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Morphine glucuronidation increases its analgesic effect in guinea pigs

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ABSTRACT

Aims: Morphine is extensively metabolized to neurotoxic morphine-3-glucuronide (M3G) and opioid agonist morphine-6-glucuronide (M6G). Due to these different roles, interindividual variability and co-administration of drugs that interfere with metabolism may affect analgesia. The aim of the study was to investigate the repercussions of administration of an inducer (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) and an inhibitor (ranitidine) of glucuronidation in morphine metabolism and consequent analgesia, using the Guinea pig as a suitable model.
Main methods: Thirty male Dunkin–Hartley guinea pigs were divided in six groups: control, morphine, ranitidine, ranitidine + morphine, TCDD and TCDD + morphine. After previous exposure to TCDD and ranitidine, morphine effect was assessed by an increasing temperature hotplate (35–52.5 °C), during 60 min after morphine administration. Then, blood was collected and plasma morphine and metabolites were quantified.
Key findings: Animals treated with TCDD presented faster analgesic effect and 75% reached the cut-off temperature of 52.5 °C, comparing with only 25% in morphine group. Animals treated with ranitidine presented a significantly lower analgesic effect, compared with morphine group ($p < 0.05$). Moreover, significant differences between groups were found in M3G levels and M3G/morphine ratio ($p < 0.001$ and $p < 0.0001$), with TCDD animals presenting the highest values for M3G, M6G, M3G/morphine and M6G/morphine, and the lowest value for morphine. The opposite was observed in the animals treated with ranitidine.
Significance: Our results indicate that modulation of morphine metabolism may result in variations in metabolite concentrations, leading to different analgesic responses to morphine, in an animal model that may be used to improve morphine effect in clinical practice.

Introduction

Morphine is one of the first-line drugs for pharmacological treatment of severe postsurgical and moderate-to-severe acute and chronic cancer-related pain (WHO, 1996). However, the set of adverse effects associated with morphine and the high interindividual variability of morphine dosage, efficacy and tolerability (Ross et al., 2005; Shi et al., 2010; Aubrun et al., 2003; Oliveira et al., 2014) are important limitations to its therapeutic effectiveness. Pain perception and response to analgesic medications are complex processes that involve multiple pathways, such as neurotransmission, inflammation, drug metabolism and drug transport, among others (Carpenter and Dickenson, 2002). Therefore, several hypotheses have been raised to explain morphine's analgesic variability, including genetic variation of opioid receptors, transporters and metabolizing enzymes (Belfer et al., 2004; Lötsch and Geisslinger, 2006; Kadiev et al., 2008; Kasai et al., 2008; Jannetto and Bratanow, 2010; Kleine-Brueggeney et al., 2010; Muralidharan and Smith, 2011).

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Variability in morphine metabolism can particularly account for different analgesic effects (Oliveira et al., 2014; Gretton et al., 2013). Morphine undergoes extensive human hepatic metabolism, especially by UDP-glucuronosyltransferase 2B7 (UGT2B7), producing two main metabolites, morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) (Carrupt et al., 1991). M6G is a potent opioid receptor agonist with higher analgesic activity than morphine (Carrupt et al., 1991; Osborne et al., 1992). On the other hand, M3G has no opioid action and it seems to cause adverse effects, namely hyperalgesia/allodynia and neurotoxicity, and to exert a functional antagonistic effect, decreasing morphine analgesia (Carrupt et al., 1991; Christrup, 1997; Holthe et al., 2002). Since M6G has been ascribed as an important mediator of the analgesic effect of morphine (Klepstad et al., 2000; Penson et al., 2005), it has been postulated that the 6-glucuronidation probably increases the analgesic effect, despite the concomitant M3G formation. However, the correlation of morphine metabolism and M6G concentration with analgesic effect is still a matter of controversy (Gretton et al., 2013; Osborne et al., 1992; Klepstad et al., 2000; Penson et al., 2005; Quigley et al., 2003; Ing Lorenzini et al., 2012; Portenoy et al., 1992; van Dongen et al., 1994), due to the variety of drugs and substrates of UGT that can interfere in M3G and M6G formation during therapy (Wittwer and Kern, 2006), and therefore the real effect on analgesic efficacy of morphine metabolism inhibition and induction is still unknown.

Although several species can metabolize morphine, remarkable interspecies differences have been found in the urinary excretion and site-selective glucuronidation of morphine (Kuo et al., 1991). On the other hand, the guinea pig presents a M3G:M6G ratio of 4:1 (Kuo et al., 1991; Aasmundstad et al., 1993), very similar to the ratio described for humans (De Gregori et al., 2012; Yue et al., 1990; Andersen et al., 2002), and therefore represents a suitable animal model to clarify the influence of morphine glucuronidation in the resulting analgesic effects. A number of compounds are known to interfere significantly with metabolic enzymes, thereby influencing drug metabolism. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent halogenated aromatic hydrocarbon that exerts its biological and toxic responses through binding to the aryl hydrocarbon receptor (AhR) (Santostefano et al., 1998). In addition to many other effects, TCDD can induce several isoforms of cytochrome P450, UGT and glutathione-*S*-transferase in humans and rodents, including guinea pigs (Erichsen et al., 2008; Münzel et al., 1999, 2003; Collier et al., 2006; Fletcher et al., 2001). Therefore it can be used to induce morphine metabolism. Besides morphine metabolism induction, its inhibition could also be of therapeutic interest. In this particular case, *in vitro* experiments with guinea pig cells have shown that ranitidine may differentially inhibit morphine glucuronidation, causing higher inhibition of the production of M3G than that of M6G (Aasmundstad and Morland, 1998). Interactions of ranitidine with morphine effect and metabolism have also been described in mice (Suh et al., 1996) and humans (McQuay et al., 1990; Aasmundstad and Storset, 1998), yielding a reduced serum M3G/M6G ratio.

