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NOTE

A simple method for simultaneous determination of *N*-arachidonoylethanolamine, *N*-oleoylethanolamine, *N*-palmitoylethanolamine and 2-arachidonoylglycerol in human cells

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Abstract The endocannabinoid system has been considered as a target for pharmacological intervention. Accordingly, inhibition of fatty acid amide hydrolase (FAAH), a degrading enzyme of the endocannabinoids *N*-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) as well as of the endocannabinoid-like substances *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA), can cause augmented endogenous cannabinoid tone. Using liquid chromatography coupled with positive electrospray ionisation mass spectrometry, we herein describe a method to simultaneously quantify levels of AEA, OEA, PEA and 2-AG in cultured cells. The procedure was developed according to the FDA guidelines for bioanalytical methods validation. The limits of quantification (LOQs) were 0.05 pmol for AEA, 0.09 pmol for OEA, 0.10 pmol for PEA and 0.80 pmol for 2-AG when molecular ion monitoring was used. In H460 human lung carcinoma cells, basal levels of all four analytes ranged between 2 and 17 pmol mg^{-1} protein with PEA showing the lowest and OEA the highest concentrations. Endocannabinoid levels observed in mesenchymal stem cells were of the same order of magnitude when compared to those in H460 human lung carcinoma cells.

Keywords LC–MS/MS · Endocannabinoids · Endocannabinoid-like substances · H460 Lung carcinoma cells · Mesenchymal stem cells

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Introduction

The endocannabinoids *N*-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) as well as the endocannabinoid-like substances *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) have been implicated in the regulation of multiple physiological and pathophysiological processes including analgesia, cancer, hypothermia and feeding [1, 2]. Whereas AEA and 2-AG have been identified as potent agonists to both CB1 and CB2 cannabinoid receptors, OEA and PEA which differ from AEA in the acyl chain size and the degree of unsaturation possess cannabimimetic activity without binding to CB1 and CB2 [1, 2]. Endogenous ethanolamines (AEA, OEA, PEA) are degraded by the enzyme fatty acid amide hydrolase (FAAH), whereas 2-AG can be hydrolysed by multiple enzymes, including FAAH and monoacylglycerol lipase with about 85 % of brain 2-AG hydrolase activity ascribed to MAGL [2]. In the last decade, inhibition of FAAH resulting in augmented endocannabinoid tone has been considered as the target for pharmacological intervention. Among its diverse potential pharmacological effects, antitumourigenic actions of FAAH inhibitors have been shown in cultured cells [3], emphasising the need for adequate methods to measure cellular endocannabinoid levels. However, apart from numerous published LC–MS/MS-based methods with validated quantifications of endocannabinoid biomarkers in different biological matrices [4 and ref. therein], less attention was paid so far for analysis of endocannabinoids in cultured cells.

The aim of this work was therefore to develop and validate a simple and selective LC–MS(MS)-based method for analysis of selected endocannabinoids in human cells. Variability of selected biomarkers was not restricted to classic representatives of the endocannabinoid member family, i.e. AEA and 2-AG, but also included the endocannabinoid-like substances

OEA and PEA. The procedure has been developed according to FDA guidelines for bioanalytical methods validation and was applied by use of H460 human lung carcinoma cells as well as mesenchymal stem cells.

Experimental

Standards and reagents

Pure standards of AEA, OEA, PEA, 2-AG and internal standard (IS) AEA-d_8 were purchased from Cayman Chemicals (Ann Arbor, MI, USA). The purity of the reference compounds was >98 %. The following GC- or LC–MS-grade solvents for lipid extraction and LC–MS were purchased: ethyl acetate, hexane, formic acid, water and tris(hydroxymethyl)aminomethane from Merck (Darmstadt, Germany), acetonitrile from Roth (Karlsruhe, Germany), 2-propanol from Backer (Griesheim, Germany) and methanol from Sigma-Aldrich (Taufkirchen, Germany). Dulbecco's modified Eagle's medium (DMEM) with 4 mmol L^{-1} L-glutamine and 4.5 g L^{-1} glucose was from Cambrex Bio Science Verviers S.p.r.l. (Verviers, Belgium). Phosphate-buffered saline (PBS) and fetal calf serum (FCS) were obtained from PAN Biotech (Aidenbach, Germany). Penicillin–streptomycin was obtained from Invitrogen (Karlsruhe, Germany). The bicinchoninic acid (BCA) protein assay kit was purchased from Fisher Scientific GmbH (Schwerte, Germany).

