Analgesic and Immunomodulatory Effects of Codeine and Codeine 6-glucuronide

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Purpose. The antinociceptive and immunosuppressive effects of codeine and codeine 6-glucuronide were determined in rats after intracerebroventricular administration.

Methods. Codeine 6-glucuronide was synthesized using a modification of the Koenigs-Knorr reaction. A lipophilic intermediate formed during synthesis, methyl [codein-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid] uronate, was also tested. Morphine was used as a positive control to compare antinociceptive potencies of these compounds.

Results. All compounds tested produced significant analgesic responses, as assessed by the tail flick model. Additionally, codeine 6-glucuronide showed significantly less immunosuppressive effects than codeine *in vitro*.

Conclusions. We conclude that codeine 6-glucuronide and related compounds may have clinical benefit in the treatment of pain in immune compromised patients.

KEY WORDS: analgesia; immune; codeine; glucuronides.

Codeine is primarily metabolized in the liver and partially in the central nervous system, kidney, lung, placenta and gut. Metabolism occurs predominantly via conjugation with endogenous glucuronic acid at the 6-position (1–3). Other pathways include O-demethylation producing morphine, with subsequent glucuronidation at the 3- and 6- positions and N-demethylation to norcodeine and its glucuronide conjugate (figure 1).

Codeine and its metabolites are excreted by the kidney, with over 95% of a single dose eliminated within 48 hours (4). Typical urinary amounts in humans, after a 30 mg dose of codeine phosphate, expressed as a percentage of the dose are: codeine (8–16%); codeine 6-glucuronide (C6G) (48–69%); morphine (0.5–1.0%), morphine 3-glucuronide (M3G) (5–8%); morphine 6-glucuronide (M6G) (0.5–2.0%); norcodeine (2–6%) and norcodeine glucuronide (1–6%) (2). In rat, recoveries of codeine, morphine and morphine 3-glucuronide in the 24 hour urine after a 2 mg/kg s.c. dose were reported as 8%, 8% and 24%, respectively (5–9). No morphine 6-glucuronide or codeine 6-glucuronide was seen in rat urine after codeine administration. Yoshimura et al. (9) reported a small amount of codeine 6-glucuronide (1%) in rat urine, while Oguri et al. (10) detected negligible amounts (0.2%) by HPLC. The absence of morphine 6-glucuronide in rat urine after codeine administration has been confirmed by a number of investigators (10,11).

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Glucuronide metabolites are generally considered to be inactive and rapidly eliminated. However, there have been some reports of analgesia produced by morphine 6-glucuronide. Yoshimura et al. reported that morphine 3-glucuronide and morphine 6-glucuronide could cross the blood brain barrier to stimulate opioid receptors in rat brain (12). Other rodent studies, have shown that morphine 6-glucuronide has a higher affinity for μ opioid receptors than morphine itself (13–16), and has 2–4 times greater analgesic potency than morphine when injected subcutaneously (17,18). Conversely, morphine 3-glucuronide, was found to have a very low affinity for μ opioid receptors in brain and no analgesic effect when administered to rats and mice. Direct clinical evidence for the analgesic activity of morphine 6-glucuronide has been reported in man (19,20). In fact, lower doses of morphine 6-glucuronide than morphine but were required for equivalent pain relief in cancer patients (18,21). Barjavel et al. (22) indicated that morphine glucuronides cross the blood brain barrier at the same rate as morphine in higher amounts. The analgesic activity of codeine 6-glucuronide has not been reported.

Immunomodulation by opiates is well known, with numerous studies documenting suppressed immune responses in morphine exposed animals. Rat natural killer (NK) cell cytolytic activity was inhibited after central administration of morphine (23). Although the mechanism is unknown, studies suggest that morphine induces its immunomodulation via specific brain regions. Hernanadez and colleagues (24) evaluated the role of opioid receptors in peripheral and central sites, observing that micro-injection of morphine into the anterior hypothalamus of rats inhibited lymphocyte proliferation without analgesia whereas micro-injection into the third ventricle inhibited lymphocyte response with analgesia.

