

RESEARCH

Open Access

# Micronized/ultramicronized palmitoylethanolamide displays superior oral efficacy compared to nonmicronized palmitoylethanolamide in a rat model of inflammatory pain

Daniela Impellizzeri<sup>1†</sup>, Giuseppe Bruschetta<sup>1†</sup>, Marika Cordaro<sup>1</sup>, Rosalia Crupi<sup>1</sup>, Rosalba Siracusa<sup>1</sup>, Emanuela Esposito<sup>1</sup> and Salvatore Cuzzocrea<sup>1,2\*</sup>

## Abstract

**Background:** The fatty acid amide palmitoylethanolamide (PEA) has been studied extensively for its anti-inflammatory and neuroprotective actions. The lipidic nature and large particle size of PEA in the native state may limit its solubility and bioavailability when given orally, however. Micronized formulations of a drug enhance its rate of dissolution and reduce variability of absorption when orally administered. The present study was thus designed to evaluate the oral anti-inflammatory efficacy of micronized/ultramicronized versus nonmicronized PEA formulations.

**Methods:** Micronized/ultramicronized PEA was produced by the air-jet milling technique, and the various PEA preparations were subjected to physicochemical characterization to determine particle size distribution and purity. Each PEA formulation was then assessed for its anti-inflammatory effects when given orally in the carrageenan-induced rat paw model of inflammation, a well-established paradigm of edema formation and thermal hyperalgesia.

**Results:** Intraplantar injection of carrageenan into the right hind paw led to a marked accumulation of infiltrating inflammatory cells and increased myeloperoxidase activity. Both parameters were significantly decreased by orally given micronized PEA (PEA-m; 10 mg/kg) or ultramicronized PEA (PEA-um; 10 mg/kg), but not nonmicronized PeaPure (10 mg/kg). Further, carrageenan-induced paw edema and thermal hyperalgesia were markedly and significantly reduced by oral treatment with micronized PEA-m and ultramicronized PEA-um at each time point compared to nonmicronized PeaPure. However, when given by the intraperitoneal route, all PEA formulations proved effective.

**Conclusions:** These findings illustrate the superior anti-inflammatory action exerted by orally administered, micronized PEA-m and ultramicronized PEA-um, versus that of nonmicronized PeaPure, in the rat paw carrageenan model of inflammatory pain.

**Keywords:** Edema, Hyperalgesia, Inflammation, Micronized, Oral administration, Pain, Palmitoylethanolamide, Ultramicronized

\* Correspondence: salvator@unime.it

†Equal contributors

<sup>1</sup>Department of Biological and Environmental Sciences, University of Messina, Viale Ferdinando Stagno D'Alcontres, no 31, Messina 98166, Italy

<sup>2</sup>Manchester Biomedical Research Centre, Manchester Royal Infirmary, University of Manchester, Manchester, Oxford Rd, Manchester M13 9WL, UK

## Background

Inflammation is fundamentally a protective cellular response aimed at removing injurious stimuli and initiating the healing process [1]. Nonetheless, there are settings in which the inflammatory response itself damages host tissue and causes organ dysfunction, such as an overly robust acute or subacute inflammatory response to pathogens or debris from damaged host cells [2]. As pointed out by Nathan and Ding [1], the problem with inflammation is not how often it starts, but how often it fails to subside. Indeed, nonresolving inflammation is one of the principal contributors to the medical burden in industrialized societies. Neuroinflammation in both the peripheral and central nervous systems plays an important role in the pathogenesis of chronic pain [3,4]. Therapeutic targeting of the inflammatory response thus continues to be an area of intense research activity.

Current approaches to treating inflammation target enzymes, ion channels, RNAs (antisense oligonucleotides) and epigenetics (for example, histone modification), among others. An alternative strategy to inhibiting inflammation would be, quoting Tabas and Glass [5], “to commandeer nature’s own anti-inflammatory mechanisms to induce a ‘dominant’ program of resolution” *Science*, p. 7. Resolution of inflammation is driven not only by selected cell types but also by the secretion or extracellular formation of soluble products [1]. Resolution may fail if expression of these factors is delayed or reduced. Such agents may include cytokines, a protease inhibitor, gaseous signals, oxygenated and nitrated lipids, a purine and a neurotransmitter [1]. In this context, the existence of peripheral lipid-mediated signaling molecules provide an intriguing avenue of investigation. These lipid mediators act to suppress the inflammatory process, restore homeostasis in injured tissues and moderate pain sensitivity by regulating the flow of nociceptive signals to the central nervous system [6]. One promising family of such molecules is the *N*-acylethanolamines, whose principal members are the endocannabinoid *N*-arachidonylethanolamine (anandamide) and its congeners *N*-stearoylethanolamine, *N*-oleoylethanolamine and *N*-palmitoylethanolamine (PEA) [7]. PEA’s ability to modulate inflammation and pain in animal studies led to the proposal of this endogenous fatty acid amide as a component of a complex homeostatic system controlling the basal threshold of both inflammation and pain. The fact that PEA is produced during inflammatory conditions supports this role. Further, data showing selective inhibition of PEA degradation to be anti-inflammatory points more directly to PEA’s involvement in the control of pain and inflammation. As an endogenous compound, PEA has no adverse effects at pharmacological doses while possessing a double therapeutic effect (that is, anti-inflammatory and antinociceptive) (see [8,9] for recent reviews).

