Evidence That Oxymorphone-Induced Increases in Micronuclei Occur Secondary to Hyperthermia

Dana L. Shuey,*1 Ramadevi Gudi,† Ljubica Krsmanovic,† and Ronald J. Gerson*

*Endo Pharmaceuticals Inc, 100 Endo Boulevard, Chadds Ford, Pennsylvania 19350; and †BioReliance, 9630 Medical Center Drive, Rockville, Maryland 20850

Received July 20, 2006; accepted October 12, 2006

Oxymorphone is a potent opioid analgesic. Oral administration of oxymorphone to rats at doses ≥ 20 mg/kg and mice at 500 mg/kg produced an increase in micronucleated polychromatic erythrocytes (MPCEs). Oxymorphone does not produce chromosome aberrations in vitro, suggesting that the increased MPCEs in vivo may involve indirect mechanisms. Opioids are known to affect thermoregulatory mechanisms. Changes in body temperature can increase the incidence of MPCEs in rodents. Studies were conducted to examine the relationship between increased MPCEs in rats given oxymorphone and changes in body temperature. Single oral doses of oxymorphone associated with increased MPCEs (20 and 40 mg/kg) also produced a marked, rapid increase in body temperature. When animals were pretreated with sodium salicylate, peak body temperature was lower and returned to baseline more quickly than when oxymorphone was given alone. MPCEs were evaluated in rats after administration of oxymorphone (40 mg/kg) alone or following pretreatment with an oral dose of sodium salicylate. Oxymorphone alone produced a statistically significant increase in the incidence of MPCEs (3.6 per 1000 polychromatic erythrocytes vs. 0.4 in controls). The number of MPCEs in animals pretreated with sodium salicylate was similar to controls. Sodium salicylate alone had no effect on the number of MPCEs. Systemic oxymorphone exposure was not affected by sodium salicylate pretreatment; maximum plasma concentration (Cmax) and area-under-the-curve values were similar after administration of oxymorphone alone or following pretreatment with sodium salicylate. These results indicate that the increased incidence of MPCEs following oxymorphone administration is directly related to increased body temperature.

Key Words: opioid; genotoxicity; thermoregulation; clastogenicity.

Oxymorphone is a potent mu-opioid agonist of the morphinan class, used in the treatment of moderate-to-severe pain. Oxymorphone has been fully tested for genetic toxicity in accordance with International Conference on Harmonization (ICH) guidelines (ICH, 1997). Oxymorphone is not mutagenic in the bacterial reverse mutation assay and does not induce chromosome aberrations in Chinese hamster ovary cells, with or without metabolic activation (Opana Package Insert). However, when administered orally, oxymorphone produced an increase in bone marrow micronucleated polychromatic erythrocytes (MPCEs) at doses of 500 mg/kg in CD1 mice, and ≥ 20 mg/kg in Sprague-Dawley rats (data presented herein). Additional studies showed that MPCEs in oxymorphone-treated mice did not contain kinetochores, indicating that oxymorphone is not aneugenic (Gudi and Krsmanovic, unpublished data).

Mixed results have been found with other opioids in in vitro cytogenetics assays and in vivo micronucleus studies. Similar to oxymorphone, morphine induces chromosome damage in peripheral blood lymphocytes and bone marrow cells of treated mice (Das and Swain, 1982; Sawant and Couch, 1995; Swain et al., 1980) but does not induce chromosome aberrations in cultured human lymphocytes (Falek et al., 1972) or mouse splenocytes (Sawant and Couch, 1995). Oxycodone produced chromosome aberrations in human peripheral blood lymphocytes in vitro and was positive in the mouse lymphoma assay, but did not increase micronuclei in the bone marrow of treated mice (OxyContin Package Insert). Fentanyl, an opioid analgesic structurally unrelated to morphinans, has shown no evidence of mutagenic activity (DURAGESIC Package Insert). Collectively, these results suggest that potent morphinan opioids produce chromosomal alterations, through either primary or secondary mechanisms. The absence of genotoxic effects in vitro suggests that the increase in MPCEs following in vivo administration of oxymorphone may be secondary to other physiological effects.

