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Intestinal anti-inflammatory activity of calcium pyruvate in the TNBS model of rat colitis: Comparison with ethyl pyruvate

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ABSTRACT

Pyruvate is a key intermediate of the carbohydrate metabolism with endogenous scavenger properties. However, it cannot be used in clinics due to its instability. Ethyl pyruvate (EP) has shown better stability as well as an antioxidant and anti-inflammatory activity. Calcium pyruvate monohydrate (CPM) is another stable pyruvate derivative that could also provide the benefits from calcium, fundamental for bone health. Considering everything, we propose CPM as a therapeutic strategy to treat diseases with an immune component in which there is also a significant dysregulation of the skeletal homeostasis. This could be applicable to inflammatory bowel disease, which is characterized by over-production of pro-inflammatory mediators, including cytokines and reactive oxygen and nitrogen metabolites that induces intestinal mucosal damage and chronic inflammation, and extra-intestinal symptoms like osteopenia and osteoporosis.

The effects of CPM and EP (20, 40 and 100 mg/kg) were evaluated on the trinitrobenzenesulfonic acid (TNBS) model of colitis in rats, after a 7-day oral treatment, with main focus on colonic histology and inflammatory mediators.

Both pyruvates showed intestinal anti-inflammatory effects in the TNBS-induced colitis. They were evident both histologically, with a recovery of the mucosal cytoarchitecture and a reduction of the neutrophil infiltration, and through the profile of inflammatory mediators (IL-1, IL-6, IL-17, IL-23, iNOS). However, CPM appeared to be more effective than ethyl pyruvate. In conclusion, CPM exerts intestinal anti-inflammatory effect on the TNBS-induced colitis in rats, although further experiments are needed to explore its beneficial effects on bone health and osteoporosis.

1. Introduction

Pyruvate, the anionic form of pyruvic acid (2-oxo-propanoic acid), is a pivotal biochemical intermediate of the carbohydrate metabolism. Almost all carbohydrates are metabolized through pyruvic acid, so a few hundreds of grams are produced by the human body every day. In addition to being an important energy-bearing metabolite, it most probably works as an endogenous scavenger of reactive oxygen species (ROS). Furthermore, many evidences support its pharmacological effect improving the cardiac function after coronary ischemia and reperfusion and critical medical conditions, like severe sepsis, acute respiratory distress syndrome, burn injury, acute pancreatitis and stroke [15]. However and despite its properties, pyruvate cannot be used in clinical practice due to its instability in solution [15].

The ethyl ester of pyruvic acid, ethyl pyruvate (EP), has shown much better stability in aqueous solutions, and to be a pharmacologically active molecule in different models of redox- and inflammation-mediated cellular or tissue injury [15], including ischemia/reperfusion [39], pancreatitis [9], sepsis [40] and intestinal

Abbreviations: CPM, calcium pyruvate monohydrate; EP, ethyl pyruvate; GSH, glutathione; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; IFNγ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LTB_4, leukotriene B_4; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase; ROS, reactive oxygen species; SAZ, sulphasalazine; TNBS, trinitrobenzenesulfonic acid.

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inflammation [10]. The mechanisms involved include the scavenging of ROS and the inhibition of NF-κB activation by scavenging ROS and also other pathways not well described [17]. It has been reported, both in vitro and in vivo, that pyruvates are not only able to inhibit the pro-inflammatory interleukin (IL)-6 and tumour necrosis factor (TNF)-α, but to increase the production of the anti-inflammatory cytokine IL-10 [42]. Furthermore, EP may also serve as a metabolic substrate that could reduce ATP depletion and mitochondrial damage.

Other pyruvate derivatives have been developed like calcium pyruvate monohydrate (CPM) that has been synthesized avoiding destabilizing reaction conditions [32]. This molecule has been used as anti-obesity or slimming aid [37] due to the fact that pyruvate is one of the smallest carbohydrate molecules existing, which does not lead to an insulin release once ingested. But it also provides the benefits from calcium, which is well known for its pivotal impact in bone health as well as in osteoporosis prevention, and beyond this, it is discussed to play a role in obesity control [12] and in lowering the risk of hypertension and colon cancer [44].

Taken together the effects described for pyruvate as a scavenger of ROS and anti-inflammatory, and the beneficial effects exerted by calcium in osteoporosis prevention, it could be interesting to think on calcium pyruvate as a therapeutic strategy to treat diseases with an immune component in which there is also a significant dysregulation of the skeletal homeostasis. This is the case of inflammatory bowel disease (IBD), in which there is an abnormal synthesis of proinflammatory mediators including cytokines and reactive oxygen and nitrogen metabolites that leads to intestinal mucosal damage and chronic tissue inflammation [38]; together with extra-intestinal manifestations [41] like osteopenia and osteoporosis in many cases [24]. Furthermore, IBD treatment is suboptimal nowadays. The therapies usually involve the use of aminosalicylates, glucocorticosteroids or immunosuppressants, including biologicals like TNF-α monoclonal antibodies [11]; however, these drugs may display limited beneficial actions and/or serious complications and side-effects, thus limiting their chronic administration in these patients [13].