Immunosuppressive effects of opiates were first observed in heroin abusers who had increased incidence of infection (25) and supression of lymphocyte proliferative responses to mitogens (26). Immunomodulatory effects of morphine on human cells have been reported in several *in vitro* systems. Chao and colleagues (27) studied the immune responses of peripheral blood mononuclear cells (PBMCs), observing that morphine suppressed the release of tumor necrosis factor-beta (TNFB) in phytohemagglutinin stimulated cells. The immunomodulatory effects of morphine or codeine glucuronides have not been studied.

The immunosuppressive potential of commonly used narcotic analgesics should be determined to establish the appropriate use of these agents in immunocompromised patients, including HIV, transplant and cancer patients. This study was designed to investigate the immunomodulatory and analgesic activities of opiate glucuronides relative to morphine and codeine.

MATERIALS AND METHODS

All chemicals were obtained as follows: codeine, morphine, morphine 6-glucuronide and silver carbonate, Sigma (St. Louis, MO); sodium methoxide, barium hydroxide and oxalic acid, Aldrich (Milwaukee, WI); methyl 2,3,4-tri-O-acetyl-1α-bromo-1-deoxy-D-glucopyranuronate, NBS Biologicals (Hatfield, Herts, U.K). Codeine 6-glucuronide was synthesized using a modification of the Koenigs-Knorr method (figure 2),

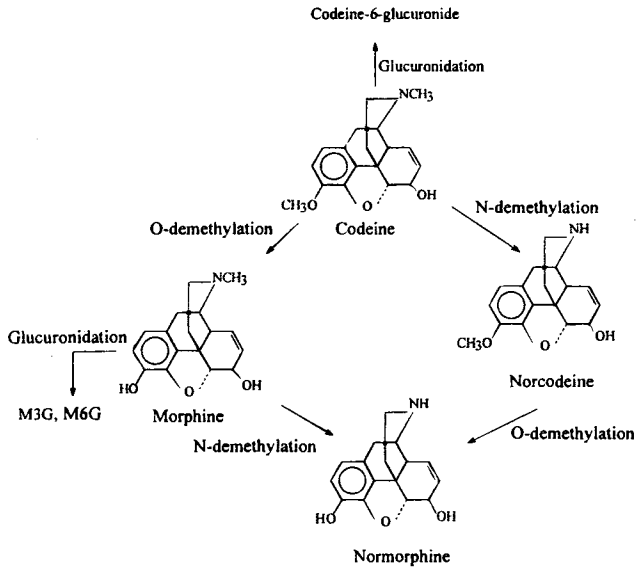

Fig. 1. The Various Metabolic Pathways of Codeine.

(28). Product identity and purity was confirmed using HPLC-UV analysis compared to an analytical standard of codeine 6-glucoronide (NIDA). A synthetic "intermediate," methyl [codein-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate, was also produced by this process. Mitogens, phytohemagglutinin (PHA) and phorbol myristate acetate (PMA), were purchased from Sigma (St. Louis, MO) and ^{3}H-thymidine from Dupont Medical Products, (Wilmington, DE).

Animals and i.c.v. Surgery

Adult, male Sprague-Dawley rats, 250–275g, (Harlan, Indianapolis, IN) were in a room with a 12 hour light-dark cycle. Animals were fed standard laboratory rat chow and tap water *ad libitum*. Rats were anesthetized with 30–40 mg/kg sodium pentobarbital i.p. and stereotaxically fitted with an intracerebroventricular guide cannula (23 gauge). Stereotaxic coordinates were: 1.4 mm lateral, 0.9 mm caudal to bregma and 4.5 mm ventral. Animals recovered for 3 days prior to experimentation. Protocols were approved by the Animal Care Committee of the University of Florida.