Given their lipidic nature and large particle size in the native state, molecules such as PEA may have limitations in terms of solubility and bioavailability. The use of micronization for dissolution enhancement of poorly water-soluble drugs is a technique frequently used in the pharmaceutical field. By application of this technique, microparticles are produced by reducing large drug crystals down to the micron range (<10 μm) [10-13]. Given that the dissolution rate of a drug is proportional to its surface area, major benefits of microcrystal formulations are enhanced rate of dissolution [14] and reduced variability of drug absorption when orally administered [15]. Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia [16] that has been extensively used in the development of anti-inflammatory drugs. In the present study, we evaluated the effects of different PEA formulations, both nonmicronized and micronized/ultramicrotonized, administered orally or parenterally in the carrageenan model. The results demonstrate that micronized/ultramicrotonized PEA has a significantly improved anti-inflammatory and anti-hyperalgesic profile compared to nonmicronized PEA when given by the oral route.

## Methods

### Materials

Unless otherwise stated, all compounds used in this study were purchased from Sigma-Aldrich (Poole, UK). PEA was obtained from the following sources: Epitech Group (Saccolongo, Italy), Tocris Bioscience (Bristol, UK), Cayman Chemical (Ann Arbor, MI, USA), JP Russell Science (Nicosia, Cyprus), LJ Pharma (Belpasso, Italy) and GalSor (Montella, Italy). All solutions used for *in vivo* infusion were prepared using nonpyrogenic saline (0.9% wt/vol NaCl; Baxter Healthcare, Thetford, UK).

### Animals

The study was carried out using male Sprague-Dawley rats (200 to 230 g; Harlan, Nossan, Italy). Food and water were available *ad libitum*. The study was approved by the University of Messina Review Board for the care of animals. Animal care was in compliance with Italian regulations on the protection of animals used for experimental and other scientific purposes (DM116192) as well as with the relevant European Economic Community (EEC) regulations (OJ of EC L 358/1 12/18/1986).

### Micronization/ultramicrotonization of palmitoylethanolamide

PEA was subjected to the air-jet milling technique, in which a coarse powder is slowly fed into a jet-mill apparatus endowed with a chamber of 300 mm in diameter that operates with “spiral technology” driven by compressed air. The high number of collisions that occur between particles as a result of the high level of

kinetic—not mechanical—energy produces micron- and sub-micron-sized crystals [17].

#### Particle size distribution

Particle size distribution (PSD) was measured by dynamic light scattering using a Mastersizer 3000 equipped with a wet dispersion unit (Malvern Instruments, Malvern, UK). Particles were dispersed in water with the aid of an in-line sonication probe for rapid agglomerate dispersion.

#### Palmitoylethanolamide microparticle morphology

The morphology of PEA microparticles was assessed with a model LEO 1525 scanning electron field emission microscope (Carl Zeiss SMT AG, Oberkochen, Germany). Particles were dispersed onto a carbon tab attached to an aluminum stub (Agar Scientific, Stansted, UK) and then coated with gold/palladium (250 Å layer thickness) using a model 108 A sputter coater (Agar Scientific).

#### High-performance liquid chromatography

The purity of the PEA preparations was determined by high-performance liquid chromatography (HPLC). PEA content was also determined and compared to commercially available PEA formulations. Analysis was carried out using a model 1260 Infinity HPLC System equipped with a degasser, a binary pump, an automatic liquid sampler injector, an ultraviolet diode array detector, a thermostatted column compartment and a thermostatic module (all from Agilent Technologies, Santa Clara, CA, USA). The column used was a Zorbax SB-C18 (5 µm, 150 × 4.6 mm; Agilent Technologies). The running conditions were as follows: column temperature set at 40°C; absorbance measured at 205 nm; eluent solution was an 85:15 mix of acetonitrile/(10 mM ammonium acetate/methanol (2:1 vol/vol)); flow rate of 1.0 ml/min; and injection volume of 10 µl. PEA retention time was approximately 5.2 minutes. Calibration was carried out using two different standard solutions, each prepared from a weighed amount of PEA working standard: standard 1 = 0.08 mg/ml PEA, standard 2 = 0.60 mg/ml PEA. The sample solution contained a concentration of about 0.12 mg/ml of equivalent PEA. Powders recovered from PeaPure and PeaVera (JP Russell Science Ltd, Netherlands) capsules were analyzed as such. Other formulations in tablet or granular form were crushed with a mortar and pestle, weighed, dissolved in methanol and diluted with the eluent solution for the HPLC assay.

#### Experimental groups

Rats were randomly allocated to the following groups:

1. *Carrageenan (CAR) + saline*: Rats subjected to CAR-induced paw edema ( $n = 10$ )

2. *CAR + PeaPure (lot 12126A; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg orally) 30 minutes before CAR ( $n = 10$ )
3. *CAR + micronized PEA-m (lot 02/10; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg orally) 30 minutes before CAR ( $n = 10$ )
4. *CAR + ultramicrosized PEA-um (lot 03/08; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg orally) 30 minutes before CAR ( $n = 10$ )
5. *CAR + PeaPure (lot 12126A; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg intraperitoneally) 30 minutes before CAR ( $n = 10$ )
6. *CAR + micronized PEA-m (lot 02/10; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg intraperitoneally) 30 minutes before CAR ( $n = 10$ )
7. *CAR + ultramicrosized PEA-um (lot 03/08; 10 mg/kg) dissolved in carboxymethylcellulose (1.5% wt/vol in saline)*: Same as CAR + saline group, but administered (10 mg/kg intraperitoneally) 30 minutes before CAR ( $n = 10$ )

The dose and the route of administration of PEA chosen were based on those used in a previous study [18]. The same batches of micronized PEA (PEA-m) and ultramicrosized PEA (PEA-um) were used for the pharmacological and chemical experiments. The sham-operated group ( $n = 10$ ) underwent the same surgical procedures as the CAR group, except that saline or drugs were administered instead of carrageenan.

