on the progression of Alzheimer's Disease.

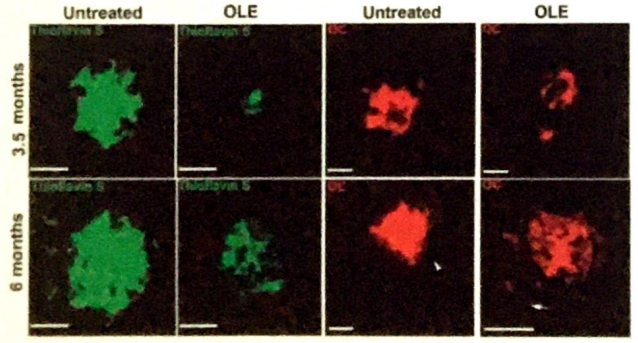

Figure. OLE modifies Ab plaque load and morphology in the brains of TgCRND8 mice. Representative photomicrographs of Thioflavin S histochemistry (green) (n = 4/group) and OC immunolabeling (red) (n = 5/group) of amyloid plaques in the cortex of untreated and OLE-fed Tg mice. In the OLE-fed Tg mice of 6 months of age several radiating plaques with ribbon-like/diffuse core and fluffy deposits (arrow) are present. Arrowhead indicates dense core amyloid plaques. Scale bars = 25 mm.

Cristina Grossi et al., 2013

2. Oleuropein aglycone prevents cytotoxic amyloid aggregation of human amylin

Here, Rigacci S. *et al.* investigated the effects on amylin aggregation and cytotoxicity of the oleuropein aglycon. It was showed that oleuropein, when present during the aggregation of amylin, consistently prevented its cytotoxicity to RIN-5F pancreatic β-cells, as determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide test and caspase-3 activity assay. A lack of interaction with the cell membrane of amylin aggregates grown in the presence of oleuropein was shown by fluorescence microscopy and synthetic lipid vesicle permeabilization. Moreover, the ThT assay, circular dichroism analysis and electron microscopy images suggested that oleuropein interferes with amylin aggregation, resulting in a different path skipping the formation of toxic prefibrillar aggregates. These results provide a molecular basis for some of the benefits potentially coming from extra virgin olive oil consumption and pave the way to further studies on the possible pharmacological use of oleuropein to prevent or to slow down the progression of type II diabetes.

The beneficial effect of Oleuropein aglycone against type II diabetes

Here, Rigacci S. *et al.* investigated the effects on amylin aggregation and cytotoxicity of the oleuropein aglycon. Amylin is a substance that is quite similar to insulin. When amylin aggregates in pancreatic cells, it generates amyloid, which is directly related to the pathogenesis of type II diabetes. Specifically, oleuropein aglycone inhibits the aggregation of amylin and its toxicity action on pancreatic cells. These results provide a molecular basis for some of the benefits potentially coming from extra virgin olive oil consumption and pave the way to further studies on the possible pharmacological use of oleuropein to prevent or to slow down the progression of type II diabetes

Oleuropein aglycone inhibits or slows down the progression of type II diabetes, by inhibiting the aggregation and toxicity of amylin in pancreatic cells, a substance that is directly related to the pathogenicity of the disease