Standard preparation

Calibration curves from 0.1 to 100 ng mL^{-1} (0.1, 0.3, 0.6, 1, 3, 10, 30, 100 ng mL^{-1}) for AEA, PEA and OEA and from 2 to 200 ng mL^{-1} (2, 5, 10, 30, 50, 100, 200 ng mL^{-1}) for 2-AG were obtained by analysis of either 1 mL 20 mM Tris–HCl buffer (pH 6.8) or PBS (pH 7.4) spiked with 10 μL of an appropriate working solution. Working solutions were prepared by dilution of the stock solutions of the analytes (1 mg mL^{-1}) in methanol. Both stock and working solutions of 2-AG were prepared in acetonitrile. Calibration standard solutions that contained different concentrations of AEA, OEA, PEA and 2-AG were prepared in 1 mL of corresponding buffer placed in ice-cold screw-capped glass tubes and spiked with 20 ng mL^{-1} of AEA-d_8. The standard solutions were extracted (2×1 mL) with a mixture of ethyl acetate/hexane (9:1, *v*/*v*). The pooled organic phase was dried under nitrogen, and the residues were reconstituted in 100 μL acetonitrile/2-propanol (60:40, *v*/*v*) containing 0.5 % formic acid for analysis.

LC–MS analysis

Extracted samples (30 to 60 μL) were analysed on a Waters HPLC 2695 Separation Module using a Multospher 120 C18 column 125×2 mm, 5-μm particle size (CS-Chromatographie Service GmbH, Langerwehe, Germany) coupled with a guard column (20×2 mm, 5-μm particle size). The endocannabinoids were resolved using mobile phase A (water containing 0.2 % formic acid) and mobile phase B (acetonitrile/2-propanol [60:40, *v*/*v*] containing 0.2 % formic acid) at a flow rate of 0.15 mL min^{-1}. The elution scheme was as follows: linear increase of mobile phase B from 65 to 80 % in 10 min, isocratic at 80 % of phase B in 3 min and linearly to 100 % phase B in the following 6 min. Finally, the system was re-equilibrated at 35 % phase A over 4 min. The HPLC column effluent was introduced into a Micromass Quatro Micro™ API mass spectrometer (Waters, Milford, USA) and analysed using electrospray ionisation in the positive mode and a single ion monitoring (SIM) modus: *m*/*z* 300.8 for PEA, *m*/*z* 326.8 for OEA, *m*/*z* 348.8 for AEA, *m*/*z* 379.8 for 2-AG and *m*/*z* 356.8 for the IS. The mass spectrometer and source parameters were set up as follows: capillary voltage 3.5 kV; cone voltage 20 and 24 V for AEA/AEA-d_8/2-AG and PEA/OEA, respectively; source temperature 120 °C; desolvation temperature 350 °C; and flow rate of desolvation gas 700 L h^{-1}. Dwell and delay times were 0.05 and 0.1 s, respectively. Alternatively, analyses were performed using electrospray ionisation in the positive-ion mode using selected reaction monitoring (SRM) to select parent–daughter transitions. The MS/MS transitions (*m*/*z*) were as follows: 300.8→61.6 (collision energy 13 V) for PEA, 326.8→61.6 (collision energy 17 V) for OEA, 348.8→61.6 (collision energy 14 V) for AEA, 379.8→287.6 (collision energy 17 V) for 2-AG and 356.8→62.6 (collision energy 13 V) for the IS (AEA-d_8). The daughter ions observed for AEA, PEA, OEA, 2-AG and AEA-d_8 are consistent with previous reports on these analytes [5, 6]. For all transitions, the dwell was 0.05 s. Argon was used for collision-induced dissociation. All instrument parameters for the monitored analytes were tuned by injecting standard solutions at a concentration of 100 ng mL^{-1} at 10 μL min^{-1} flow rate by a syringe pump. The data were acquired using MassLynx software version 4.1 (Micromass Ltd., Manchester, UK).

