Biochemical Pharmacology, Vol. 38, No. 1, pp. 207–209, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

Effect of glutathione depletion on morphine toxicity in mice

(*Received* 6 *January* 1988; *accepted* 15 *June* 1988)

Heroin addition has been associated with cardiac [1], renal [2], and hepatic [3, 4] pathologies in humans. However, interpretation of many clinical studies examining the toxicology of opiates is difficult due to the polydrug use, nutritional status, and disease state of the individual.

Several investigators have demonstrated hepatic toxicity from the administration of the opiate, morphine, to mice. Hepatic enlargement, lipid infiltration and changes in serum transaminases have been reported [5–7]. It appears, however, that the observed toxicity is due to a biologically transformed product of morphine rather than to morphine itself. In addition to the major metabolite, morphine-3-glucuronide, morphine has been shown to be metabolized to morphinone [8], 7,8-epoxide morphine [9], and 2-hydroxymorphine which can be metabolized further to 2,3-quinone [10], all of which have the potential to generate the highly reactive free oxide radicals or to bind to glutathione (GSH), a naturally occurring superoxide radical scavenger. The conjugation and subsequent depletion of GSH would then allow the endogenous accumulation of free radicals. The reactive electrophils formed either directly or indirectly from the metabolism of morphine could subsequently cause cellular toxicity by enzyme inactivation, DNA damage and/or lipid peroxidation [11].

Indeed, investigators have shown that morphine causes a rapid and dose-dependent depletion of rat hepatic GSH content *in vivo* which is blocked by opiate receptor antagonists [6, 12]. Morphine-induced GSH depletion with subsequent transaminase elevation was also demonstrated in isolated perfused rat liver [13]. Carbon tetrachloride, whose toxicity is believed to be due to generation of free oxide radicals, potentiates morphine toxicity in mice [5].

Evidence implicates the morphine metabolite, morphinone, which is eight times more toxic than morphine in mice [13]. Nagamatsu *et al.* [14] found that pretreatment of mice with GSH or cysteine significantly increases the survival rates of mice given a lethal dose of morphinone, whereas morphinone lethality is potentiated markedly by diethyl maleate, which depletes GSH [14]. In addition, they have also isolated a morphinone-glutathione conjugate in kidney, lung, and liver in mice. Furthermore, the formation of the morphinone-GSH conjugate parallels a reduction of hepatic GSH [13]. In addition, Correia *et al.* [12] have concluded that the observed morphine-induced depletion of hepatic GSH and the increases of serum transaminases from isolated rat liver preparations are due to the bioactivation of the (−)3-hydroxy-*N*-methylmorphinan moiety of morphine to a reactive electrophil.

Therefore, the effect of GSH depletion (by buthionine sulfoximine) on the potential hepatic and renal toxicity of morphine administration to mice was investigated by examining changes in organ weights, selected serum chemistries, and histology.

Materials and methods

Morphine sulfate was obtained from Eli Lilly, Indianapolis, IN. L-Buthionine-*SR*-sulfoximine (mol. wt = 222.31) was purchased from the Chemical Dynamics Corp., Plainfield, NJ.

This study was conducted using forty C57B1/B6By female mice (weighing 20–30 g) divided into four treatment groups of ten mice each. Mice were housed (five per cage) in solid-bottomed plastic cages containing pine bedding for a minimum of 15 days. Pine shavings used as bedding have been shown to be an inducer of microsomal cytochrome P-448 and, therefore, potentially increase metabolite formation. Indeed, housing mice on pine shavings has been shown to potentiate morphine-induced increases in aspartate transaminase activity [7].

Half of the mice (groups 2 and 4) received 1 mmol of L-buthionine sulfoximine (BSO) per kg of body weight 2 hr prior to the administration of morphine or saline. It has been shown that GSH depletion by BSO (a selective and potent inhibitor of gamma-glutamylcysteine synthetase) is at a maximum at 2 hr after administration [15]. Morphine was injected at a maximally tolerated dosage of 400 mg/kg to mice in groups 3 and 4. The intraperitoneal route was chosen to optimize the generation of metabolites (via the first pass effect). Mice in groups 1 and 2 received a volume of saline equal to the largest volume of morphine administered (26.7 ml/kg).