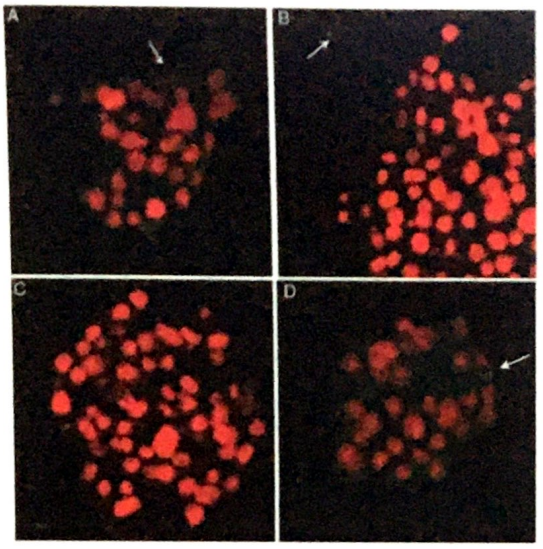

Fig. Immunofluorescence analysis of RIN-5F cells treated with hIAPP aggregates. The cells were treated with 30 min-aged hIAPP aggregates (final concentrations: 200 nM hIAPP, 1.8 μMoleuropein). After 5 h, the cells were fixed and stained with rabbit anti-amylin and Alexafluor 488-labeled anti-rabbit antibodies. Nuclei were stained with propidium iodide. (A) Cells treated with hIAPP. (B) Cells treated with hIAPP incubated with oleuropein. (C) Control, untreated cells. (D) Cells treated with hIAPP that was aged without oleuropein and given to cells together with oleuropein.

Stefania Rigacci et al., 2009

3. Extra-virgin olive oil polyphenols inhibit HER2 (erbB-2)-induced malignant transformation in human breast epithelial cells: Relationship between the chemical structures of extra-virgin olive oil secoiridoids and lignans and their inhibitory activities on the tyrosine kinase activity of HER2

Menendez *et al.* in their study explored the ability of oleuropein aglycone to modulate HER2 tyrosine kinase receptor-induced in vitro transformed phenotype in human breast epithelial cells. Using MCF10A normal breast epithelial cells it was further determined the relationship between chemical structure of oleuropein aglycone and its inhibitory activities on the tyrosine kinase activity of the HER2 oncoprotein. When compared with untreated cells, MCF10A/HER2 cells, treated with oleuropein aglycone, grew less dense, were significantly bigger in volume and showed a profound reorganization of cell-cell contacts with the appearance of multiple extrusions. Oleuropein aglycone was one of the most active inhibitors of HER2 expression in MCF10A/HER2 cells, with a reduction 63%, and IC50 64μM. HER2 overexpression further promoted an exacerbated sensitivity to the apoptotic effects of oleuropein aglycone. These findings molecularly support epidemiological evidence revealing that oleuropein aglycon anti-breast cancer effects primarily affect the occurrence of breast tumors overexpressing the type I receptor tyrosine kinase HER2 but further suggest that its stereochemistry might provide an excellent and safe platform for the design of new HER2 targeted anti-breast cancer drugs.

The effect of oleuropein aglycone on breast cancer

Menendez *et al.* in their study explored the ability of oleuropein aglycone to modulate HER2 tyrosine kinase receptor-induced, which is present in large amounts in breast cancer cells and causes their uncontrolled proliferation. Oleuropein aglycone was one of the most active inhibitors of HER2 expression in cells, with a reduction 63%, even in very small doses and eventually the cancer cells were led to programmed cell death. These findings provide an excellent and safe platform for the design of new anti-breast cancer drugs.

Oleuropein aglycone inhibits the growth of breast cancer. It causes a reduction in the number of HER2 tyrosine kinase receptors, which are presented in large amounts in breast cancer cells and lead to their uncontrolled proliferation.