Validation

The validation of the method was performed according to FDA guidelines and recommendations for bioanalytical methods validation [7]. The following parameters were evaluated: sensitivity (limits of detection (LODs) and limits of quantification (LOQs)), linearity, precision, accuracy, specificity, recovery and matrix effects.

LODs and LOQs

The sensitivity of the method was tested by comparing the signals obtained for each analyte in blank samples (five samples) with those obtained from samples spiked at a concentration corresponding to the LOQ. The LOD was defined as the lowest concentration with a signal-to-noise ratio (*S*/*N*) >3. The LOQ was defined as the lowest concentration at which the corresponding analyte signal was ten times over the signal of the blank response. Both precision (relative standard deviation (RSD)) and accuracy (relative error, RE) were <20 %.

Linearity

Calibration standards were prepared as described above. Calibration curves (a zero sample and at least seven non-zero samples covering the ranges of 0.1–100 ng mL^{-1} for AEA, OEA and PEA and 2–200 ng mL^{-1} for 2-AG) were plotted as the peak area ratio of the analyte over a single IS (AEA-d_8) against the nominal concentration of the calibrator. To assess linearity, the line of best fit was determined by least square linear regression.

Recoveries and matrix effect

Recoveries were calculated at the low (LOQ), middle and high concentration levels: 0.3, 10 and 100 ng mL^{-1} for AEA; 0.5, 10 and 100 ng mL^{-1} for OEA and PEA; and 5, 50 and 200 ng mL^{-1} for 2-AG, each in five replicates. Relative and absolute recoveries were expressed as percentage values by comparing the peak area ratios of the analyte/internal standard in the extracted samples against either that from buffer blanks spiked post-extraction or a neat solution of the corresponding compound. Internal standards were post-spiked into the extracted samples. Matrix effect (ME) calculated as percentage was evaluated for each endocannabinoid by comparing the peak area ratios of the analyte/internal standard in the buffer blanks spiked post-extraction against a neat solution of the corresponding compound [8].

Precision and accuracy

Precision (RSD) and accuracy (RE) were calculated at three concentration levels assayed in sets of five replicates. The experimental precision was expressed in percents as the relative standard deviation (RSD=standard deviation/mean× 100). Inter-day precision was obtained from the areas of 15 spiked samples of each concentration analysed over three different days (five per day). The accuracy was calculated as the relative error expressed in percents by comparing the concentration relative to the mean peak area calculated by the calibration curve equation against the theoretical concentration.

Stability

Three aliquots of samples at the low (LOQ), middle and high concentration levels—0.3, 10 and 100 ng mL^{-1} for AEA; 0.5, 10 and 100 ng mL^{-1} for OEA and PEA; and 5, 50 and 200 ng mL^{-1} for 2-AG—were subjected to three cycles of freezing at −80 °C and thawing at room temperature. Short-term stability tests were carried out after keeping the samples of the same concentration levels at room temperature for 6 h. Long-term stability was tested after keeping samples at the temperature of −80 °C for a period of 60 days, unless otherwise stated. Additionally, both short- and long-term stability of the four analytes was tested either in 20 mM Tris–HCl buffer (pH 6.8) or PBS.