#### Carrageenan-induced paw edema

Changes in paw volume was measured as previously described [19]. Briefly, paw volume was measured with a plethysmometer (Ugo Basile, Comerio, Italy) immediately prior to the injection of carrageenan and at hourly intervals thereafter for 6 hours. Edema was expressed as the increase in paw volume in milliliters after carrageenan injection relative to the preinjection value for each animal. Results are expressed as paw volume change in milliliters.

#### Behavioral analysis

Behavioral testing was done with the experimenter blinded to treatment conditions. Hyperalgesic responses to heat were determined by using the Plantar Test (Hargreaves method; Ugo Basile) [20] with a cutoff latency of 20 seconds to prevent tissue damage. Rats were individually confined to Plexiglas chambers and allowed to habituate. A mobile unit consisting of a high-intensity projector bulb

was positioned to deliver a thermal stimulus directly to an individual hind paw from beneath the chamber. The withdrawal latency period of injected paws was determined with an electronic clock circuit and thermocouple. Results are expressed as paw withdrawal latencies.

### Histological analysis

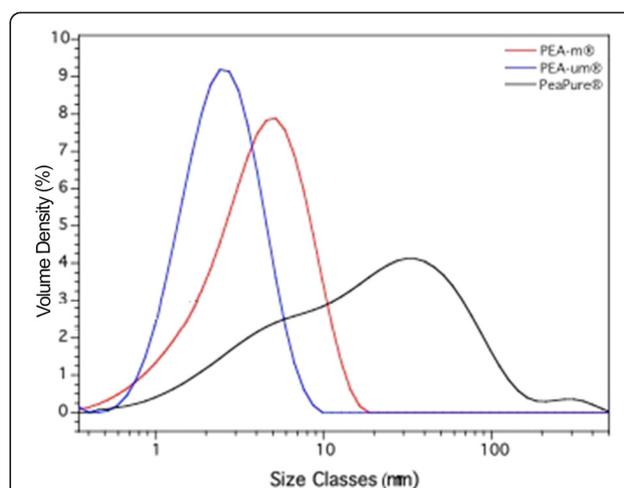
Paw biopsies were taken 6 hours following intraplantar injection of carrageenan. Tissue from the hind paw pads was removed with a scalpel. The tissue slices were fixed in Dietric solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid) for 1 week at room temperature, dehydrated by a graded series of ethanol and embedded in Paraplast (Sherwood Medical). Sections (7- $\mu$ m thickness) were deparaffinized with xylene, stained with hematoxylin and eosin (H&E) and observed with a Dialux 22 Leitz microscope. Stained sections were scored by two investigators blinded to the treatment condition. The degree of inflammation was evaluated according to a previously described method [21] and assigned a score from 0 to 5, defined as follows: 0 = no inflammation, 1 = mild inflammation, 2 = mild/moderate inflammation, 3 = moderate inflammation, 4 = moderate/severe inflammation and 5 = severe inflammation.

### Myeloperoxidase activity

Myeloperoxidase (MPO) activity, which is an index of polymorphonuclear leukocyte accumulation, was determined as previously described [22]. Paw tissues, collected at 6 hours after carrageenan induction, were homogenized in 0.5% hexadecyltrimethylammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7.0) and centrifuged for 30 minutes at 20,000 *g* at 4°C. An aliquot of the supernatant was allowed to react with a solution of 1.6 mM tetramethylbenzidine/0.1 mM H<sub>2</sub>O<sub>2</sub>. The rate of change in absorbance was measured with a spectrophotometer at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 mM of peroxide within 1 minute at 37°C and was expressed in units per gram weight of wet tissue.

### Statistical analysis

All values in the figures and text are expressed as mean  $\pm$  standard error of the mean (SEM) of *n* observations. For *in vivo* studies, *n* represents the number of animals used. In experiments involving histology or immunohistochemistry, the figures shown are representative of at least ten slices analyzed from three experiments performed on different days. Results were analyzed by one-way analysis of variance followed by a Bonferroni *post hoc* test for multiple comparisons. Significance was set at  $P < 0.05$ .



**Figure 1 Particle size distribution profile of palmitoylethanolamide.** Graph of particle sizes of micronized palmitoylethanolamide (PEA-m, lot 02/10), ultramicronized PEA (PEA-um, lot 03/08) and nonmicronized PEA (PeaPure, lot 12126A) obtained by dynamic light scattering. See Methods for further details.

