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Ibuprofen hepatic encephalopathy, hepatomegaly, gastric lesion and gastric pentadecapeptide BPC 157 in rats

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ABSTRACT

Chronic ibuprofen (0.4 g/kg intraperitoneally, once daily for 4 weeks) evidenced a series of pathologies, not previously reported in ibuprofen-dosed rats, namely hepatic encephalopathy, gastric lesions, hepatomegaly, increased AST and ALT serum values with prolonged sedation/unconsciousness, and weight loss. In particular, ibuprofen toxicity was brain edema, particularly in the cerebellum, with the white matter being more affected than in gray matter. In addition, damaged and red neurons, in the absence of anti-inflammatory reaction was observed, particularly in the cerebral cortex and cerebellar nuclei, but was also present although to a lesser extent in the hippocampus, dentate nucleus and Purkinje cells. An anti-ulcer peptide shown to have no toxicity, the stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419, 10 μg, 10 ng/kg) inhibited the pathology seen with ibuprofen (i) when given intraperitoneally, immediately after ibuprofen daily or (ii) when given in drinking water (0.16 μg, 0.16 ng/ml). Counteracted were all adverse effects, such as hepatic encephalopathy, the gastric lesions, hepatomegaly, increased liver serum values. In addition, BPC 157 treated rats showed no behavioral disturbances and maintained normal weight gain. Thus, apart from efficacy in inflammatory bowel disease and various wound treatments, BPC 157 was also effective when given after ibuprofen.

1. Introduction

An understanding of the hepatic encephalopathy that occurs following overdosing with NSAIDs (i.e., paracetamol overdose is the leading cause of acute liver failure (Fontana, 2008) with a sudden onset of encephalopathy) still needs a more adequate therapeutic approach, and experimental studies can help in elucidating the mechanisms behind its development. Hepatotoxicity is a common adverse finding following overdosing with non-steroidal anti-inflammatory drugs (NSAIDs)(Manov et al., 2006) although it can present with widely varying severity and histopathological type of liver lesions, which are depending on the mode of action: direct hepatotoxicity inducing necrosis — paracetamol; biliary toxicity inducing cholestasis – alpha-naphthylisothiocyanate (ANIT), ibuprofen; steatosis – tetracyclins, hypertrophia, hyperplasia, hepatitis, etc. (Schoonen et al., 2007). Some of these mechanisms don't easily follow dose response relationships (Manov et al., 2006).

In order to improve the understanding of the mode of action of NSAIDs, we focused on ibuprofen, as a representative NSAID showing moderate hepatotoxicity in rats, compared to other hepatotoxic agents. It was shown that ibuprofen induced histopathological lesions in rats liver with a dose of 100 mg/kg comparable in severity to those induced by paracetamol at a dose of 1800 mg/kg (however, paracetamol induced necrotic lesions and greater increase in enzymes), and more severe lesions than chlorpromazine at 125 mg/kg, with comparable clinical chemistry (Schoonen et al., 2007). In this respect we have used ibuprofen-induced hepatic encephalopathy in rats as a representative toxin of the class of NSAIDs. Ibuprofen is one of the NSAIDs that are commonly known to produce hepatotoxicity through inducing cholestatic hepatitis (Manov et al., 2006), but it is not the most potent hepatotoxin of the class of NSAIDS (for a review see Manov et al., 2006). There is some evidence that ibuprofen could be hepatoprotective (Hamburger and McCay, 1990; Denda et al., 1997). In the studies of hepatotoxicity of ibuprofen, the treatment in mice (100–400 mg/kg for two weeks) resulted in hepatomegaly which was characterized microscopically by hepatocellular hypertrophy and hyperplasia (Bendele et al., 1993). In addition, ibuprofen was also found to be the most active in impairing gluconeogenesis from lactate, and in impairing albumin synthesis in vitro (Castell et al., 1988).

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Therefore, in order to better demonstrate ibuprofen hepatic encephalopathy, we investigated the effects of a chronic exposure to ibuprofen following a protocol known to result in hepatomegaly in mice (Bendele et al., 1993). We additionally studied the effect of co-administration of the stable gastric pentadecapeptide, BPC 157, an orally active anti-peptic ulcer drug stable in human gastric juice (Sikiric et al., 1994, 1996, 1997a,b, 1999a,b, 2006; Prkacin et al., 2001b; Dobric et al., 2007; Xue et al., 2004a, 2004b; Veljaca et al., 1994; Wood, 2004) that has also been suggested as having a moderating effect on many central nervous system disturbances (Sikiric et al., 1999a; Jelovac et al., 1999; Jelovac et al., 1998; Boban et al., 2005, 2006; Blagaic et al., 2004; Tohyama et al., 2004). In experimental and clinical studies BPC 157 have been shown to be effective in the treatment of inflammatory bowel disease (Ruenzi et al., 2005; Veljaca et al., 2003) and in a diverse range of wound treatments (Novinscak et al., 2008; Sebecic et al., 1999; Seiwerth et al., 1997; Lazic et al., 2005; Krivic et al., 2006; Seveljevic-Jaran et al., 2006; Sikiric et al., 2003; Staresinic et al., 2003, 2006; Tkalcevic et al., 2007; Bilic et al., 2005; Skorjanec et al., 2009), with no reported toxicity (Ruenzi et al., 2005; Veljaca et al., 2003) and which in addition showed a degree of hepatoprotection (Prkacin et al., 2001a, 2002; Sikiric et al., 1993b).