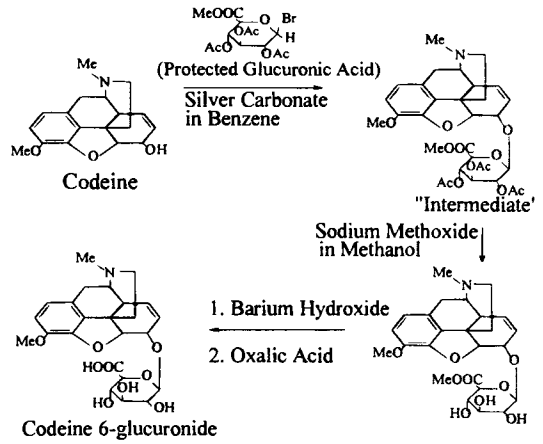

Fig. 2. Synthesis of Codeine 6-Glucuronide by a Modification of the Koenigs-Knorr Reaction.

Drug Administration

Drugs were administered in 5 μl physiological saline (pH 4.5–5.7) directly into the lateral ventricles via the indwelling cannula at the following doses: codeine, 100 μg; codeine 6-glucuronide and "intermediate", 10μg; and morphine, 5 μg.

Experimental Design

One group of six rats was monitored after surgery without drug/saline to assess effects of the surgical procedure on the pharmacodynamic measurements. Each compound was then administered to separate groups of six rats and tail flick response times were noted at the following times: 0, 5, 10, 20, 30, 60, 90, 120 and 180 minutes. Each animal, acting as its own control, then received a dose of saline followed by measurement of pharmcodynamic parameters.

Tail Flick Method

Tail withdrawal time was measured after application of a focused beam of light (29), before drug administration (baseline latency) and at regular intervals thereafter, using a Model 33 Tail Flick Analgesia Meter (Iitc Inc., Landing, NJ). Beam intensity and sensitivity were set at 75 and 8, respectively. In the absence of a response, a cut-off period of 40 seconds was used to prevent tissue damage and was considered as the maximal suppression of pain. Due to variability in the baseline response times, the data were standardized by calculating the % of maximum possible effect (% MPE).

Immune Studies

Peripheral lymphocytes from healthy volunteers were isolated from heparinized blood after Ficoll Hypaque density gradient centrifugation. Mitogen stimulation: lymphocytes 1×10^5 cells/ml were cultured with either (PMA) (50 ng/ml) or (PHA) (5 μg/ml) in RPMI-1640 medium containing 5% human AB serum and antibiotics for 48 hours at 37°C in a 5% CO_2 humidified atmosphere. Third party mixed lymphocyte reactions (MLR) were performed as follows: responder lymphocytes at 1×10^5 cells/ml were cultured with an equal number of irradiated stimulator lymphocytes from another donor with different HLA antigens in RPMI-1640 medium containing 5% human AB serum and antibiotics for 48 hours at 37°C in a 5% CO_2 humidified atmosphere. After 48 hours (PMA, PHA) and 120 hours (MLR), the cells were labeled with ^{3}H-thymidine (1μCi). Cells were harvested and ^{3}H-thymidine incorporation into DNA was measured in a scintillation counter. Drugs were added to tissue culture plates dissolved in RPMI-1640 at concentrations ranging from 0.156 to 10 μg/ml immediately after cell stimulation. Percentage inhibition of proliferation was calculated: [(cells without drug cpm−cells with drug cpm)/ cells without drug cpm] × 100. Assays were performed in triplicate and experiments were evaluated for statistical analysis using student's paired t-test.

RESULTS

Intracerebroventricular administration of codeine, codeine 6-glucuronide (C6G), "intermediate" and morphine produced

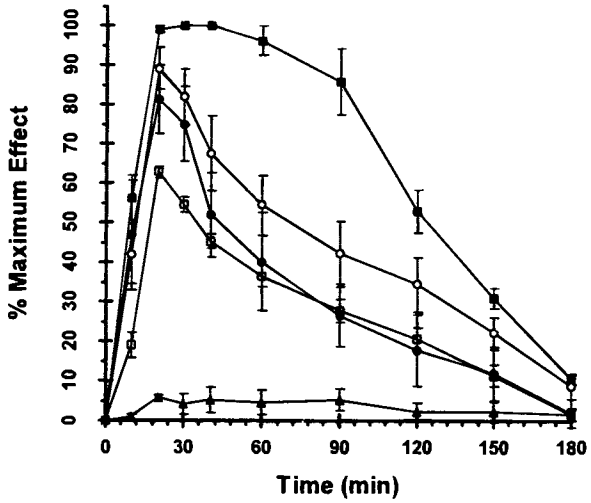

Fig. 3. Analgesic Responses of Rats to ICV Administration of Saline (△), Codeine (□), Intermediate (●), Codeine-6-glucuronide (○) and Morphine (■).