## Results

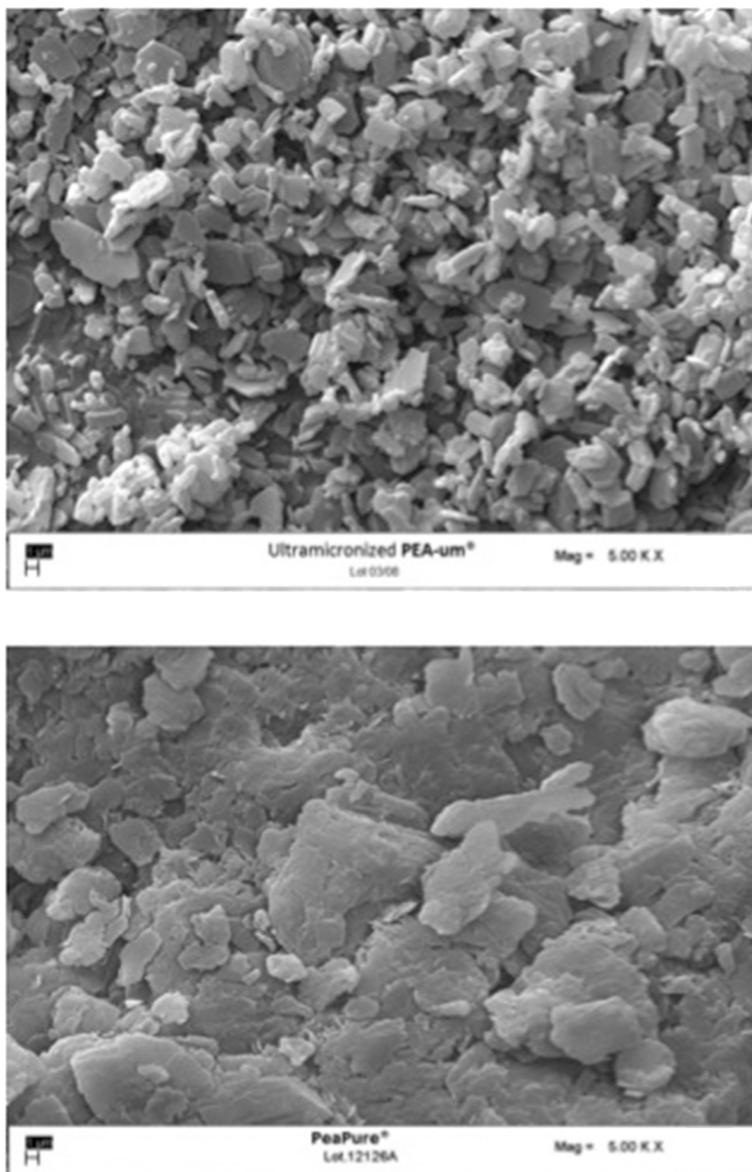
### Physicochemical characterization of micronized/ultramicronized palmitoylethanolamide

Lipidic molecules such as PEA can present challenges in terms of solubility and bioavailability when administered orally. Because increasing a drug's surface area enhances its rate of dissolution [14] while reducing variability of absorption [15], we investigated the influence of micronization/ultramicronization on PEA action in carrageenan-induced inflammation in the rat paw. Figure 1 shows the PSD profile of micronized and ultramicronized PEA in comparison to a commercial formulation of nonmicronized PEA. A clear shift toward lower particle sizes is evident for PEA upon micronization/ultramicronization. The percentage of particles below a given size is summarized in Table 1. The particle size distinction between nonmicronized and ultramicronized PEA is illustrated morphologically in the scanning electron microscopic images in Figure 2. Tables 2 and 3 report, respectively, PEA purity and content determined by HPLC across a number of currently available PEA formulations. A considerable degree of divergence is evident from these analyses, in particular

**Table 1 Particle size distribution of different palmitoylethanolamide sources<sup>a</sup>**

Particle size	PEA-m (lot 02/10)	PEA-um (lot 03/08)	PeaPure (lot 12126A)
D10	1.57 $\mu$ M	1.19 $\mu$ M	3.19 $\mu$ M
D50	4.5 $\mu$ M	2.57 $\mu$ M	21.1 $\mu$ M
D90	9.36 $\mu$ M	4.96 $\mu$ M	82.9 $\mu$ M

<sup>a</sup>PEA-m, Micronized PEA; PEA-um, Ultramicronized PEA. D10, D50 and D90 refer to the percentage of particles below the indicated size according to ASTM International standards for particle size analysis.



**Figure 2 Scanning electron microscopic images of PEA.** Images show ultramicrosized palmitoylethanolamide (PEA-um, lot 03/08; top) and nonmicronized palmitoylethanolamide (PeaPure, lot 12126A; bottom).

**Table 2 High-pressure liquid chromatography purity analysis of different palmitoylethanolamide sources<sup>a</sup>**

Product	PEA-m	PEA-um	PEA	PEA	PeaPure 400 mg	PeaVera 400 mg
Supplier	Epitech Group	Epitech Group	Cayman	Tocris Bioscience	JP Russell	JP Russell
Lot no.	02/10	03/08	152055-51	3A/151360	12126A	14A09H
Purity claimed	>98%	>98%	>98%	>98%	100%	100%
Purity found	100.13%	101.08%	98.97%	101.13%	87.69%	88.60%

<sup>a</sup>PEA-m, Micronized palmitoylethanolamide; PEA-um, Ultramicrosized palmitoylethanolamide. Values exceeding 100% are due to the precision of the analytical methods used. Assay specification limits are 98% to 102%.