Opioid analgesics have complex effects on thermoregulation that vary by species, dose, ambient temperature, restraint, and route and duration of dosing (reviewed in Clark, 1979; Martin, 1983). In lightly restrained mice, many opioid agonists, including morphine and oxymorphone, produce a biphasic response (hyperthermia at low doses, hypothermia at high doses) at low ambient temperature (20°C) but only a dose-dependent hyperthermia at high ambient temperature (30°C) (Rosow et al., 1980). Morphine produces a similar biphasic effect on body temperature in restrained rats but only hyperthermia in freely moving animals (Martin and Papp, 1979). The effects of opioids on body temperature appear to involve both centrally mediated effects.

1 To whom correspondence should be addressed. Fax: (484) 840-4291. E-mail: shuey.dana@endo.com.
on the hypothalamus and effects on peripheral “thermisensors” that alter the set point for thermoregulation (Clark, 1979).

Chemically and environmentally induced changes in body temperature, both hyperthermia and hypothermia, have been associated with increased micronuclei in rodents. Several compounds that do not have clastogenic effects in vitro produce an increased incidence of micronuclei in vivo at doses that also produce hyperthermia. In these studies, control of environmental conditions to prevent hyperthermia also prevents the increase in the incidence of micronuclei (Asanami and Shimono, 1997a, 2000; Asanami et al., 1998). Hyperthermia induced using high ambient temperature has also been shown to increase micronuclei in mice (Asanami and Shimono, 1997b; Chrisman and Baumgartner, 1980; King and Wild, 1983). However, no examples of chemically induced hyperthermia and micronuclei induction were found.

To evaluate the relationship of increased MPCEs and effects on body temperature following oxymorphone administration, a series of studies were performed to monitor body temperature changes following administration of oxymorphone at doses associated with increased MPCEs and then to develop a paradigm to regulate body temperature changes in oxymorphone-treated rats and evaluate the impact on oxymorphone-induced MPCEs.

MATERIALS AND METHODS

Animals and husbandry. Crl:CD (SD) IGS BR rats were obtained from Charles River Laboratories (Raleigh, NC). Rats were 6–8 weeks of age at the time of dosing. ICR mice were obtained from Harlan Sprague Dawley, Inc (Frederick, MD). Mice were 6–8 weeks of age at the time of dosing.

Animals were group housed, by sex, with up to three per cage (rats) or five per cage (mice) in polycarbonate cages, covered with filter material. Heat-treated hardwood chips were provided as bedding. Rooms were environmentally controlled with target temperature and humidity of 72 ± 3 °F and 50 ± 20%, respectively, and a 12 h-light/dark cycle. Animals had free access to municipal tap water and feed (Harlan 2018C Certified Global Rodent Diet [rats], Harlan Teklad certified Rodent 7012C [mice]). Tap water met U.S. Environmental Protection Agency drinking water standards and is monitored at least annually for levels of organophosphorus pesticides, metals, coliform bacteria, and other contaminants. Study procedures were reviewed and approved by an Institutional Animal Care and Use Committee.

Test articles and treatment. Oxymorphone hydrochloride was obtained from Mallinckrodt Inc. (St Louis, MO) Cyclophosphamide monohydrate (positive control; CAS #6055-19-2), sodium salicylate (CAS #54-21-7), naproxen sodium (CAS #26159-34-2), and acetaminophen (CAS #103-90-2) were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). All compounds were dissolved in sterile distilled water just before dosing and administered as a single dose by oral gavage. Oxymorphone formulations were adjusted for purity (98.3–99.3%) and salt and water content such that doses represent free base equivalents. Oxymorphone is a Schedule II controlled substance. Appropriate handling precautions were taken. All unused materials were disposed off in accordance with US Drug Enforcement Administration regulations.

