Supplementary Information

Modulation of Tau Protein Fibrillization by Oleocanthal

Maria Chiara Monti, Luigi Margarucci, Raffaele Riccio, and Agostino Casapullo*

Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, Via Ponte don Melillo, 84084 Fisciano, Italy

Tau-441 Modified Peptides after Oleocanthal (1) Treatment.

Tau441 Peptides*	[M-H]+	ΔMw 92 Da	Modified Lysine
6 23	2054,1367		
6 24	2182,2485		
6-24 ox	2198,2678		
24-44	2294,2883		
25-44	2165,2239		
45-67	2391,2114		
68-87	1955,2219		
88-126	3956,8425		
131-155	2533,4072		
133-143	1550,0264		
133-143	1213,1298	x	K140 or K141
133-150	1778,6976		
141-163	2249,2732		
144-170	2620,959		
156-170	1423,9475		
156-174	1833,0383		
156-180	2424,6321		
164-190	2696,5581		
181-194	1411,8999		
181-209	2786,1504		
195-209	1394,8037		
195-221	2684,686		
210-211	1309,9122		
210-224	1664,1287		
210-225	1792,25		
210-230	2316,2471		
212-224	1420,9701		
222-242	2361,4546		
225-242	1895,2795		
225-242	1986,8715	x	K225 or K234 or K240
231-242	1242,8788		
235-242	911,02	x	K240
241-257	1986,8715	x	K254
241-257ox	1910,2379		
243-259ox	1883,235		
258-280	2525,5098	x	K259 or K267 or K274
260-280	2217,2493		
260-280	2310,1184	x	K267 or K274
260-281	2437,5579	x	K267 or K274 or K280
268-280	1467,8977	x	K274
281-298	1977,2791		
299-317	1979,1586		
312-343	3406,2439	x	K317 or K321 or K331 or K340
318-343	2675,8945		
322-340	1917,2693		
341-349	1137,7769		
344-347	522,245		
354-369	1580,0566		
354-370	1707,0985		
371-383	1586,9957		
384-395	1331,9078		
384-406	2413,2583		
386-406	2215,3679		
407-438	3244,38		
* 100% Sequence coverage			

Figure S1. Proteolytic map of tau-441:**1** complex at 37 °C.

Reaction Profile of Oleocanthal (1) with Basic Amino Acids. The reactivity of **1** towards the basic amino acids l-lysine and l-arginine has been carried out by HRESIMS, HPLC-ESIMS and MS^n experiments. Each amino acid (final concentration 250 μM) was dissolved in sodium acetate 20 mM at a neutral pH and an appropriate volume of **1**, dissolved in CH_3CN, was added (final concentration 25 μM). The amount of CH_3CN was kept lower than 5%. The mixture was incubated at 37 °C for 40 min and analyzed by RP-HPLC-MS on a Phenomenex C_{18} narrow-bore column by means a linear gradient from 10 % to 80% aqueous acetonitrile over 30 min The mass spectrometer was a Q-TOF Premiere from Waters, Co.

In order to assess the nitrogen side chain as the sole nucleophilic center on the above-mentioned amino acids, the reactivity of l-alanine was tested towards **1**. LC-MS runs of the reaction mixture after 40 min did not show any adduct between l-alanine and **1**, confirming the lack of reactivity of the α-amino group.