**Table 3 High-pressure liquid chromatography analysis of palmitoylethanolamide content in different commercially available palmitoylethanolamide products<sup>a</sup>**

	Normast 300 mg (tablet)	Normast 600 mg (tablet)	Normast 600 mg (microgranular)	PeaPure	PeaVera	Peanase	Sinerga
Supplier	Epitech Group	Epitech Group	Epitech Group	JP Russell	JP Russell	LJ Pharma	Galsor
Lot no.	D09813	D012A4	D067F3	12126A	14A09H	SK629	11283
PEA content/dose claimed	300 mg	600 mg	600 mg	400 mg	400 mg	300 mg	150 mg
PEA content found	300.8 mg	611.5 mg	614.5 mg	344.7 mg	347.8 mg	248.5 mg	71.4 mg

<sup>a</sup>PEA-m, Micronized palmitoylethanolamide; PEA-um, Ultramicronized palmitoylethanolamide. Values exceeding 100% are due to the precision of the analytical methods used. Assay specification limits are 98% to 102%.

for several of the commercially available PEA sources, including PeaPure and PeaVera.

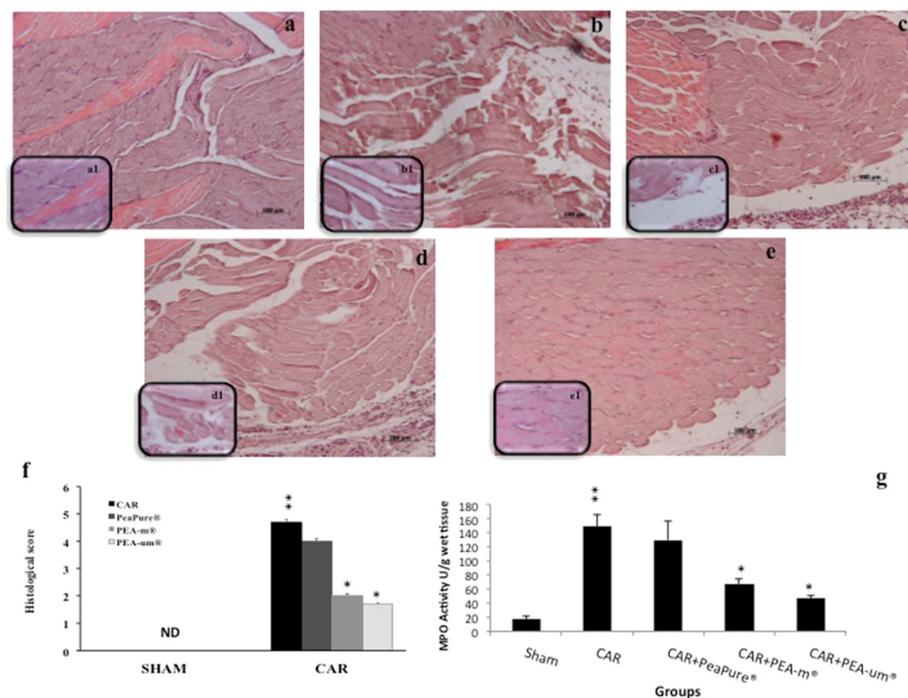
### Histological analyses of rat paw tissue

To evaluate histologically the anti-inflammatory effect of different formulations of PEA, samples of paw tissue from each experimental group were examined by H&E staining. No histologic damage was found in sham-operated rats (Figure 3a and inset a1). In contrast, carrageenan injection into the right hind paw led to a marked accumulation of infiltrating inflammatory cells (Figure 3b and inset b1) compared to control. Inflammatory cell infiltration was

significantly decreased with treatment of micronized PEA-m (Figure 3d and inset d1) or ultramicronized PEA-um (10 mg/kg) (Figure 3e and inset e1). In contrast, treatment with PeaPure (10 mg/kg) (Figure 3c and inset c1) did not result in a significant reduction in histological scores of the carrageenan-treated animals. Histological scores for the various treatment groups are given in Figure 3f.

### Effects of palmitoylethanolamide on myeloperoxidase activity

The development of histological damage was associated with increased infiltration of neutrophils, as shown by



**Figure 3 Anti-inflammatory effects of orally administered palmitoylethanolamide formulations following intraplantar injection of carrageenan into the rat hind paw: histological and biochemical analyses.** Histological evaluation was performed by hematoxylin and eosin staining. (a) Control. (b) Intraplantar injection of carrageenan (CAR) into the rat hind paw. (c) through (e) Intraplantar injection of carrageenan CAR with nonmicronized palmitoylethanolamide (PeaPure) (c); micronized PEA-m (d) and ultramicronized PEA-um (e). Insets a1 through e1 are higher-resolution images of the respective panels. All PEA formulations (10 mg/kg for each) were administered orally 30 minutes before CAR injection, and all animals were killed 6 hours after CAR injection. (f) Histological scores for the various treatment groups. (g) Myeloperoxidase (MPO) activity in paw tissues from the various treatment groups. Micronized PEA-m and ultramicronized PEA-um produced significant improvements in both measurements. See Methods for further details. Values are means  $\pm$  SEM. \*\* $P < 0.01$  vs sham and \* $P < 0.05$  vs CAR.

an increase in MPO activity, a peroxidase enzyme released by neutrophils and considered a marker of neutrophilic infiltration (Figure 3g) [23]. The administration of either micronized PEA-m or ultramicrosized PEA-um (10 mg/kg) significantly reduced MPO activity (Figure 3g). However, PeaPure (10 mg/kg) was unable to produce a significant reduction in neutrophil infiltration in the paw tissues (Figure 3g).

#### Effects of palmitoylethanolamide on the time-course of carrageenan-induced paw edema

Intraplantar injection of carrageenan in rats led to a significant and time-dependent increase in paw volume that was maximal after 5 hours (Figures 4a and 4b). The carrageenan-induced paw edema was markedly and significantly reduced by treatment with micronized PEA-m and ultramicrosized PEA-um at each time point compared to PeaPure, although there was a trend in this last group (Figure 4a). Moreover, no significant differences were observed between treatment groups when the PEA formulations were administered intraperitoneally (Figure 4b).