Analysis of biological samples

Mesenchymal stem cells were prepared as described elsewhere [9] and kept at −80 °C prior to use. H460 human lung carcinoma cells were purchased from ATCC-LGC [American Type Culture Collection (ATCC) number: HTB-177] and maintained as described previously [10]. For one sample or donor, H460 or mesenchymal stem cells were seeded in 6 or 8 x 10 mL-petri dishes at a density of 2×10^6 or 1.2×10^6 cells per well and grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum (FCS), 4 mM glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. After 18 h or 48 h H460 or mesenchymal stem cells were washed once with Dulbecco's PBS, incubated additionally for 24 h or 9 h in FCS-free DMEM and washed again with Dulbecco's PBS. The cell pellets obtained after centrifugation (10 min, 2000×*g*, 4 °C) were frozen in liquid nitrogen and stored at −80 °C prior to analysis. For the determination of endocannabinoids, cell pellets were further re-suspended in 1 mL of 20 mM Tris–HCl buffer (pH 6.8) spiked with 20 ng mL^{-1} of AEA-d_8 and lysed using a Sonopols U-tip sonifier (Bandelin, Berlin, Germany) three times with a 15×5-s pulse at 75 % power followed by a 60-s pause. The lysates were transferred to ice-cold screw-capped glass tubes. In parallel with standard solutions, samples were extracted and analysed as described above. An aliquot of each lysate (10 μL) was applied for quantitation of total protein using a BCA protein assay kit.

Results and discussion

Extraction procedure and analytic conditions

In the current work, a simple LC–MS(MS)-based method for simultaneous analysis and quantification of 2-AG and the

three ethanolamines (AEA, OEA, PEA) in human cells was developed. PBS/BSA solution may be used as an appropriate matrix for preparation of calibration standards to quantify endocannabinoids in biological tissues [11]. The latter has been reported to have little or no matrix effect [12]. Both selected ion monitoring (SIM) and selected reaction monitoring (SRM) were used as methods of choice and compared. Although the selected reaction monitoring (SRM) mode seems to be more specific than SIM to capture trace amounts of analytes in extracts of biological samples that may contain high levels of impurities, the yield of the parent ion can be significantly decreased due to formation of adducts that are resistant to collision-induced dissociation [13]. A mixture of ethyl acetate–hexane (9:1, *v*/*v*) was found to provide optimal extraction yields of the four endocannabinoids. The share of 1-AG under this procedure did not exceed 7–8 % which is in accordance with previous observations for this analyte [6]. The chromatographic resolution of isomeric 2-AG and 1-AG and suppression of the protonated adducts $[M+Na]^+$ was also considered. The best ionisation of 2-AG and the three ethanolamines was achieved by use of an acetonitrile/2-propanol (60:40, *v*/*v*) mixture as mobile phase rather than pure acetonitrile or methanol.

Linearity, LODs and LOQs

The calibration curves were found to be linear in the calibration range, and the correlation coefficients (r^2) were always >0.999 both in monitoring the molecular ion (SIM) and transitions specified in the "Experimental" section SRM (Table 1). Extracted LC–MS(MS) chromatograms of the four analytes (AEA, OEA, PEA, 2-AG) at the LOQs and the IS in spiked buffer (upper panels) vs. blank buffer (lower panels) obtained in both MS/MS (Electronic Supplementary Material (ESM) Fig. S1A) and MS modus (ESM Fig. S1B) are shown. Analysis of the blank extracts revealed trace amounts of an impurity, which was identical by its MS/MS spectrum and retention time with the authentic PEA standard (ESM Fig. S1A, lower panel). Although traces of PEA have been shown to be present mainly in different brands of chloroform [14], contamination through extraction solvents and/or at any other sample preparation stage cannot be excluded. The PEA signal of the blank response was considered for all further calculations. The LOQ level of PEA was calculated to be ten times over the PEA signal peak area of the blank sample. LODs and LOQs for 2-AG and ethanolamines are listed in Table 1. With respect to published data [5, 13], the LOQs presented in this work were comparable to those reported for AEA, OEA and PEA but somewhat lower for 2-AG, which, due to its chemical properties, generally yields a weaker ESI/MS response as compared to that of ethanolamines [13 and ref. therein]. For better understanding, the LOQs are expressed in absolute values per amount of the analyte applied for SIM/SRM LC–MS: 0.05/0.05, 0.09/0.03, 0.10/0.03, and 0.80/0.80 pmol for AEA, OEA, PEA and 2-AG, respectively.