significant antinociceptive responses in rats. All compounds tested produced a peak response about 20 min after administration. Each rat was also administered saline which produced minimal changes to baseline responses. The effect of the i.c.v. surgery on response time, was consistent over a 3 hour period. Morphine was the most effective compound producing a maximal suppression of pain, i.e., 100% of the maximum possible effect (MPE). Codeine 6-glucuronide and "intermediate" also exhibited marked increases in antinociceptive responses, up to 89 ± 5 and 81 ± 9 % of MPE, respectively (figure 3). Codeine also produced analgesia, with a peak effect of 62 ± 2% of MPE. The % MPE at peak response time for all compounds is summarized in table 1.

The area under the effect curve (AUEC) was determined from individual % MPE versus time graphs, from 0 to 180 min, by trapezoidal calculation (table 2). Using this method, the rank order of effectiveness was morphine > codeine 6-glucuronide ≃ intermediate > codeine.

All four agents tested (morphine, morphine 6-glucuronide, codeine and codeine 6-glucuronide) demonstrated immunomodulatory effects in the *in vitro* systems. Cell viability was 98% using tryphan blue staining. In comparing the immunosuppressive effects of morphine and morphine 6-glucuronide in mitogen stimulated lymphocytes, both demonstrated the greatest inhibition in the PHA stimulated cells. Inhibitory effects were significantly lower for the glucuronide than the parent compound in the concentration range of 0.156–2 μg/ml ($p < 0.01$). Conversely, in PMA stimulated lymphocytes, morphine and morphine 6-glucuronide demonstrated negligible inhibition at lower concentrations (<1.25 μg/ml) but marked inhibition at concentrations above 5 μg/ml. There was no statistical difference between the two agents in the inhibition of cell proliferation. Morphine and morphine 6-glucuronide also showed inhibition of the allogeneic model or the mixed lymphocyte reaction (MLR). The 6-glucuronide of morphine showed significantly less immunosuppression compared to morphine over the entire concentration range (0.156–10μg/ml). Decreased immunosuppressive effects of morphine 6-glucuronide compared to morphine were apparent at concentrations below 0.625 μg/ml ($p < 0.01$).

Table I. % of Maximum Possible Effect for Various Compounds at the Peak Response Time

Compound	% MPE
Morphine (5 μg)	100 ± 0
Codeine (100 μg)	62 ± 2
Codeine 6-glucuronide (10 μg)	89 ± 5
Intermediate (10 μg)	81 ± 9

Table II. Total $AUEC_{0-3\,h}$ Values for Various Compounds

Compound	Total $AUEC_{0-3\,h}$
Morphine (5 μg)	11545 ± 744
Codeine (100 μg)	4462 ± 546
Codeine 6-glucuronide (10 μg)	8156 ± 1744
Intermediate (10 μg)	6416 ± 2602

Similar to the immunosuppressive effects of morphine and morphine 6-glucuronide, codeine and its 6-glucuronide demonstrated the greatest inhibition in the PHA stimulated lymphocytes, with maximal differences in cell inhibition of the parent compound and its metabolite in the concentration range of 0.156–5 μg/ml ($p < 0.05$) (figure 4). As with morphine 6-glucuronide, codeine 6-glucuronide showed less inhibition of cell proliferation compared to codeine. In PMA stimulated lymphocytes, the glucuronide showed negligible inhibition in lower concentrations compared to codeine (figure 5). Codeine 6-glucuronide demonstrated significantly less inhibition than codeine at all concentrations. Codeine and codeine 6-glucuronide inhibited MLR in a dose-dependent manner, however there was no statistical difference between the effects of the two agents.