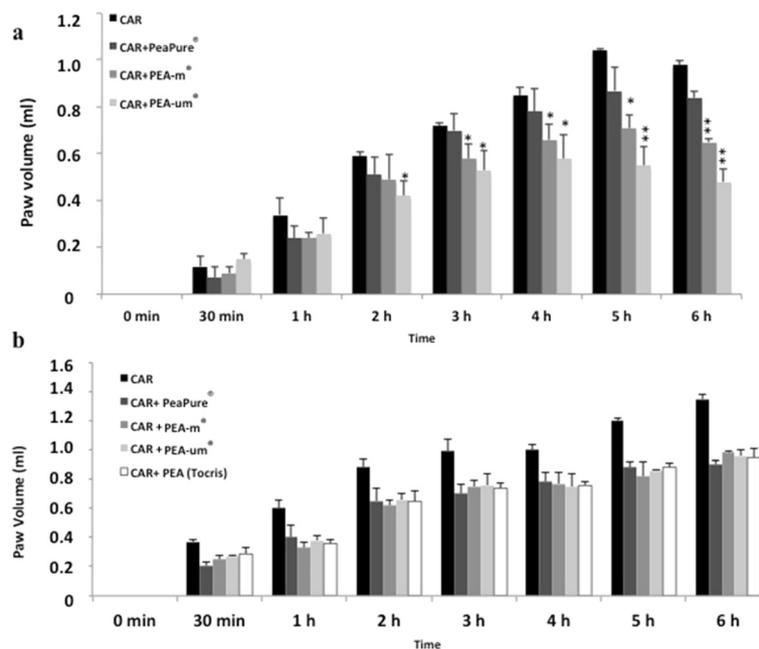
#### Effects of palmitoylethanolamide on the time-course of carrageenan-induced thermal hyperalgesia

Intraplantar injection of carrageenan led to a time-dependent development of thermal hyperalgesia that

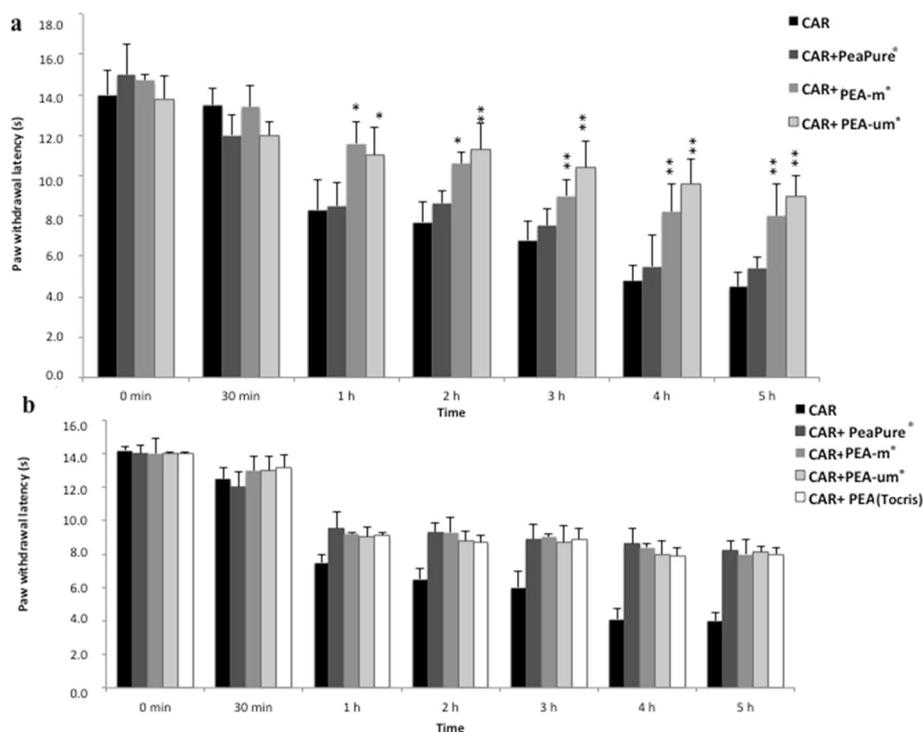
peaked at 3 hours and was sustained through 5 hours (Figures 5a and 5b). Oral administration of ultramicrosized PEA-um given 30 minutes before carrageenan produced a clear and significant inhibition of the development of carrageenan-induced thermal hyperalgesia (Figure 5a). Similarly, oral treatment with micronized PEA-m (10 mg/kg) was also efficacious in significantly attenuating the carrageenan-induced hyperalgesic response. In contrast, treatment with PeaPure (10 mg/kg) failed to show a significant reduction, although there was a trend (Figure 5). Moreover, no significant differences were observed between treatment groups when the PEA formulations were administered intraperitoneally (Figure 5b).