Recovery and matrix effect

While developing a method for quantitation of endocannabinoids in cultured cells, we selected and tested two BSA-free buffers (PBS and Tris–HCl) for both preparation of calibration solutions and cell lysis. In fact, the average total protein concentration obtained in the lysed cell samples constituted only 5 % of that tested by Nguyen and co-workers in the artificial PBS/BSA matrix [12]. Salt/matrix effects of the two buffers (PBS, pH 7.4, and 20 mM Tris–HCl, pH 6.8) were evaluated by plotting the concentration-dependent responses of analytes to those recorded from pure solution standards. Matrix effects were most pronounced for PBS with LOQs of all four analytes ranging between 85 and 144 % when AEA-d_8 and a SIM were used (ESM Table S1). The results obtained for PBS using SRM modus were comparable with those obtained for Tris–HCl buffer using single ion monitoring. Slight enhancement was detected at LOQs and slight or no suppression was observed at other concentrations (Table 2) for all four analytes tested. The latter observation suggested that Tris–HCl buffer may be more appropriate for endocannabinoid analysis in cells. Relative and absolute recoveries were in general not below 75 % in all cases.

Accuracy and precision

For each day of the validation procedure, five repetitions of the blank level were carried out to establish the response

Table 1 Regression data. LODs and LOQs for selected analytes obtained either in SIM or SRM modus

Compound	SIM modus				SRM modus			
	r^2	Equation	LOD, ng mL^{-1}	LOQ, ng mL^{-1}	r^2	Equation	LOD, ng mL^{-1}	LOQ, ng mL^{-1}
AEA	0.9998	y=0.1065x−0.0057	0.1	0.3	0.9998	y=0.4536x+0.0453	0.1	0.3
OEA	0.9999	y=0.4372x+0.0797	0.17	0.5	0.9999	y=4.1043x−0.3842	0.05	0.15
PEA	0.9999	y=0.4181x+0.0183	0.17	0.5	0.9999	y=7.2059x+0.6590	0.05	0.15
2-AG	0.9994	y=0.0135x−0.0086	1.7	5	0.9991	y=0.0237x+0.0319	1.7	5

Table 2 Recovery and matrix effects for AEA, OEA, PEA and 2-AG obtained from Tris–HCl buffer. Values represent means± SEM of n=5 determinations

Compound	Concentration, ng mL^{-1}	20 mM Tris–HCl buffer (pH 6.8)		
		Relative recovery (%)	Absolute recovery (%)	Matrix effect (%)
AEA	0.3	94.1±8.6	89.4±5.5	94.7±5.8
	10	82.2±5.4	80.8±3.2	99.7±6.0
	100	81.7±3.1	80.5±4.7	98.5±3.0
OEA	0.5	73.4±1.4	79.3±5.5	109.7±7.3
	10	78.0±0.7	78.6±3.6	103.2±2.0
	100	83.8±5.7	79.7±2.4	92.8±2.5
PEA	0.5	75.0±3.7	88.9±5.7	111.7±4.8
	10	77.9±2.4	76.5±2.2	99.1±2.7
	100	83.2±4.3	78.1±4.2	93.9±3.2
2-AG	5	88.9±10.9	112.4±3.8	116.9±2.0
	50	90.5±6.5	91.1±8.7	101.3±3.2
	200	94.3±6.2	83.8±6.5	88.7±4.2

corresponding to the PEA contamination level. This basal concentration was considered in all calculations related to the validation procedure. The intra-day and inter-day precision (RSD) and accuracy (RE) of the method were suitable for analysis of all four endocannabinoids studied. Very good precision around the mean value was achieved for measurements of the three ethanolamines and 2-AG in Tris–HCl buffer, with RSD values being always ≤12 % even at LOQ level. Inaccuracy ranged around the actual value being within ±20% of the recommended tolerance for the LOQs and ±15% for other concentration levels (Table 3).