Within 14–16 hr after morphine or saline injection, the mice were bled from the orbital sinus and samples were analyzed for aspartate transaminase activity (SGOT), alanine transaminase activity (SGPT), gamma-glutamyl transpeptidase activity (SGGT), urea nitrogen (BUN), alkaline phosphatase, and creatinine concentrations [16]. Animals were fasted overnight prior to necropsy. Liver and kidney tissues were evaluated for histopathological changes at the level of light microscopy. Statistical analyses consisted of weighted analysis of variance with subsequent pairwise comparisons (alpha = 0.05).

Results and discussion

The effect of glutathione depletion by buthionine sulfoximine on morphine toxicity was investigated. Toxicological assessment was based on changes in organ weights, selected serum chemistries, and histological assessment.

In this test system, the administration of BSO to saline-treated mice had a small effect on hepatic and renal function. The observed decreases of SGPT (Table 1) and BUN (77.8%, P = 0.02) after BSO administration when compared to control values may reflect a perturbation of the amino acid pool or transport. Decreases in serum transaminase activities can also be meaningful indicators of hepatic damage. For example, drugs reducing the pyridoxal-5-phosphate pool as well as hydrazines, known to be toxic, can depress transaminase activities *in vivo* [17]. The observed decrease in BUN may reflect an impaired ability of the liver to synthesize urea and, therefore, reflect hepatic injury. Furthermore, the administration of BSO caused an increase in serum creatinine (Fig. 1), which may indicate renal damage.

Buthionine sulfoximine is a potent and specific inhibitor of gamma-glutamylcysteine synthetase which results in GSH depletion [15]. BSO does not inhibit gamma-glutamyl cyclotransferase, gamma-glutamyl transpeptidase, or glutathione synthetase. However, it may inhibit gamma-glutamyl amino acid transport in the kidney. L-BSO has been shown to deplete *in vivo* liver and kidney GSH to 7 and 9% of control values, respectively, after 2 hr. The GSH depletion remains up to 25 hr [15].

The administration of morphine caused an increase of SGOT (Table 1), alkaline phosphatase (Fig. 2) activities, and creatinine concentration (Fig. 1), as well as severity of centrilobular hepatocyte swelling when compared to saline controls (Table 1). Centrilobular necrosis has also been shown to be induced by acetaminophen [18], carbon tetrachloride [19], or bromobenzene [20]. This appears to be a result of the centrizonal concentration of the enzyme sys-

Table 1. Effects of BSO pretreatment on morphine-induced changes of SGOT and SGPT activities and on hepatic histology

Group No.	Treatment	Mean severity*	Sporadic lesions (number/total)	SGOT activity† (I.U./L)	SGPT activity† (I.U./L)
1	Saline	1.8 ± 0.20	Slight C.N.‡ (1/10)	110.6 ± 17.64	48.9 ± 7.51
2	BSO-Saline	1.5 ± 0.31	Bile duct hyperplasia (2/10)	86.6 ± 13.4	27.3 ± 2.26
3	Morphine	2.8 ± 0.49	Peliosis (1/10)	151.7 ± 28.22	28.3 ± 4.17
4	BSO-Morphine	3.6 ± 0.22	Moderate C.N. (2/10)	234.7 ± 26.48	96.8 ± 28.74

* Mean severity of hepatocytic swelling ± S.E.

† Mean activity ± S.E. Mice were pretreated with BSO 2 hr prior to morphine treatment. Liver and serum samples were taken 16–18 hr later for histological assessment. Each treatment group consisted of ten mice.

‡ Coagulation necrosis.

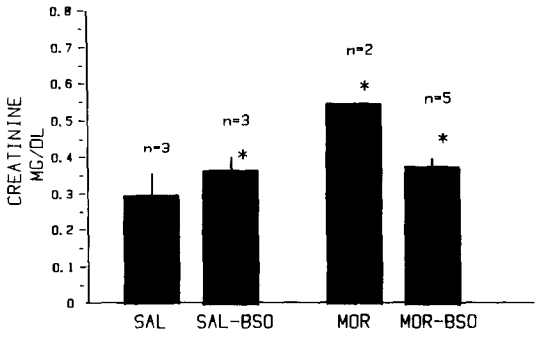

Fig. 1. Effect of BSO administration on creatinine concentrations in morphine- or saline-treated mice. Values are means ± SE except for the morphine group where the average for two mice is given. Key: (*) Significant effects: saline vs BSO-saline ($P = 0.007$); saline vs morphine ($P = 0.0004$); morphine vs BSO-morphine ($P = 0.0003$); and BSO-saline vs BSO-morphine ($P = 0.0004$).