#### Discussion

Nonresolving inflammation is a major driver of disease. Perpetuation of inflammation is an inherent risk, because inflammation can damage tissue and necrosis can provoke inflammation. However, when prolonged, inflammation overrides the bounds of physiological control and eventually becomes destructive. Inflammation increasingly surfaces as a key element in the pathobiology of chronic pain, neurodegenerative diseases, stroke, spinal cord injury and possibly even neuropsychiatric disorders [24-28]. It is not surprising, then, that the inflammatory response is today a focus of intense research activity. Here we show that



**Figure 4** Effects of palmitoylethanolamide formulation on the time course of carrageenan-induced paw edema following intraplantar injection of carrageenan into the rat hind paw. All palmitoylethanolamide (PEA) formulations (10 mg/kg for each) were administered orally or intraperitoneally 30 minutes before carrageenan (CAR) injection. Paw edema was assessed at the time points indicated. **(a)** Micronized PEA-m and ultramicrosized PEA-um produced significant improvements in both scores in comparison to PeaPure. **(b)** Intraperitoneal administration of PEA did not show a significant difference between treatment groups for any of the PEA formulations tested. See Methods for further details. Values are means  $\pm$  SEM. \* $P < 0.05$  and \*\* $P < 0.01$  vs CAR.



**Figure 5 Effects of palmitoylethanolamide formulation on the time course of carrageenan-induced thermal hyperalgesia following intraplantar injection of carrageenan in the rat hind paw.** All palmitoylethanolamide (PEA) formulations (10 mg/kg for each) were administered orally or intraperitoneally 30 minutes before carrageenan (CAR) injection. Hyperalgesia was assessed at the time points indicated. **(a)** Micronized PEA-m and ultramicrozoned PEA-um produced significant improvements in both scores in comparison to PeaPure. **(b)** Intraperitoneal administration did not show a significant difference between treatment groups. See Methods for further details. Values are means  $\pm$  SEM. \* $P < 0.05$  and \*\* $P < 0.01$  vs CAR.

micronized/ultramicrozoned PEA is orally efficacious in limiting edema and thermal hyperalgesia in rats receiving intraplantar injection of carrageenan.

We now know that inflammatory processes may be counteracted by a program of resolution that includes the production of lipid mediators able to switch off inflammation [29]. One interesting class of such natural mediators are the *N*-acyl ethanolamines, which are composed of a fatty acid and ethanolamine. Fatty acid ethanolamine family members include the endocannabinoid *N*-arachidonylethanolamine (anandamide) and its congeners *N*-stearoylethanolamine, *N*-oleoylethanolamine and PEA [7]. PEA may function to maintain cellular homeostasis by acting as a mediator of resolution of inflammatory processes and by modulating the behaviors of mast cells and microglia [9], two of the principal cell types in neuroinflammatory processes [30,31]. For example, mast cell amines play a role in the edema induced by zymosan and carrageenan in rats [32].

Oral delivery of a drug continues to be the most popular route of administration because of its versatility, ease of administration and, probably most importantly, patient compliance. In this context, the physicochemical characteristics of PEA (its lipidic nature and large particle size in

the native state) can become a therapeutic issue in terms of solubility and bioavailability. Reducing large drug crystals down to the micron or submicron range for dissolution enhancement is a technique frequently used in the pharmaceutical field [10-13], keeping in mind that the dissolution rate of a drug is proportional to its surface area [14]. The absorption rate of poorly water-soluble drugs is especially sensitive to particle size because their bioavailability is dissolution rate-controlled in most cases [15]. Moreover, the rate of absorption of small drug particles is not influenced by the hydrodynamics in the gastrointestinal tract—an important factor in reducing variability of drug absorption when orally administered [15,33]. Using the air-jet milling technique, micronized and ultramicrozoned formulations of PEA were produced and shown to possess superior pharmacological action against carrageenan-induced inflammatory pain. This was in contrast to a preparation of nonmicronized PEA (PeaPure), which failed to show efficacy when given orally in this model.

In the course of our analyses, it became apparent that there exists a rather wide degree of variability among available PEA formulations in terms of both purity and actual content of active ingredients. Investigators should

thus exercise due caution when selecting a source of PEA for their experiments.

## Conclusions

In this study, we have demonstrated the beneficial effects of PEA in reducing edema formation and thermal hyperalgesia in carrageenan-induced inflammation in the rat paw. These results show the differential effects exerted on the degree of inflammation by micronized PEA-m and ultramicrosized PEA-um, vs nonmicronized PeaPure, the latter formulation being ineffective in this model when given orally.

## Abbreviations

CAR: Carrageenan; H&E: Hematoxylin and eosin; HPLC: High-performance liquid chromatography; MPO: Myeloperoxidase; PEA: Palmitoylethanolamide; PSD: Particle size distribution; SEM: Standard error of the mean.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

EE and CS participated in research design. ID, CM, BG, CR and SR conducted the experiments. BG, ID and CS performed data analysis. ID, EE and CS contributed to the writing of the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

The authors would like to thank Giovanni Leotta for his excellent technical assistance during this study and Valentina Malvagni for editorial assistance with the manuscript.