Stability

The stability of each analyte was investigated at three different concentrations. Extracted samples were shown to be stable in acetonitrile at room temperature (6 h), after storage at −80 °C for 60 days and after three freeze–thaw cycles (data not shown). To reduce isomerisation of 2-AG to 1-AG, all samples were stabilised with formic acid [13]. Analytes were considered to be also stable in the lysis buffer (Tris–HCl) since no significant degradation (<10 %) was recorded over a 6-h time period and during long-term storage (60 days) at −80 °C for AEA, OEA and PEA and 30 days at −80 °C for 2-AG (ESM Table S2).

Analysis of biological samples

The sample preparation and LC–MS(MS) conditions reported here were applied to measure basal levels of the three ethanolamines and 2-AG in both mesenchymal stem cells and human lung carcinoma cells (H460) using either monitoring

Table 3 Intra- and inter-day precision (expressed as RSD%), and accuracy (RE%) values for quantification of AEA, OEA, PEA and 2-AG. Values represent means±SEM of n=5 determinations

Analyte	Concentration, ng mL^{-1}	Intra-day		Inter-day 1		Inter-day 2		Inter-day 3	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
AEA	0.3	7.0	5.3±4.2	8.9	8.2±6.9	8.6	3.4±1.0	8.9	6.4±6.6
	10	2.3	1.6±0.8	4.3	6.0±3.8	1.3	11.5±1.4	7.5	8.1±2.5
	100	7.3	5.4±1.4	1.7	2.4±1.6	3.9	3.5±1.6	1.5	11.2±1.3
OEA	0.5	0.1	7.0±1.2	7.6	8.4±3.6	0.3	16.9±2.1	1.1	5.5±4.6
	10	0.3	5.6±0.9	5.2	4.8±1.8	0.3	2.4±1.0	4.9	8.3±4.2
	100	0.8	9.6±4.5	3.5	10.0±3.8	0.5	1.9±0.5	2.2	1.72±2.3
PEA	0.5	1.7	7.3±2.0	3.1	4.1±2.9	2.9	4.1±0.3	9.5	2.3±3.0
	10	3.9	5.4±1.5	0.6	3.8±0.6	3.5	6.8±3.8	3.7	11.6±3.4
	100	0.3	4.9±0.3	3.4	2.7±0.6	0.9	2.8±0.8	2.6	0.3±2.6
2-AG	5	0.4	10.5±0.3	3.3	7.4±3.9	7.9	8.5±3.9	7.9	5.7±2.4
	50	2.1	3.3±1.8	2.4	6.2±1.3	8.2	4.0±2.5	6.5	1.0±6.3
	200	2.0	7.9±2.2	2.6	8.7±2.9	2.8	5.9±2.5	5.4	3.5±5.1