The lack of a good analgesic response in some patients, the variability of the relative amount of glucuronides formed and uncertainty of their contributions on the total analgesic effect prompted us to formulate a controlled study of both induction, using TCDD, and inhibition, using ranitidine, of morphine metabolism, and pain assessment in an adequate animal model, the Guinea pig.

Methods

Ethics commitment

All experimental procedures followed the regulations of local authorities in handling laboratory animals, as well as the European Directive 2010/63/EU and the ethical guidelines for the study of pain in experimental animals (Zimmermann, 1983). The study was also approved by the Ethical Internal Commission of Faculty of Medicine of University of Porto/São João Hospital.

Reagents and standards

Commercial formulations of morphine (morphine sulfate, MST® 10 mg) and ranitidine (ranitidine hydrochloride, Zantac® 25 mg/mL) were obtained in a local pharmacy. TCDD was obtained from Sigma-Aldrich (St. Louis, MO). Morphine was dissolved in saline solution and TCDD in corn oil (Merck Darmstad, Germany) for the intraperitoneal (IP) administrations. For the quantification of morphine and metabolites, standards of morphine hydrochloride, M3G hydrochloride and M6G hydrochloride were obtained from Lipomed (Arlesheim, Switzerland). Phenacetin (internal standard, IS), triethylamine, sodium dodecyl sulfate and hydrochloric acid were obtained from Sigma-Aldrich (St. Louis, MO). Methanol, acetonitrile, sodium dihydrogen phosphate and phosphoric acid were obtained from Merck (Darmstad, Germany). OASIS® weak cation exchange (WCX) cartridges, 60 mg, 3 mL were obtained from WATERS (Milford, MA) and Bond Elut® C18 cartridges, 100 mg, 1 mL were obtained from Agilent. All chemicals and reagents were of analytical grade or from the highest available grade.

Animals and experimental design

Animals

Thirty male Dunkin–Hartley guinea pigs (Harlan Laboratories, Spain) weighing 250–300 g were used. Animals were kept under constant photoperiod conditions (12-hour alternating light-dark cycles) at 22 °C and 40–50% relative humidity with food and water *ad libitum*. In order to minimize fear-motivated behaviors, all animals were handled daily and habituated to all testing procedures before the onset of the experiments. In all behavioral tests, the evaluator was unaware of the animal's experimental group.

Experimental protocol

Thirty animals were randomly distributed in six experimental groups ($n = 5$): (i) control group (C); (ii) morphine group (M); (iii) ranitidine group (R); (iv) ranitidine + morphine group (RM); (v) TCDD group (T); and (vi) TCDD + morphine group (TM) (Table 1). After the period of habituation, the experimental protocol was held for 3 days (Fig. 1 and Table 1). The enzymatic inducer was administered twice, 48 and 24 h before the behavioral assessment, whereas the inhibitor was administered three times (48, 24 and 2 h before the hot plate test). Behavioral assessment was performed immediately before and 15, 30, 45 and 60 min after saline or morphine administration. Morphine (10 mg/kg), TCDD (1 μg/kg) and ranitidine (200 mg/kg) doses were defined according to the literature (Enan et al., 1996; Orishiki et al., 1994; Olster, 1994; Flecknell, 1984; Collier et al., 1961) and all solutions were administered IP between 9 and 11 A.M.

Assessment of hot plate thermal analgesia

The hot-plate test was performed in a computer-controlled hot/cold plate analgesia meter (Bioseb, Vitrolles, France). The animals were placed on a metal surface (16.5 cm × 16.5 cm), surrounded by a Plexiglas box (36.5 cm height). The initial surface temperature was 35 °C and a cut-off temperature of 52.5 °C was defined, to prevent tissue damage. After a short adaptation period (20–30 s), an increasing thermal gradient of 9 °C/min was applied. This heating rate was chosen in order to avoid unnecessary stress in the animals (maximal assay duration *ca.* 2 min, as previously described) (Tjolsen et al., 1991). The temperature (in °C) to elicit genitalia licking was recorded (Leite-Panissi et al., 2001).

Sample collection

Immediately after the end of the behavior assessment, anesthesia was induced with isoflurane. Animals were placed in the *decubito supino* position and the thorax was opened by two lateral transversal incisions and one central longitudinal incision. Blood was collected from the heart, with heparinized needles, into EDTA containing tubes and then