DISCUSSION

Our results show that, like morphine 6-glucuronide, codeine 6-glucuronide also possesses analgesic activity. In fact, codeine 6-glucuronide appears to have a greater analgesic activ-

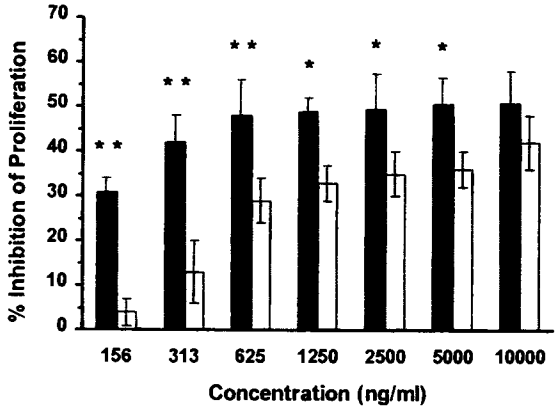

Fig. 4. The % Inhibition of Proliferation of PHA stimulated T Lymphocytes after the addition of Codeine (■) and Codeine 6-glucuronide (□). * and ** Indicate p-values < 0.05 and 0.01, respectively.

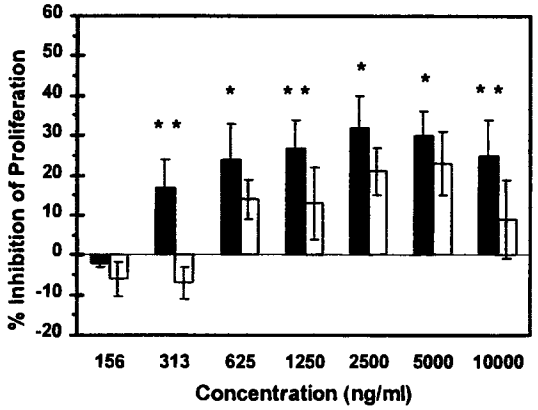

Fig. 5. The % Inhibition of Proliferation of PMA stimulated T Lymphocytes after the addition of Codeine (■) and Codeine 6-glucuronide (□). * and ** Indicate p-values < 0.05 and 0.01, respectively.

ity than codeine in rats. The "intermediate" formed during the synthetic procedure was isolated and characterized. It contains a glucuronic acid moiety attached to the 6-position of codeine with intact acetyl/methyl groups rendering it more lipophilic than codeine 6-glucuronide and more capable of crossing the BBB. This compound also exhibited antinociceptive activity similar to that of codeine 6-glucuronide.

Both morphine and codeine showed greater immunosuppressive effects than either of their 6-glucuronide metabolites at physiological concentrations. Morphine 6-glucuronide showed a concentration-dependent inhibition profile similar to morphine. Both agents demonstrated greatest inhibitory effects in the calcium and interleukin-2 driven pathways simulated by PHA experiments. This was also supported by their ability to inhibit more non-specific stimulatory pathways induced in the allogeneic model of the MLR. Due to the low activity seen in the PMA stimulated lymphocytes, morphine and its 6-glucuronide metabolite do not suppress the immune system through protein kinase C. These findings are in agreement with previously reported data (24). Similar to morphine 6-glucuronide, codeine 6-glucuronide possesses less immunosuppressive activity than its parent compound, codeine. Codeine and codeine 6-glucuronide appear to exert their immunomodulatory effects through interleukin-2 and calcium dependent pathways stimulated by PHA. Additionally, they suppress non-specific pathways induced in the MLR model. Compared to morphine and morphine 6-glucuronide, codeine and codeine 6-glucuronide have more activity in the PMA stimulated cells suggesting their immunosuppressive activity may include inhibition of protein kinase C.

The analgesic effect of codeine 6-glucuronide, the major plasma and urinary metabolite in man (30, 31), has yet to be evaluated in humans. Given our preliminary findings that codeine 6-glucuronide has similar analgesic activity to codeine with less immunosuppressive action, this and related compounds deserve further study as potential analgesic agents for immunocompromised patients.

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