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A novel protective formulation of Palmitoylethanolamide in experimental model of contrast agent induced nephropathy

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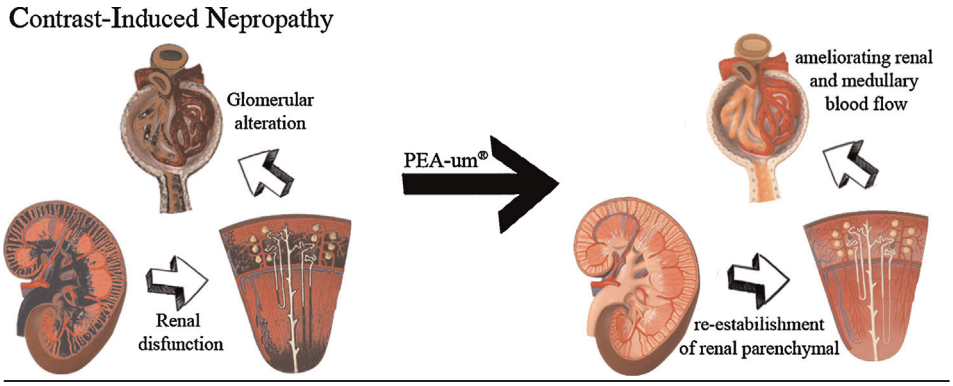
HIGHLIGHTS

- CIN is an acute reduction in renal functions secondary to iodinated contrast media.
- The prevalence of CIN is high in diabetic patients with renal impairment.
- PEA is a food component with analgesic effect and anti-inflammatory activity.
- PEA-um® prevent CIN and the alteration of different parameters after nephrectomy.

GRAPHICAL ABSTRACT



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ABSTRACT

Contrast-induced nephropathy (CIN) is a complication in patients after administration of iodinated contrast media. Several risk factors contribute to the development and progression of CIN, including hypertension, diabetes, and dyslipidemia. Animal models of CIN by surgical intervention to reproduce its clinical and pathology has been developed, and thus, therapeutic methods tested. Palmitoylethanolamide (PEA) is a member of the fatty acid ethanolamine family with analgesic and anti-inflammatory effects. In this study, we analyzed streptozotocin-induced diabetes model and in an another set of experiment a surgical remotion of the kidney with the aim of evaluating effect of ultramicronized Palmitoylethanolamide (PEA-um®) on contrast induced renal disfunction and glomerular morphology alteration. In a first step of our study, we demonstrated that PEA-um® significantly reduced CIN-mediated glomerular dysfunction, modulates Na^+ and K^+ levels in plasma and decreased urine and plasma NGAL levels and α-GST urine levels. Moreover, in a second set of experiment we investigated how PEA-um® reduced creatinine and BUN plasma levels after nephrectomy, ameliorate renal and medullary blood flow and re-established renal parenchymal after CIN induction as well as after nephrectomy. Take together our results demonstrated that PEA-um® are able to preventing CIN in diabetic rats and alteration of biochemical parameters after nephrectomy.

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1. Introduction

The use of contrast media is becoming one of the most important technique in clinical diagnostic and interventional procedures (Asif and Epstein, 2004; Wang et al., 2013). As a result, contrast-induced nephropathy (CIN) is a complex form of acute kidney injury (AKI) that is defined as an acute reduction in renal functions secondary to iodinated contrast media administration (Inal et al., 2014). It has been reported that the prevalence of CIN may occur in 1–6% of hospitalized patients. It is especially the case that incidences of this condition may rise as high as 50% in patients at high-risk for such disorders as renal impairment and diabetic (Gruberg et al., 2000). Specifically, in an attempt to assess the influence of diabetic and pre-diabetic state on the development of CIN in chronic kidney disease patients, demonstrate that patients with diabetes mellitus are at a higher risk of developing CIN, but patients with pre-diabetes mellitus are not at as high a risk for developing CIN as diabetes patients (Toprak et al., 2007). The pathophysiological cellular mechanism of the development of CIN is not fully elucidated. Development of AKI involves different complementary pathophysiological processes. Persson and Tepel (2006) have demonstrated that contrast agents induce renal vasoconstriction, accompanied by shunting of blood flow from the medulla to the cortex, a consequence of reducing renal blood flow to the medulla which is followed by renal medulla ischemia. Moreover, Tesch and Allen (2007) have described and compared the current protocols being used to establish models of diabetic nephropathy in rat and mouse, including nephrectomy. It has been demonstrated that hypoxia can promote further ischemic renal injury by the increase of oxygen free radicals through oxidative stress (Brezis and Rosen, 1995; Fisman et al., 2006). In fact, after the administration of contrast media, ROS enhance, leading to lipid peroxidation and cytotoxic damage. Free radicals react with nitric oxide to produce peroxynitrite, reducing the bioavailability of nitric oxide, thereby increasing tissue damage and inflammation (Ari et al., 2012). Despite the use of various prophylactic therapies and low-osmolar contrast agents with lesser adverse effects, intravenous isotonic fluid infusion is the only method that has been proven effective in preventing CIN in clinic practice (Barrett and Parfrey, 2006).