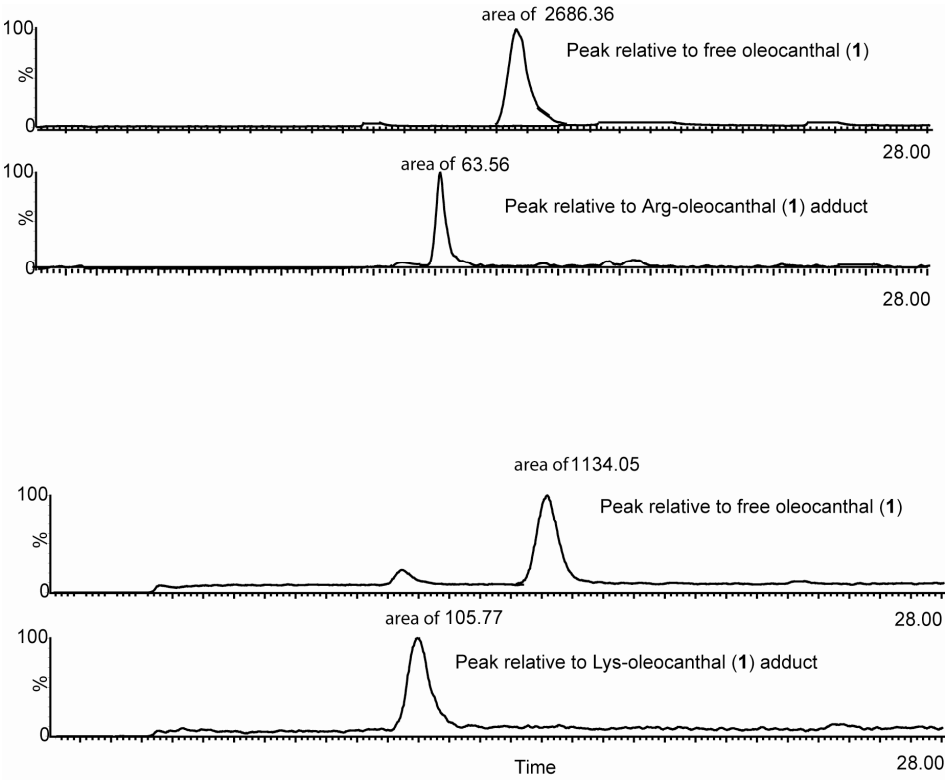

Figure S2. RP-HPLC-MS of reaction mixtures of **1** with l-Lys (below) and l-Arg.

Reaction Profile of Oleocanthal (1) with l-Lysine. The LC-MS analysis performed on a **1**:l-Lys reaction mixture showed the formation of a single adduct at *m/z* 433.3, corresponding to the imine of **1** generated by the nucleophilic attack of the l-Lys ε-amino group on the oleocanthal carbonyl residue at C-3. The relative areas of the peaks at *m/z* 433.3 and 327.3, corresponding to the sodiated oleocanthal species, revealed that only a small fraction of **1** reacted with l-Lys (Figure S2). The imine nature of the **1** adduct was confirmed by a displacement experiment with hydroxylamine (NH_2OH). Addition of NH_2OH to the reaction mixture produced a **1**-oxyme and free l-Lys, as detected by LC-MS.

Reaction Profile of Oleochantal (1) with l-Arginine. The reaction of **1** with l-Arg followed a pathway similar to that of l-Lys, revealing the formation of a single adduct at *m/z* 461.3. As in the above-discussed case, this species corresponded to the imine of **1** generated by the nucleophilic attack of the arginine guanidinium group onto the C-3 aldehyde carbonyl of **1**. The relative areas of the peaks at *m/z* 461.3 and 327.3 (sodiated oleocanthal species) revealed a low reactivity of **1** with l-Arg (Figure S2). As in the case of l-Lys, the treatment of the reaction mixture with hydroxylamine produced a 1-oxyme and free l-Arg, confirming the imine nature of the adduct.

Reaction Profile of Oleocanthal (1) with Myoglobin, Lysozyme and Human Synovial PLA_2. The covalent reactivity of **1** towards several proteins used as standards was tested by MALDI-MS on a MALDI-MICRO (Waters Co). Briefly, each protein at 3 μM concentration in PBS 1X was incubated at 37 °C for 40 min in the presence of **1** (20 fold molar excess). Then, 1 μL of the reaction mixture was loaded on a MALDI plate using α-cyano-4-hydroxycinnamic acid dissolved in 1:1 CH_3CN-H_2O-0.1% TFA as matrix. As shown in Figure S3, no covalent adducts were measured for these proteins.

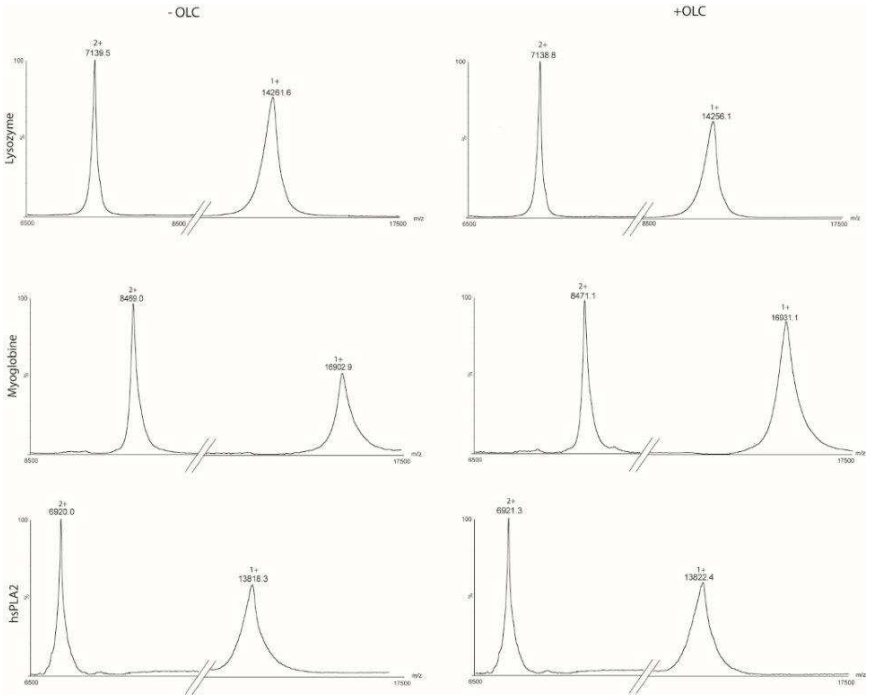

Figure S3. MALDI-MS analysis of myoglobin, lysozyme and human synovial PLA_2 incubated with oleocanthal (1).