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A morphine conjugate vaccine attenuates the behavioral effects of morphine in rats

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ABSTRACT

Vaccines for opioid dependence may provide a treatment that would reduce or slow the distribution of the drug to brain, thus reducing the drug's reinforcing effects. We tested whether a conjugate vaccine against morphine (keyhole limpet hemocyanin-6-succinylmorphine; KLH-6-SM) administered to rats would produce antibodies and show specificity for morphine or other heroin metabolites. The functional effects of the vaccine were tested with antinociceptive and conditioned place preference (CPP) tests. Rats were either vaccinated with KLH-6-SM and received two boosts 3 and 16 weeks later or served as controls and received KLH alone. Anti-morphine antibodies were produced in vaccinated rats; levels increased and were sustained at moderate levels through 24 weeks. Antibody binding was inhibited by free morphine and other heroin metabolites as demonstrated by competitive inhibition ELISA. Vaccinated rats showed reduced morphine CPP, tested during weeks 4 to 6, and decreased antinociceptive responses to morphine, tested at week 7. Brain morphine levels, assessed using gas-chromatography coupled to mass spectrometry (GC–MS) on samples obtained at 26 weeks, were significantly lower in vaccinated rats. This suggests that morphine entry into the brain was reduced or slowed. These results provide support for KLH-6-SM as a candidate vaccine for opioid dependence.

1. Introduction

Opioid dependence is a serious health problem in the United States and world-wide (UNODC, 2010). Relapse rates remain high even though there are approved pharmacological treatments for this disorder (Veilleux et al., 2010). Medications currently approved to treat opioid dependence include methadone, buprenorphine, and naltrexone (Maxwell and Shinderman, 2002; Stotts et al., 2009). These agents act at opioid receptors as an agonist, a partial agonist, or an antagonist, respectively. While each of these treatments shows some therapeutic success, there are numerous limitations to their use, including high cost, limited availability, problems with compliance, and, in the case of the agonists, diversion (Kahan et al., 2001; Kreek et al., 2010; Stotts et al., 2009). An alternative strategy to developing a treatment for opioid dependence is an anti-opioid vaccine (Shen et al., 2012).

The approach for the development of drug addiction vaccines differs from the approach used in the development of the other medications. Whereas the opioidergic treatment agents were designed to target opioidergic effector systems in the brain through pharmacodynamic mechanisms, vaccines act as "pharmacokinetic" antagonists. Upon administration, the vaccine stimulates the production of drug-specific antibodies that can bind to the drug in the circulating blood and extracellular fluid when the drug is ingested. This action should reduce or slow the distribution of the drug to brain and theoretically attenuate the drug's reinforcing or addictive effects. Support for the vaccine approach has been shown by the promising results obtained in clinical trials for vaccines designed for the treatments of cocaine and nicotine addiction (Haney et al., 2010; Hatsukami et al., 2011; Martell et al., 2009).

Abbreviations: CPP, conditioned place preference; KLH, keyhole limpet hemocyanin; BSA, bovine serum albumin; 6-SM, 6-succinylmorphine; KLH-6-SM, keyhole limpet hemocyanin-6-succinylmorphine; alum, aluminum hydroxide gel; 6-AM, 6-acetyl morphine; 3-GM, morphine-3-glucuronide; 6-GM, morphine-6-glucuronide; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; sulfo-NHS, N-hydroxysulfosuccinimide; ELISA, enzyme-linked immunosorbent assay; GC–MS, gas-chromatography coupled to mass spectrometry; IC50, 50% inhibition of maximum binding; MPE, maximal possible effect.

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Heroin, which is arguably the most serious of all opioid addictions, is a pro-drug; that is, its effects occur mainly through its metabolites. Heroin is readily hydrolyzed by serum and liver esterases to the more stable compounds, 6-acetylmorphine (6-AM) and morphine. Morphine itself can then undergo further metabolism into morphine-3-glucuronide (3-GM) and morphine-6 glucuronide (6-GM) via enzymes in liver and kidney (Inturrisi et al., 1983; Selley et al., 2001). In fact, 6-AM is considered to cause the immediate euphoric effects of heroin administration. Thus, a vaccine that can produce antibodies to heroin, morphine, and 6-AM is desirable.

While vaccines for stimulant addictions have been developed fairly recently, vaccines directed at morphine were first tested in animals 40 years ago (Berkowitz and Spector, 1972; Bonese et al., 1974). The vaccine was prepared by conjugating a morphine hapten to bovine serum albumin (BSA) through a 6-succinylmorphine (6-SM) linkage to lysine residues on BSA. These early studies demonstrated that this vaccine could produce antibodies with specificity for heroin and 6-AM, as well as for morphine. Other studies showed that binding specificity differed depending upon the hapten used (Koida et al., 1974; VanVunakis et al., 1972; Wainer et al., 1973). The study of vaccines for opioid dependence likely fell out of favor after these initial studies due to the introduction of methadone as an effective treatment agent. However, as discussed above, there are limitations and problems with the standard medications for opioid dependence. Thus, the vaccine approach for opioid dependence is being reinvestigated (Anton and Leff, 2006; Anton et al., 2009; Ma et al., 2006; Stowe et al., 2011). For example, a bivalent morphine–heroin vaccine developed using tetanus toxoid as the carrier protein produced antibodies and prevented the acquisition of heroin self-administration in rats (Anton and Leff, 2006; Anton et al., 2009). However, this vaccine required four boosts over 60 days, and biweekly boosts over a year in order to maintain adequate titers. In another study, rats administered a heroin vaccine based on a hapten structure where the linker was attached to the nitrogen of nor-heroin showed reduced heroin-induced antinociception and acquisition of heroin self-administration (Stowe et al., 2011). However, rats administered a vaccine that was also based on nor-morphine did not show changes in heroin self-administration even though both vaccines generated antibodies. This failure to show functional effects of the vaccine may reflect that it had no affinity for 6-AM (Stowe et al., 2011).

