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Molecular foundations of OMDM198, dual-target FAAH inhibitor and TRPV1 antagonist, analgesic potential: role of TRPV1 upregulation and sensitization in osteoarthritis

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Introduction: Osteoarthritis (OA) is a joint disease leading to chronic pain. FAAH inhibition was proved to exert antinociception in various preclinical models of chronic pain but failed to induce analgesia in clinical manifestations of OA pain. This is most likely related to TRPV1 receptor stimulation. Our results discuss the benefits of polypharmacology over single-acting compounds for OA treatment.

Methods: OA was induced in Wistar rats by intra-articular injection of 3 mg of monoiodoacetate (MIA) diluted in 50mL 0.9% saline. Animals were deeply anesthetized with 5% isoflurane in 100% O_2 (4,5L/min). Single target compounds (URB597 - FAAH inhibitor, 5mg/kg; SB366,791 - TRPV1 antagonist, 2mg/kg) and dual-acting compound OMDM198 (FAAH inhibitor/TRPV1 antagonist, 1mg/kg) were used in present studies. All reagents were dissolved in DMSO, Tween 80, ethanol and carboxymethyl cellulose in 0.9% saline. At day 21 after OA induction rats were sacrificed 1h after i.p. drugs' administration. Total number of 10 animals per treatment group was used in the experiment. Molecular alternations in expression of mRNA after treatment were evaluated in lumbar spinal cord (SC) by RT-qPCR. RNA isolation was performed according to Chomczynski's method. Results are presented as a fold change proportional to the expression level in intact animals. Data was analyzed using one way analyses of variance with a Bonferroni post hoc test.

Results:

Contra	Vehicle	OMDM198	SB366,791	URB597
Cnr1	5,34396 ±0,34 (8) ***	6,02753 ±0,35 (10) ***	6,20558 ±0,19 (10) ***	5,0704 ±0,34 (8) ***
Cnr2	4,075169 ±0,49 (8) ***	2,604934 ±0,1 (8) *	3,388387 ±0,42 (10) ***	2,717181 ±0,5 (9) **
Trpv1	1,98928 ±0,12 (8) ***	1,8344 ±0,11 (10) ***	1,85948 ±0,07 (9) ***	1,9315 ±0,19 (9) ***
Faah	4,63649 ±0,49 (8) ***	3,49026 ±0,27 (9) ***	4,23487 ±0,28 (10) ***	3,38967 ±0,55 (9) ***
Mapk3	3,69186 ±0,28 (8) ****	3,52777 ±0,22 (9) ****	3,72732 ±0,19 (10) ****	3,98384 ±0,54 (8) ****
Mapk14	3,87233 ±0,45 (8) ****	3,02531 ±0,29 (10) **	3,80339 ±0,27 (10) ****	3,69974 ±0,67 (9) ****
Prkaca	3,81766 ±0,19 (7) ****	4,14995 ±0,32 (8) ****	3,92648 ±0,34 (10) ****	3,53792 ±0,53 (9) ****
Prkcg	5,34829 ±0,63 (8) ****	4,36753 ±0,28 (9) ****	4,52286 ±0,5 (10) ****	5,16141 ±1,02 (9) ****
alox12	2,722801 ±0,17 (8) ****	1,917924 ±0,13 (9)	2,020144 ±0,2 (10) *	2,324423 ±0,3 (9) **
Ptgs2	4,27398 ±0,32 (8) ****	3,89243 ±0,2 (9) ****	4,05906 ±0,14 (10) ****	3,86385 ±0,46 (9) ****

Ipsi	Vehicle	OMDM198	SB366,791	URB597
Cnr1	4,36 ±0,33 (10) ***	5,73934 ±0,28 (10) *** #	5,62706±0,21 (10) *** #	4,16213±0,37 (7) ***
Cnr2	2,77949 ±0,21 (9) **	2,829189 ±0,4 (10) ***	1,76802 ±0,14 (9)	2,166964 ±0,29 (7)
Trpv1	1,75039 ±0,11 (9) ***	1,86664 ±0,15 (10) ***	1,78189 ±0,12 (10) ***	1,80975 ±0,15 (6) ***
Faah	3,67581 ±0,35 (10) ***	3,91468 ±0,43 (10) ***	3,08223 ±0,17 (9) ***	2,63209 ±0,36 (7) ***
Mapk3	2,847 ±0,23 (10) ****	3,20392 ±0,19 (10) ****	3,16103 ±0,21(10) ****	3,30138 ±0,36 (7) ****
Mapk14	3,38744 ±0,46 (10) ****	2,56705 ±0,08 (8)	3,01161 ±0,1 (8) **	2,69419 ±0,22 (6)
Prkaca	4,13981 ±0,4 (10) ****	4,66819 ±0,35 (6) ****	4,40495 ±0,35 (6) ****	3,33948 ±0,3 (7) ****

Prkcg	4,10269 ±0,37 (10) ***	1,95838 ±0,41 (9)	4,25047 ±0,45 (6) ****	3,18495 ±0,3 (7)
alox12	2,045551 ±0,18 (10) *	1,519128 ±0,11 (10)	1,876361 ±0,2 (10) *	1,866132 ±0,18 (7)
Ptgs2	3,81417 ±0,31 (10) ****	4,15244 ±0,36 (10) ****	4,03079 ±0,13 (10) ****	3,5141 ±0,24 (7) ****

Following MIA administration, we observed strong mRNA upregulation of endocannabinoid receptors (*Cnr1, Cnr2, Trpv1*) and their metabolic enzymes (*Faah, Ptgs2, Alox12)* together with TRPV1 sensitizing kinases (*Mapk3, Mapk14, Prkcg, Prkaca)* in lumbar spinal cord. Treatment with OMDM198 further increased *Cnr1* expression exclusively on the ipsilateral side, whereas it has not affected levels of *Cnr2*. Furthermore treatment with OMDM198, SB366,791 or URB597 did not change *Trpv1, Faah Mapk3, Prkaca* and *Ptgs2* expression levels but both FAAH inhibitors, OMDM198 and URB597 abolished the expression of *Mapk14, Prkcg* and *Alox12* in the ipsilateral part of spinal cord.*p<0.05 vs Intact; #p<0.05 vs Vehicle

Conclusions: Our results shed a light on the inflammatory component of OA that is related to TRPV1 sensitization as presented by changes in *Trpv1* and kinases mRNA expressions together with increase in *Alox12* expression. Apparent interplay between articular, immunological and nervous system in pathophysiology of OA is a strong justification for novel multi-target drug strategies targeting ECS.*Supported by National Science Centre, Poland by grants: OPUS UMO-2014/13/B/NZ7/02311, SONATABIS/NCN/2012/07/E/NZ7/01269 and statutory funds. All experiments were approved by the Local Bioethics Committee of the Institute of Pharmacology (approval number 938/2012).*