Considering the increasing necessity of utilization of iodinated contrast agents in patients with risk factors, the recognition that new contrast agents, despite their lower toxicity, do not prevent CIN, and the better knowledge about the pathophysiology of the renal damage following the intravenous administration of these compounds, several drugs have been tested aiming at attenuating or avoid radiocontrast-induced nephropathy. Among these drugs, the following could be mentioned: dopamine, mannitol, endothelin, calcium channel blockers, free-radicals sweepers, and phenoldopam (Morcos, 1998; Baker and Baker, 2001). Therefore, a novel kidney-protecting treatment model is required for nephropathy contrast-mediated.

Palmitoylethanolamide (PEA) is a fully saturated, and endogenous *N*-acylethanolamine, first discovered in the late 1950s and produced "on demand" by mammalian cells (Mattace Raso et al., 2013). It is a food component that has been shown to inhibit peripheral inflammation and mast-cell degranulation, as well as to exert neuroprotective and analgesic effect and anti-allergic and anti-inflammatory activity (Esposito and Cuzzocrea, 2013a; Esposito et al., 2014; Paterniti et al., 2014). PEA is additionally able to inhibit NADPH oxidase and the associated generation of ROS (Chung et al., 2012). PEA may occur through three possible mechanisms: the first mechanism suggests that PEA acts by down-regulating mast-cell degranulation via an "Autacoid Local Inflammation Antagonism" (ALIA) effect (Aloe et al., 1993); the second mechanism is founded on the ability of PEA to directly stimulate the nuclear peroxisome proliferator-activated receptor-α (PPAR-α), (Esposito and Cuzzocrea, 2013a); the last mechanism, PEA could modulate the nuclear factor NF-κB pathway (Di Paola et al., 2012).

These actions are mediated by PPAR-α activation and are accompanied by a decrease in nitric oxide production, neutrophil influx, and expression of pro-inflammatory proteins such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Esposito and Cuzzocrea, 2013b).

In our recent review we discussed how PEA treatment could represent a new therapeutic approach for the treatment of chronic kidney disease (CKD) and a recent work additionally demonstrated that long-term treatment with PEA on conditions associated with kidney disease, such as hypertension, through a reduction of glomerulosclerosis, and tubulointerstitial fibrosis, modulating key enzymes in ROS/RNS synthesis and/or scavenging and angiotensin II (AT) receptors homeostasis could have a potential beneficial effects (Mattace Raso et al., 2013; Impellizzeri et al., 2014b).

The purpose of this study was to investigate the potential therapeutic role of ultramicronized PEA-um®, on nephropathy induced by Iomeprol 400, a nonionic monomeric contrast medium with a iodine concentrations of 400 mg/ml (in the text to follow will be called only Iomeprol) in diabetic rats and on nephropathy induced by Omnipaque™ a low-osmolar contrast medium with a iodine concentrations of 180 mg/ml after uninephrectomy.

2. Materials and methods

2.1. Animals

Male Wistar rats (150–200 g; Harlan, Nossan, Italy) were housed in a controlled environment, maintained on a 12-h light/dark cycle (lights on at 07:00 AM), and 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 protection of animals used for experimental and other scientific purposes (D.M.116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

2.2. Ultramicronization of PEA

PEA-*um*® (Epitech, Padova, Italy) 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, which operates with "spiral technology" driven by compressed air. The high number of collisions that occurs among particles, as a result of the high level of kinetic – not mechanical – energy, produces micron- and sub-micron-sized crystals (Impellizzeri et al., 2014a).

2.3. Particle size distribution

Particle size distribution (PSD) was measured by dynamic light scattering using a Mastersizer 3000, equipped with wet dispersion unit (Malvern Instruments Ltd, Malvern, UK). Particles were dispersed in water with the aid of an in-line sonication probe for rapid agglomerate dispersion (Impellizzeri et al., 2014a).

2.4. Induction of diabetes

After 12 h of fasting, the animals received a single 60 mg/kg intravenous injection of streptozotocin (STZ, Sigma–Aldrich, St. Louis, MO) dissolved in 10 mM sodium citrate buffer, pH 4.5. After 24 h, animals with blood glucose levels greater than 250 mg/dl were considered diabetic. Diabetic state was evaluated daily