The purpose of this study was to evaluate a morphine vaccine consisting of a KLH-6-SM conjugate and aluminum (alum) in rats. We investigated the immunogenicity of KLH-6-SM at its optimal dose for eliciting effective antibodies and tested its ability to attenuate opioid-induced behavioral effects. These behavioral effects included the analgesic and rewarding effects of morphine using hot plate, tail-flick, and place conditioning procedures. In addition, a competitive inhibition ELISA was used to assess antibody binding to free morphine, 6-AM, 3-GM, and 6-GM.

We also hypothesized that the vaccine-induced antibodies would sequester the morphine in the blood, thereby increasing in blood levels and reducing brain levels. This was tested in the present study using gas chromatography combined with mass spectrometry.

2. Methods

2.1. Animals and housing

Male, Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, USA) at about 8 weeks of age and 250 g body weight. They were group-housed (3 per cage) under temperature- and humidity-controlled conditions with a 12:12 h light/dark cycle (lights on from 0600). Food and water were available ad libitum. Procedures were approved by the Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (NIH, 1996).

2.2. Groups

A total of 54 rats were employed in the studies. Half of the rats were vaccinated with KLH-6-SM as described below and the other half served as naïve, non-vaccinated controls. The 27 vaccinated rats consisted of three groups (n = 9 ea) that were administered one of three amounts of vaccine conjugate at the second boost session (see below). One group (n = 9) of vaccinated rats was tested for antinociception and compared to a sub-group (n = 9) of naïve rats and both of these groups were used at the termination of the protocol to provide blood and tissue samples. All of the vaccinated and naïve rats were used for the conditioned place preference (CPP) study.

2.3. Drug

Morphine sulfate was purchased from Noramco Inc. (Wilmington, DE). 6-AM, 3-GM, and 6-GM were obtained from the National Institute on Drug Abuse (Chemistry and Physiological Systems Research Branch, Bethesda, MD). All drug doses and concentrations were expressed as salt free base. Morphine was administered at a dose of 2 mg/kg (S.C.) in a volume of 5 ml/kg for the hot plate and tail flick tests. A dose of 1 and 2 mg/kg (S.C.) in a volume of 4 ml/kg was used for the CPP study.

2.4. Vaccine preparation and administration

2.4.1. Preparation of protein conjugates

6-succinyl morphine (6-SM) was prepared according to a previously published method (Simon et al., 1972). To prepare the KLH conjugate, 14 mg (0.07 mmol, 1.2 M equivalents over 6-SM) of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich, Milwaukee WI) and 20 mg (0.09 mmol, 1.5 M equivalents over 6-SM) of N-hydroxysulfosuccinimide (sulfoNHS Sigma-Aldrich, Milwaukee WI) were added to 0.5 mL phosphate-buffered saline (PBS) and the solution stirred for 20 min at room temperature. 6-SM (23 mg, 0.06 mmol) was added and stirring continued for 1 h. KLH (2 mL,10 mg/mL) was prepared by adding 2 mL H_2O and 89 mg NaCl to a vial containing freeze-dried Imject KLH/PBS (Thermo-Fisher Scientific, Rockford, Ill). The solution of activated 6-SM was added to the KLH solution and the pH adjusted to 7.4 with 2 M NaOH. The resulting clear solution was stirred overnight and purified over a NAP-25 column (GE Healthcare, Piscataway, NJ) equilibrated with PBS, following the manufacturer's instructions. The final KLH-6-SM conjugate (3.5 mL, 5.7 mg/mL) was filtered through a 0.45 μm filter (Millipore, Cork, Ireland) and stored at 4 °C until use. The BSA-6-SM conjugate was synthesized using the same method as for KLH, except that NaCl was not added to the 2 mL of 10 mg/mL Imject KLH/PBS BSA. The morphine conjugate structure is depicted in Fig. 1.

2.4.2. Vaccination schedule

The experimental procedure schedule is shown in Fig. 2. Rats were vaccinated via intramuscular (IM) injection with 100 μg of KLH-6-SM mixed with 1500 μg alum (Sigma-Aldrich) at week 0. All rats were boosted with 100 μg of the KLH-6-SM conjugate at 3 weeks. At 16 weeks, rats received another boost injection using 100 μg, 200 μg, and 300 μg of KLH-6-SM morphine conjugate with 1500 μg, 3000 μg or 4500 μg alum, respectively (n = 9 ea). The control rats received an injection of KLH alone at the same time as the vaccinated rats. The CPP study was conducted between weeks 4 to 6 and the hot plate and tail flick tests were performed at week 7 as shown in Fig. 2. Finally, blood and brain tissue samples were obtained at week 26.