Thus, the aim of the present study is to evaluate the potential use of the stable and pure CPM in the treatment of IBD, and compare it with ethyl pyruvate, which has previously shown anti-inflammatory effects in acute and chronic murine colitis [10]. CPM has been also tried as a form of calcium supplementation for the treatment of osteoporosis in postmenopausal women showing a good bioavailability and tolerability [35]. We have evaluated the intestinal anti-inflammatory properties of both compounds in the trinitrobenzenesulfonic acid (TNBS) model of colitis in rats, a well established model of intestinal inflammation that mimics many histopathological and immune characteristics of human IBD.

2. Methods

2.1. Chemicals and reagents

Calcium pyruvate monohydrate (CPM) was provided by Phyto-Lab GmbH & Co. KG (Vestenbergsgreuth, Germany). All other chemicals, including ethyl pyruvate, were obtained from Sigma–Aldrich Quimica (Madrid, Spain), unless otherwise stated.

2.2. TNBS model of rat colitis

This study was carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the National Institute of Health and the protocols approved by the Ethic Committee of Laboratory Animals of the University of Granada (Spain) (Ref. No. CEEA-2010-286). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [20,27]. Female Wistar rats (180–200 g) obtained from Janvier (St Berthevin Cedex, France) were housed in makrolon cages, maintained in an air-conditioned atmosphere with a 12 h light–dark cycle, and provided with food and tap water *ad libitum*. They were randomly assigned to nine groups ($n = 10$). Three groups received treatment with CPM (20, 40 and 100 mg kg^{-1}); other three received treatment with ethyl pyruvate (20, 40 and 100 mg kg^{-1}); and the remaining was treated with sulphasalazine (30 mg kg^{-1}). All compounds were dissolved in 1 ml of carboxymethylcellulose (0.2%) in water solution, and administered daily by oral gavage. An untreated TNBS control group and a non-colitic group were included for reference, which received the vehicle used to administer the test compounds. Colonic inflammation was induced in control and treated groups as previously described [8]. Briefly, rats were fasted overnight, anesthetized with halothane and given 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v v^{-1}) by means of a Teflon cannula inserted 8 cm through the anus. During and after TNBS administration, the rats were kept in a head-down position until they recovered from anaesthesia, and then returned to their cages. Rats from the non-colitic group were administered intracolonically 0.25 ml of phosphate buffered saline instead of TNBS. The treatments were given from the day of the colitis induction until the sacrifice of the rats with an overdose of halothane, seven days later. Animal body weights, occurrence of diarrhoea, and water and food intake were recorded daily throughout all the experiments. Once the animals were sacrificed, the colon was removed aseptically and placed on an ice-cold plate, longitudinally opened and cleaned from their luminal contents with cold saline. Afterwards, it was weighed and its length measured under a constant load (2 g). Each colon was scored for macroscopically visible damage on a 0–10 scale by two observers unaware of the experiment, according to the criteria described before [5].

Colon samples (0.5 cm^2) containing all the layers were taken from a region of the inflamed colon corresponding to the adjacent segment to the gross macroscopic damage and were fixed in 4% buffered formaldehyde for the histological studies. Equivalent colonic segments were also obtained from the non-colitic group. The colon was subsequently minced, aliquoted and kept frozen at −80 °C until biochemical determinations and RNA extraction was performed.

2.3. Histological studies

Cross-sections were selected and embedded in paraffin. Full-thickness sections of 5 μm were obtained at different levels and stained with haematoxylin and eosin. The histological damage was evaluated by a pathologist observer, who was blinded to the experimental groups, according to the criteria previously described [4].

Immunohistochemistry evaluation of the myeloid marker CD11b was performed in colonic tissue sections from the different experimental groups. Briefly, deparaffinised and rehydrated tissue sections were treated in a steamer for 20 min in citrate buffer for antigen retrieval. After blockade of endogenous peroxidase and unspecific protein binding, anti-CD11b antibody (NB110-89474SS; Novus Biologicals, Littleton, CO, USA) was used at 1:500 dilution for one hour. Presence of specific binding was detected by brown precipitate using the DAB detection method following manufacturer instructions (ab80437 EXPOSE Rabbit specific HRP-DAB Detection IHC Kit v2; Abcam, Cambridge, MA, USA), and haematoxylin was used as counterstain.

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