Of note, although hepatotoxicity in general can permit toxins to reach the brain and thereby affect neural function, the particular effect of NSAIDs on hepatic encephalopathy was been less well recognized than for other hepatotoxins (Bilir, and Bilir, 1997). For example, indomethacin has been shown to improve locomotor deficit and to reduce the concentrations of neuroinhibitory steroids in the brain of rats following portacaval anastomosis (Ahboucha et al., 2008) with little detrimental effect (Chan et al., 2006). It has also been shown not to enhance, nor protect, the histopathologic severity of hepatic damage and encephalopathy, defined as defects in motor activity, seen in rats with bile duct ligation (Chu et al., 2005). In a study of rats with chronic liver failure, ibuprofen completely restored their ability to learn (Cauli et al., 2007).

On the other hand, the possibility that chronic ibuprofen treatment, in addition to causing hepatomegaly (Bendele et al., 1993) might be inducing hepatic encephalopathy was generally supported by the evidence that high doses of ibuprofen, possibly through a pharmacological action, could induce drowsiness, dizziness, headache, tinnitus, nystagmus, severe seizures and coma (Vale and Meredith, 1986). Although neuroprotective effects of ibuprofen were demonstrated in therapeutic dosage regimen (40 mg/kg), one cannot exclude possibility that encephalopathy is the result of direct action of high-dose (400 mg/kg) toxicity of ibuprofen on central nervous system.

Thereby, to counteract the possible ibuprofen-induced hepatic toxicity and encephalopathy (Bendele et al., 1993), together with the gastric ulcers, induced by the chronic high dose regimen, the anti-ulcer stable gastric pentadecapeptide, BPC 157, was co-administered to maintain gastrointestinal mucosal integrity (Veljaca et al., 1995; Sikiric et al., 1993a, 2004) and to alleviate NSAIDs-induced gastrointestinal lesions (Sikiric et al., 1996, 1997a, 1999b; Xue et al., 2004a, 2004b). Evidence exists that BPC157 also protected against the liver lesions induced by CCl_4, restraint stress, bile duct and hepatic artery ligation, and chronic alcohol application (Sikiric et al., 1993b; Prkacin et al., 2001a, 2001b). The mode of action of BPC 157 in alleviating the damage induced by chronic ethanol administration may be NO-related (Lovric-Bencic et al., 2004). At therapeutic doses BPC 157 caused no behavioral changes when given alone and was without toxicity. It has also been shown to have anti-convulsive activity (Sikiric et al., 1999a; Jelovac et al., 1998, 1999; Boban et al., 2005, 2006; Blagaic et al., 2004; Tohyama et al., 2004).

Thus, the ibuprofen regimen was administered at doses reported previously to induce encephalopathy (Bendele et al., 1993). In order to study the potential therapeutic value of the pentadecapeptide, BPC 157, it was given using previously shown, effective protocols (Sikiric et al., 1993a, 2004) via the intraperitoneal or per-oral route in drinking water.

2. Materials and methods

2.1. Animals

Male Albino Wistar (200 g) rats, were used in all of the experiments, approved by the Local Ethic Committee, assessed by observers unaware of the given treatment groups. Animals were kept in individual cages and given food and water ad libitum.

2.2. Drugs

Medication, without carrier or peptidase inhibitor, included pentadecapeptide BPC 157 a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline; peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity, manufactured by Diagen, Ljubljana, Slovenia, GEPPPGKPADDAGLV, M.W. 1419) (Sikiric et al., 1993a, 2004) and ibuprofen (Sigma, USA) prepared as described before (Sikiric et al., 1994).

Ibuprofen (0.4 g/kg) was given intraperitoneally once daily for 4 weeks. In order to study the effects of BPC157 as a potential antidote for ibuprofen toxicity, it was applied as a stable gastric pentadecapeptide BPC 157 formulation (10 μg/kg, 10 ng/kg) given (i) intraperitoneally daily immediately after the ibuprofen dose, or (ii) in drinking water (0.16 μg/ml, 0.16 ng/ml) from the beginning of the experiment until the end of the 4 week time period while controls received an equivolume of saline (5 ml/kg intraperitoneally) or drinking water only.

2.3. Gastric assay

2.3.1. Gastric lesions

The severity of gastric injury was assessed grossly immediately after sacrifice. The sum of the longest lesion diameters were assessed as described earlier (Sikiric et al., 1994), and gastric tissue was fixed in 10% neutral buffered formalin and processed for routine light microscopy analysis as described previously (Sikiric et al., 1994).

2.4. Liver assay

2.4.1. Enzyme activity

To determine serum values (IU/l) of aspartate aminotransaminase (AST), and alanine aminotransaminase (ALT), blood samples were centrifuged for 15 min at 3000 rpm, immediately after death. All tests were performed on an Olympus AU2700 analyzer with original test reagents (Olympus Diagnostica, Lismeehan, Ireland) (Novinscak et al., 2008).

2.4.2. Weight assessment

Animals were weighed before ibuprofen protocol initiation and before sacrifice and normal non-treated rats were used as an additional control. Following sacrifice the liver was removed intact and weighed. The absolute and relative liver weight was assessed.

2.4.3. Liver lesions

Liver tissue was immediately placed in 10% neutral buffered formalin for 24 h, dehydrated in graded ethanols, cleared in xylene/toluene and embedded in paraffin. Hematoxylin-eosin stained sections were analyzed on three high power fields (400×). The number of nuclei, as well as their diameter was measured using the ISSA program (Vamstec, Zagreb, Croatia) and the number of binucleated cells was also counted.

2.5. Brain assay

2.5.1. Brain lesions

The brain (whole brain slices of 5 μm) was fixed in 10% neutral buffered formalin for 2 days. After fixation, the brain was grossly