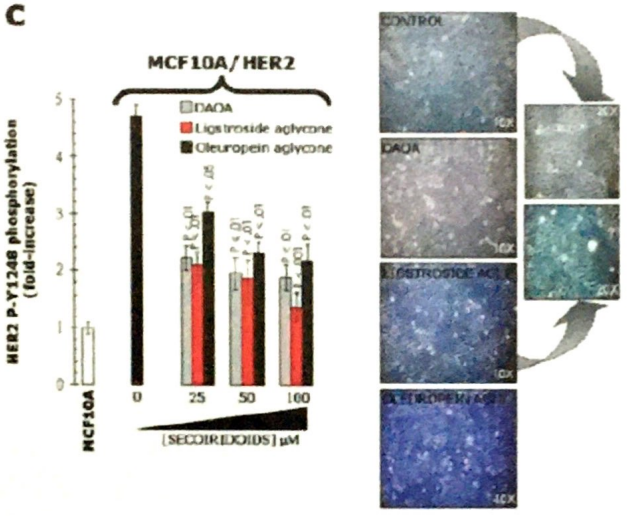

Figure. Effects of EVOO secoiridoids (c) on the activation status of HER2 tyrosine kinase. Overnight serum-starved MCF10A/HER2 cells were cultured in DMEM/F12 medium- 0.1% horse serum in the absence or presence of increasing concentrations of EVOO phenolics for 6 and 24 h. Assessment of the active/inactive status of the HER2 tyrosine kinase receptor was performed by semi-quantitatively determining the degree of degree of phosphorylation of the 1248 tyrosine residue (Tyr1248) of HER2 by using the FACE ErbB-2 (Y1248) kit as described in 'Materials and methods'. The total HER2 antibody supplied in the FACE ErbB-2 kit allows determining HER2 phosphorylation relative to the total HER2 protein found in the cells. Data were plotted after correction for cell number (performed through use of crystal violet staining) and the measurement of phosphor-HER2 (Y1248) in untreated HER2-negative MCF10A cells was arbitrarily designed as 1.0-fold. Data are the mean (columns) and 95% confidence intervals (bars) of three independent experiments performed in duplicate. One-factor ANOVA was used to analyze differences in the relative levels of phosphor-HER2 (Y1248) in MCF10A/HER2 cells following 6 h treatment with EVOO phenolics. Statistically significant differences (one-factor ANOVA analysis) between experimental conditions and unsupplemented control cells are labeled. All statistical tests were two-sided. N.S, Not statistically significant. Figure also shows the impact of exogenous supplementation with EVOO phenolics on cell morphology of MCF10A/HER2 cells as assessed by phase contrast microscopic analysis.

JAVIER A. MENENDEZ et al., 2008

4. Oleuropein aglycone: A polyphenol with different targets against amyloid toxicity.

It has been reported that the aglycone form of Oleuropein (OleA) interferes in vitro and in vivo with amyloid aggregation of a number of proteins/peptides involved in amyloid, particularly neurodegenerative, diseases avoiding the growth of toxic oligomers and displaying protection against cognitive deterioration. Leri M et al carried out a cellular and biophysical study on the relationships between the effects of OleA on the aggregation and cell interactions of the D76N β2-microglobulin (D76N b2m) variant associated with a familial form of systemic amyloidosis. The results indicate that OleA protection against D76N b2m cytotoxicity results from two mechanisms. First, through a modification of the conformational and biophysical properties of its amyloid fibrils and second through a modification of the cell bilayer surface properties of exposed cells. The present study reveals that OleA remodels not only D76N b2m aggregates but also the cell membrane interfering with the misfolded proteins-cell membrane association.

Leri M et al, Biochim Biophys Acta. 2018

5. Oleuropein Aglycone Protects against MAO-A-Induced Autophagy Impairment and Cardiomyocyte Death through Activation of TFEB

Miceli C. et al explored the effects of OA in cardiomyocytes with overexpression of monoamine oxidase-A (MAO-A). They observed that OA treatment counteracted the cytotoxic effects of MAO-A through autophagy activation. Moreover, the decrease in autophagosomes and the increase in autolysosomes suggested a restoration of the defective autophagic flux. Most interestingly, they found that the ability of OA to confer cardioprotection through autophagy induction involved nuclear translocation and activation of the transcriptional factor EB (TFEB). These data provide strong evidence of the beneficial effects of OA, suggesting its potential use as a nutraceutical agent against age-related pathologies involving autophagy dysfunction, including cardiovascular diseases.

Miceli et al, Oxidative Medicine and Cellular Longevity 2018

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