Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst132P.pdf

Characterization of oleamide hydrolysis by human recombinant fatty acid amide hydrolase-2 (FAAH-2) expressed in HEK293T cells

J.T. Dongdem[1,2], S.P. Dawson[1], S.P.H. Alexander[1.] [1]School of Life Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, UK, [2]School of Medicine and Health Sciences, University for Development Studies, Tamale-Campus, Ghana

Fatty acid amide hydrolase (FAAH) is the primary enzyme responsible for the degradation of the endocannabinoid anandamide and related fatty acid amides (1). Although there is apparently one gene encoding FAAH activity in rodents, a recently identified alternative gene (FAAH-2) is found in other species including man (2). FAAH-2 also has the ability to inactivate a broad range of bioactive lipids, albeit with a distinct rank order of affinity, and is also sensitive to inhibitors of FAAH. Selected non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to inhibit FAAH activity from mouse and rat preparations (3). More recently, the metabolism of endocannabinoids by COX-2 was suggested to be differentially regulated by NSAIDs (4). Thus in this study, we investigated the inhibition of FAAH-2 metabolism of oleamide using selected NSAIDs.

HEK293T cells were transfected with a pcDNA3.1-FAAH-2 construct by the polyethyleneimine method and incubated 24, 48, 72 hr at 37 ºC after which cells were harvested and homogenized. FAAH-2 activity of cells transfected and mock transfected with the FAAH-2 expression plasmid was assessed by a fluorescent OPA-based detection method for quantification of ammonia generated from the primary amide substrates (N = 4 of triplicate assessments) (5).

HEK293T-FAAH-2 activity hydrolysed oleamide with a K_m value of 51 ± 7 and V_{max} of 0.09 ± 0.01 nmol min^{-1} (mg protein)$^{-1}$ and was inhibited by FAAH inhibitor, URB597 with pIC_{50} = 6.9 ± 0.1 (N = 4). Of the NSAIDs screened at 500 μM, only meclofenamate produced complete inhibition of FAAH-2 activity. Carprofen, sulindac, sulindac sulphone and diflunisal evoked an inhibition to < 50 % of control while indomethacin and diclofenac exhibited modest inhibition of FAAH-2 activity to between 60 and 70 % of control. However, ibuprofen, ketorolac, dipyrone, salicylic acid, acetyl salicylic acid, acetaminophen and

salicyluric acid (103.5 – 122.0 % of control) did not inhibit FAAH-2 oleamide hydrolysis.

Meclofenamate (pIC_{50} = 3.86 ± 0.04), sulindac (3.62 ± 0.06), carprofen (3.57 ± 0.04), diflunisal (3.53 ± 0.05), sulindac sulphone (3.39 ± 0.06) and indomethacin (3.26 ± 0.03) exhibited concentration-dependent inhibition of oleamide hydrolase activity with significant potency. In the presence of meclofenamate or indomethacin, Michaelis-Menten analysis suggested a reduction in the V_{max} of oleamide, without significant alteration in substrate affinity, indicative of a non-competitive action of these inhibitors.

	Control (- inhibitor)	+ 200 µM indomethacin	+100 µM meclofenamate
K_m (µM)	42 ± 5	46 ± 5	40 ± 3
V_{max} nmol min^{-1} mg $protein^{-1}$	0.09 ± 0.01	0.08 ± 0.01	0.05 ± 0.01

Expressed as Mean ± SEM, N = 5 of triplicate assessments

We therefore confirm the ability of URB597 to inhibit HEK293T-FAAH-2 oleamide activity with high potency and established significant inhibitory action of meclofenamate, carprofen, diflunisal, sulindac, sulindac sulphone and indomethacin to inhibit FAAH-2 activity. Our results thus, suggest potential for study of these NSAIDs as combined FAAH-2-COX inhibitors.

References:

1. Deutsch DG and Chin SA (1993). *Biochem Pharmacol* **46:** 791 – 796.
2. Wei BQ *et al.* (2006). *J Biol Chem* **281:** 36569 – 36578
3. Fowler CJ *et al*. (1997). *J Pharmacol Exp Ther* **283:** 729 – 734.
4. Duggan *et al*. (2011). *Nat Chem Biol* **7:** 803 – 809.
5. Garle MJ *et al*. (2006). pA2 online **3:** 114P –