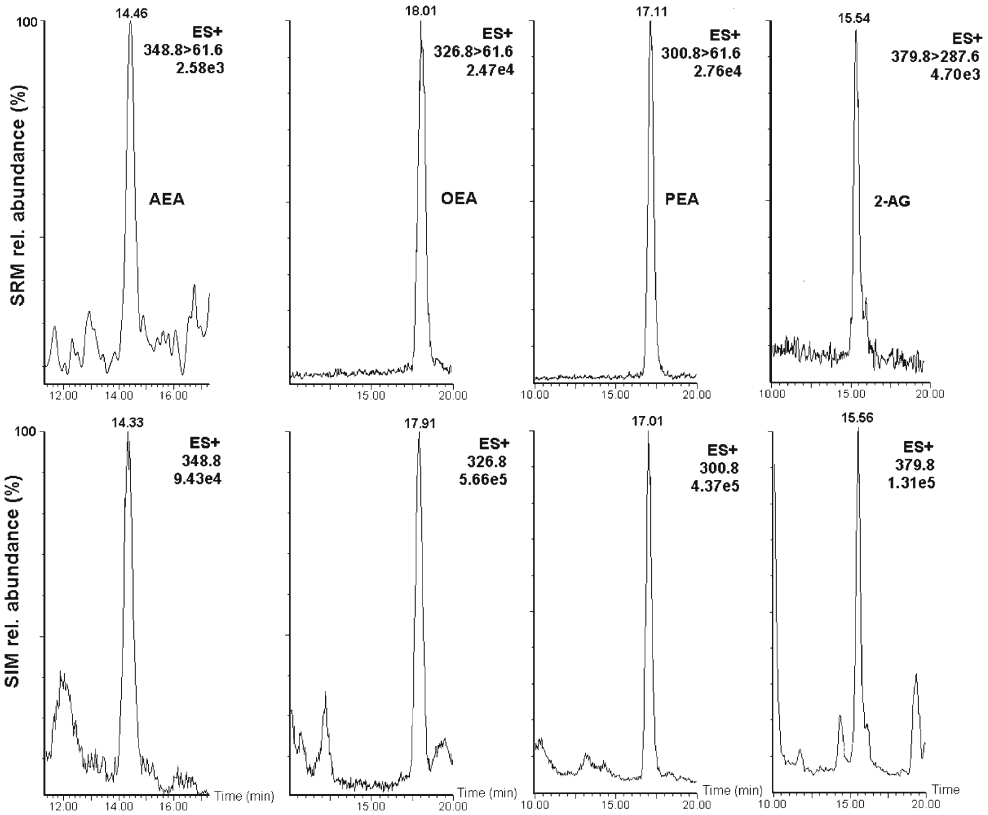

Fig. 1 LC–MS/MS (*upper panel*) and LC–MS (*lower panel*) chromatograms from the endocannabinoid analysis of mesenchymal stem cells

of the single ion or recording transitions specific for each particular endocannabinoid (Fig. 1). As illustrated in Fig. 2b, basal levels of all four endocannabinoids were measured in H460 human lung carcinoma cells, ranging between 3 and 17 pmol mg^{-1} protein, whereby the concentration of PEA was the lowest and that of OEA the highest. The basal level of PEA in H460 human lung carcinoma cells was below the LOQ but above the LOD levels for this analyte in the SIM modus. In turn, in the SRM modus, the concentration of PEA was found to be higher than the LOQ. The basal levels of the ethanolamines observed in mesenchymal stem cells were comparable to each other among the three different donors tested, whereby detectable 2-AG levels (above LOD) were found only in mesenchymal stem cells of one donor (Figs. 1 and 2a). A very close correlation was observed between the results obtained from MS and MS/MS methods (data not shown).

Conclusions

Herein, we describe a selective LC–MS(MS) methodology allowing simultaneous analysis of several endocannabinoids,

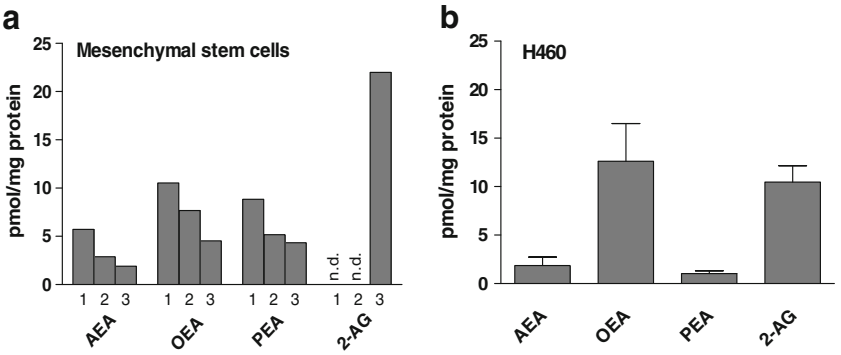

Fig. 2 Endocannabinoid concentrations found in **a** mesenchymal stem cells obtained from three different donors and **b** H460 human lung carcinoma cells. Experimental data obtained for H460 cells are presented as mean value±SEM for $n=3$ samples. For mesenchymal cells, values of three donors are presented separately. All data shown are normalised to the protein content

acting as bioactive mediators and potential endocannabinoid system markers, including AEA, OEA, PEA and 2-AG in cells. An excellent linearity, greater than 0.999, good accuracy, precision and specificity have been demonstrated for determination of the above-mentioned endocannabinoids using a simple LC–MS method. The accuracy and precision of the method were established by examining the three standards over an extended period of time. The lower limit of quantitation was sufficient to capture the basal levels of the ethanolamines measured in mesenchymal stem cells and all four analytes in H460 human lung carcinoma cells. The MS data obtained found a good support by the MS/MS methodology following a single parent–daughter transition. These results suggest that a simple LC–MS method may be sufficient to quantify the above-mentioned endocannabinoids in cultured cells.