Received: 27 June 2014 Accepted: 25 July 2014

Published online: 28 August 2014

## References

1. Nathan C, Ding A: Nonresolving inflammation. *Cell* 2010, **140**:871–882.
2. Castellheim A, Brekke OL, Espevik T, Harboe M, Mollnes TE: Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. *Scand J Immunol* 2009, **69**:479–491.
3. Moalem G, Tracey DJ: Immune and inflammatory mechanisms in neuropathic pain. *Brain Res Rev* 2006, **51**:240–264.
4. Myers RR, Campana WM, Shubayev VI: The role of neuroinflammation in neuropathic pain: mechanisms and therapeutic targets. *Drug Discov Today* 2006, **11**:8–20.
5. Tabas I, Glass CK: Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* 2013, **166**:166–172.
6. Piomelli D, Sasso O: Peripheral gating of pain signals by endogenous lipid mediators. *Nat Neurosci* 2014, **17**:164–174.
7. Pacher P, Bátkai S, Kunos G: The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006, **58**:389–462.
8. Petrosino S, Iuvone T, Di Marzo V: N-palmitoylethanolamine: biochemistry and new therapeutic opportunities. *Biochimie* 2010, **92**:724–727.
9. Skaper SD, Facci L, Giusti P: Glia and mast cells as targets for palmitoylethanolamide, an anti-inflammatory and neuroprotective lipid mediator. *Mol Neurobiol* 2013, **48**:340–352.
10. Olusanmi D, Jayawickrama D, Bu D, McGeorge G, Sailes H, Kelleher J, Gamble JF, Shahd UV, Tobyn M: A control strategy for bioavailability enhancement by size reduction: effect of micronization conditions on the bulk, surface and blending characteristics of an active pharmaceutical ingredient. *Powder Technol* 2014, **258**:222–233.
11. Rasenack N, Müller BW: Micron-size drug particles: common and novel micronization techniques. *Pharm Dev Technol* 2004, **9**:1–13.
12. Joshi JT: A review on micronization techniques. *J Pharmaceutical Sci Technol* 2011, **3**:651–681.
13. Chaumeil JC: Micronization: a method of improving the bioavailability of poorly soluble drugs. *Methods Find Exp Clin Pharmacol* 1998, **20**:211–215.

14. Bisrat M, Nyström C: Physicochemical aspects of drug release. VIII. The relation between particle size and surface specific dissolution rate in agitated suspensions. *Int J Pharm* 1988, **47**:223–231.
15. Oh DM, Curl RL, Yong CS, Amidon GL: Effect of micronization on the extent of drug absorption from suspensions in humans. *Arch Pharm Res* 1995, **18**:427–433.
16. Winter CA, Riskey EA, Nuss GW: Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med* 1962, **111**:544–547.
17. Paterniti I, Impellizzeri D, Di Paola R, Navarra M, Cuzzocrea S, Esposito E: A new co-ultramicrosized composite including palmitoylethanolamide and luteolin to prevent neuroinflammation in spinal cord injury. *J Neuroinflammation* 2013, **10**:91.
18. Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G: Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. *Br J Pharmacol* 2002, **135**:181–187.
19. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG: Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br J Pharmacol* 1996, **118**:829–838.
20. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988, **32**:77–88.
21. Bang JS, da Oh H, Choi HM, Sur BJ, Lim SJ, Kim JY, Yang HI, Yoo MC, Hahm DH, Kim KS: Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1 $\beta$ -stimulated fibroblast-like synoviocytes and in rat arthritis models. *Arthritis Res Ther* 2009, **11**:R49.
22. Cuzzocrea S, Mazzon E, Esposito E, Muia C, Abdelrahman M, Di Paola R, Crisafulli C, Bramanti P, Thiemeermann C: Glycogen synthase kinase-3 $\beta$  inhibition attenuates the development of ischaemia/reperfusion injury of the gut. *Intensive Care Med* 2007, **33**:880–893.
23. Finley A, Chen Z, Esposito E, Cuzzocrea S, Sabbadini R, Salvemini D: Sphingosine 1-phosphate mediates hyperalgesia via a neutrophil-dependent mechanism. *PLoS One* 2013, **8**:e52555.
24. Giovannini MG, Scali C, Prosperi C, Bellucci A, Vannucchi MG, Rosi S, Pepeu G, Casamenti F:  $\beta$ -Amyloid-induced inflammation and cholinergic hypofunction in the rat brain *in vivo*: involvement of the p38MAPK pathway. *Neurobiol Dis* 2002, **11**:257–274.
25. Dauer W, Przedborski S: Parkinson's disease: mechanisms and models. *Neuron* 2003, **39**:889–909.
26. Tenorio G, Kulkarni A, Kerr BJ: Resident glial cell activation in response to perispinal inflammation leads to acute changes in nociceptive sensitivity: implications for the generation of neuropathic pain. *Pain* 2013, **154**:71–81.
27. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O: Neuroinflammation and psychiatric illness. *J Neuroinflammation* 2013, **10**:43.
28. Iadecola C, Anrather J: The immunology of stroke: from mechanisms to translation. *Nat Med* 2011, **17**:796–808.
29. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP: The resolution of inflammation. *Nat Rev Immunol* 2013, **13**:59–66.
30. Skaper SD, Giusti P, Facci L: Microglia and mast cells: two tracks on the road to neuroinflammation. *FASEB J* 2012, **26**:3103–3117.
31. Skaper SD, Facci L, Giusti P: Mast cells, glia and neuroinflammation: partners in crime? *Immunology* 2014, **141**:314–327.
32. Damas J, Remacle-Volon G: Mast cell amines and the oedema induced by zymosan and carrageenans in rats. *Eur J Pharmacol* 1986, **121**:367–376.
33. Scholz A, Abrahamsson B, Diebold SM, Kostewicz E, Polentarutti BI, Ungell AL, Dressmann JB: Influence of hydrodynamics and particle size on the absorption of felodipine in Labradors. *Pharm Res* 2002, **19**:42–47.

doi:10.1186/s12974-014-0136-0

Cite this article as: Impellizzeri et al.: Micronized/ultramicrosized palmitoylethanolamide displays superior oral efficacy compared to nonmicronized palmitoylethanolamide in a rat model of inflammatory pain. *Journal of Neuroinflammation* 2014 **11**:136.