Monitoring of body temperature. To monitor body temperature, IPTT-200 transponders (Implantable Programmable Temperature Transponders) were implanted subcutaneously in the interscapular region 1 day prior to dosing. These transponders are small, hermetically sealed electronic transponder chips that store the animal number and a record of animal body temperature internally.

Data are transmitted via a specially designed probe. They were used as part of the DAS-5001 monitoring system (BioMedic Data System, Seaford, DE). Temperature was monitored following guidelines obtained from the manufacturer. For all studies, body temperature was recorded just prior to dosing and 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h after dosing.

Evaluation of MPCEs. For all studies, MPCEs were evaluated in bone marrow using established procedures (Hayashi et al., 1994; Heddele, 1973; Mavournin et al., 1990) and in accordance with ICH guidelines (ICH, 1997). In the initial in vivo micronucleus studies in rats and mice, bone marrow was collected 24 h (all groups) and 48 h (control and high dose) after oxymorphone administration. For all subsequent studies in rats, bone marrow was collected only at 24 h after oxymorphone administration because the greatest effect was observed at this time point. Males and females were evaluated in initial studies. Only males were evaluated in subsequent studies because they generally showed a more robust increase in MPCEs.

Bone marrow was collected from the femur following CO2 asphyxiation and smears were prepared using standard procedures. Slides were fixed in methanol, stained with May-Gruenwald-Giemsa, and permanently mounted. Slides were coded such that the evaluator was blinded to treatment group. For each animal, 2000 polychromatic erythrocytes (PCEs) were scored for the presence of micronuclei. The number of PCEs per 1000 erythrocytes was also recorded to provide an indication of bone marrow toxicity. Statistical significance was determined using the Kastenbaum-Bowman tables, which are based on the binomial distribution (Kastenbaum and Bowman, 1970).

Toxicokinetics. In the initial in vivo micronucleus study in rats, blood samples were collected from 5 animals/sex/group at 1, 4, and 24 h after dosing (all animals were sampled at each time point). In the micronucleus study of oxymorphone with sodium salicylate, blood samples were collected from six satellite animals per group. Each animal was sampled at three time points (0.5, 2, and 8 h or 1, 4, and 24 h; three animals were bled at each time point). Blood was collected into tubes containing ethylenediaminetetra acetic acid, plasma was isolated, and oxymorphone concentrations were determined using a validated LC/MS/MS assay. Mean plasma concentration was calculated for each time point. The reported Cmax is the maximal mean plasma concentration observed. Tmax is the time at which the maximal mean plasma concentration was observed, unless otherwise noted (i.e., by definition, must be one of the sampling time points in a given study). Area under the plasma concentration vs. time curve (AUC) was calculated by a noncompartmental model using WinNonlin program, version 3.2 (Pharsight Corporation, Mountain view, CA). In the initial micronucleus study, an AUC was calculated for each animal and the reported AUC represents a mean of individual animal values. In the micronucleus study with sodium salicylate pretreatment, a composite AUC was calculated based on mean plasma concentration at each time point.

RESULTS

Initial In Vivo Micronucleus Studies of Oxymorphone in Rats and Mice (Table 1)