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References

1. Piomelli D, Sasso O (2014) Peripheral gating of pain signals by endogenous lipid mediators. Nat Neurosci 17:164–174
2. Fezza F, Bari M, Florio R, Talamonti E, Feole M, Maccarrone M (2014) Endocannabinoids, related compounds and their metabolic routes. Molecules 19:17078–17106
3. Proto MC, Gazzerro P, Di Croce L, Santoro A, Malfitano AM, Pisanti S, Laezza C, Bifulco M (2012) Interaction of endocannabinoid system and steroid hormones in the control of colon cancer cell growth. J Cell Physiol 227:250–258
4. Zoerner AA, Gutzki FM, Batkai S, May M, Rakers C, Engeli S, Jordan J, Tsikas D (2011) Quantification of endocannabinoids in biological systems by chromatography and mass spectrometry: a comprehensive review from an analytical and biological perspective. Biochim Biophys Acta 1811:706–723
5. Jian W, Edom R, Weng N, Zannikos P, Zhang Z, Wang H (2010) Validation and application of an LC-MS/MS method for quantitation of three fatty acid ethanolamides as biomarkers for fatty acid hydrolase inhibition in human plasma. J Chromatogr B 878:1687–1699
6. Zoerner AA, Batkai S, Suchy MT, Gutzki FM, Engeli S, Jordan J, Tsikas D (2012) Simultaneous UPLC-MS/MS quantification of the endocannabinoids 2-arachidonoyl glycerol (2AG), 1-arachidonoyl glycerol (1AG), and anandamide in human plasma: minimization of matrix-effects, 2AG/1AG isomerization and degradation by toluene solvent extraction. J Chromatogr B Analyt Technol Biomed Life Sci 883–884:161–171
7. Smith G (2010) Bioanalytical method validation: notable points in the 2009 draft EMA guideline and differences with the 2001 FDA guidance. Bioanalysis 2:929–935
8. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 75: 3019–3030
9. Schmuhl E, Ramer R, Salamon A, Peters K, Hinz B (2014) Increase of mesenchymal stem cell migration by cannabidiol via activation of p42/44 MAPK. Biochem Pharmacol 87:489–501
10. Ramer R, Heinemann K, Merkord J, Rohde H, Salamon A, Linnebacher M, Hinz B (2013) COX-2 and PPAR-γ confer cannabidiol-induced apoptosis of human lung cancer cells. Mol Cancer Ther 12:69–82
11. Williams J, Wood J, Pandarinathan L, Karanian DA, Bahr BA, Vouros P, Makriyannis A (2007) Quantitative method for the profiling of the endocannabinoid metabolome by LC-atmospheric pressure chemical ionization-MS. Anal Chem 79:5582–5593
12. Nguyen HP, Li L, Nethrapalli IS, Guo N, Toran-Allerand CD, Harrison DE, Astle CM, Schug KA (2011) Evaluation of matrix effects in analysis of estrogen using liquid chromatography-tandem mass spectrometry. J Sep Sci 34:1781–1787
13. Sergi M, Battista N, Montesano C, Curini R, Maccarrone M, Compagnone D (2013) Determination of the two major endocannabinoids in human plasma by μ-SPE followed by HPLC-MS/MS. Anal Bioanal Chem 405:785–793
14. Skonberg C, Artmann A, Cornett C, Hansen SH, Hansen HS (2010) Pitfalls in the sample preparation and analysis of N-acylethanolamines. J Lipid Res 51:3062–3073