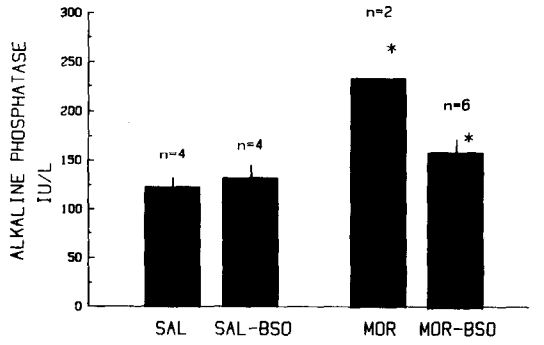

Fig. 2. Effect of BSO administration on alkaline phosphatase activities in morphine- or saline-treated mice. Values are means ± SE except for the morphine group where the average for two mice is given. Key: (*) Signfiicant effects: saline vs morphine ($P < 0.05$); and morphine vs BSO-morphine ($P < 0.05$).

tems responsible for the conversions of the agents to hepatotoxic metabolites. In addition, significant decreases of SGPT and BUN (74.9%, $P = 0.012$) were also observed after morphine administration. The observed elevation in alkaline phosphatase is probably due to the increase in bile duct pressure commonly seen upon morphine administration [21].

Pretreatment with BSO potentiated the hepatotoxicity of morphine, as evidenced by significant ($P < 0.05$) increases in SGOT and SGPT and in severity of the centrilobular hepatocyte swelling, when compared to both the morphine-treated and the BSO-saline-treated mice. This supports the hypothesis of the formation of hepatotoxic morphine metabolite that either conjugates with GSH or generates reactive oxides that results in GSH depletion. Investigators have provided evidence that incubation of morphine with isolated rat hepatocytes causes a GSH depletion that is due to the formation of GSH conjugates rather than to the direct generation of free oxide radicals [22].

Increases in serum creatinine concentrations were observed in the BSO–saline vs saline groups, the morphine vs saline groups, the morphine vs BSO-morphine groups, and the BSO-morphine vs BSO-saline-treated mice. Morphine has been shown to cause a decrease in the glomerular filtration rate in humans [23]. There was no evidence of increases of SGGT activities (which reflect renal function in mice [17]) or renal histopathological changes in any of the treatment groups. The apparent lack of renal toxicity may be because the specific activities of the mixed-function oxidase system are much lower in the kidney than in the liver [24], thereby reducing the generation of toxic metabolites.

This study provides evidence of a morphine-induced hepatotoxicity that is potentiated by glutathione depletion, as shown by changes of hepatic histology and perturbations of serum chemistries in mice. This suggests that endogenous GSH conjugates with morphine or (more likely) the metabolites of morphine to prevent a toxic interaction with hepatic cells. Therefore, the co-administration of an opiate, such as morphine or heroin, with other GSH depletors, such as cocaine [25], may lead to a potentiation of the hepatotoxicity in humans.

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Biochemical Pharmacology, Vol. 38, No. 1, pp. 209–213, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

The interaction of temelastine with cytochrome P_{450} mixed-function oxidase enzymes *in vivo* and *in vitro*

(*Received* 18 *April* 1988; *accepted* 3 *August* 1988)

Temelastine, 2-[4-(5-bromo-3-methylpyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one, is a drug which has been shown to have selective histamine H_1-receptor antagonist activity in a number of *in vitro* and *in vivo* tests in animals and also in man [1]. It has been shown not to penetrate the blood–brain barrier of the rat to any appreciable extent [2]. It is currently undergoing development at SK&F Research Ltd, The Frythe, Welwyn, as a potential non-sedating antihistamine compound for use in the treatment of allergic rhinitis, allergic skin disorders etc., in man. The presence of substituted pyridine rings in temelastine suggests the potential of the molecule to bind to the mixed-function oxidase enzymes referred to as cytochrome P_{450}. The binding constants of a number of substituted pyridines to this hemoprotein have been compared and ranged between 1 and 2250 μM [3]. The most potent of these compounds, metyrapone, induces a type II binding spectrum with liver microsomes and is a stronger ligand for phenobarbital-induced forms of cytochrome P_{450} than for those forms of cytochrome P_{450} induced by polyaromatic hydrocarbons [4]. The studies described in this investigation were designed to evaluate the interaction of temelastine with cytochrome P_{450} *in vitro* and *in vivo* in the rat.