Oxymorphone was tested for induction of MPCEs in bone marrow following a single oral dose to rats (10, 20, and 40 mg/kg) or mice (125, 250, and 500 mg/kg). Administration of 40 mg/kg oxymorphone to rats produced mortality (2/15 males, 1/15 females). Lethargy occurred at all doses, and piloerection was observed at 40 mg/kg. The ratio of PCE to total erythrocytes was unaffected by oxymorphone, indicating no bone marrow toxicity. The number of MPCEs was statistically significantly increased 24 h after dosing in both males and females given 20 mg/kg and males given 40 mg/kg. The number of MPCEs was also higher than controls in males given 10 mg/kg but did not
reach statistical significance. At 48 h after administration of
40 mg/kg the number of MPCEs was similar to controls. Toxico-
kinetic parameters from the rat study are summarized in Table 3.
In mice, administration of 500 mg/kg produced mortality
(2/15 males, 1/15 females). Opioid-related clinical signs, in-
cluding hyperactivity and Straub tail, occurred at all doses. The
ratio of PCEs to total erythrocytes was reduced 19–40% in all
oxymorphone-treated groups, indicating bone marrow toxicity.
A statistically significant increase in MPCEs was observed 24 h
after dosing in females at 250 mg/kg and in both males and
females at 500 mg/kg ($p \leq 0.05$). MPCEs remained significantly
higher than controls 48 h after administration of 500 mg/kg in
males, although this value was within the vehicle historical
control range. The incidence of MPCEs in females given 250
mg/kg was also within the vehicle historical range and thus not
considered of biological significance. Cyclophosphamide in-
duced statistically significant increases in MPCEs in male and
female rats and mice.

**Effects of Oxymorphone Administration on Body
Temperature in Rats and Mice**

Studies were conducted to determine if administration of
oxymorphone at doses associated with increased MPCEs in
male rats (20 and 40 mg/kg) and mice (250 and 500 mg/kg) also
produce changes in body temperature. In rats, a rapid increase in
body temperature occurred at both doses, which was detectable
within 30 min after dosing (Fig. 1). Body temperature remained
near its peak for 4 h, after which it slowly declined to baseline.
Dose-related increases in body temperature also occurred in
male mice after administration of oxymorphone (individual
peak temperature up to 39.5°C), although the effect was more

**TABLE 1**
Results of Initial Micronucleus Studies With Oxymorphone

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Sex</th>
<th>N</th>
<th>24 h Postdose</th>
<th>48 h Postdose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PCE/total</td>
<td>MPCE per 1000 PCEs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>erythrocytes</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (vehicle control)</td>
<td>M</td>
<td>10a</td>
<td>0.663 ± 0.06</td>
<td>0.7 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10a</td>
<td>0.662 ± 0.04</td>
<td>0.7 ± 0.67</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>5</td>
<td>0.649 ± 0.02</td>
<td>1.4 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.663 ± 0.02</td>
<td>0.5 ± 0.61</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>5</td>
<td>0.653 ± 0.04</td>
<td>1.7 ± 0.57*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.685 ± 0.05</td>
<td>1.6 ± 0.65*</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>10ab</td>
<td>0.664 ± 0.04</td>
<td>2.6 ± 0.42*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10ab</td>
<td>0.693 ± 0.04</td>
<td>1.1 ± 0.42</td>
</tr>
<tr>
<td>Cyclophosphamide (40 mg/kg)</td>
<td>M</td>
<td>5</td>
<td>0.599 ± 0.03</td>
<td>29.8 ± 9.26*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.604 ± 0.03</td>
<td>18.5 ± 6.20*</td>
</tr>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (vehicle control)</td>
<td>M</td>
<td>10a</td>
<td>0.523 ± 0.02</td>
<td>0.5 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10a</td>
<td>0.505 ± 0.04</td>
<td>0.4 ± 0.22</td>
</tr>
<tr>
<td>125</td>
<td>M</td>
<td>5</td>
<td>0.413 ± 0.07</td>
<td>0.5 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.353 ± 0.03</td>
<td>0.9 ± 0.22</td>
</tr>
<tr>
<td>250</td>
<td>M</td>
<td>5</td>
<td>0.365 ± 0.02</td>
<td>1.2 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.381 ± 0.05</td>
<td>1.3 ± 0.27*</td>
</tr>
<tr>
<td>500</td>
<td>M</td>
<td>10ab</td>
<td>0.343 ± 0.06</td>
<td>5.4 ± 1.29*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10ab</td>
<td>0.410 ± 0.08</td>
<td>3.8 ± 3.27*</td>
</tr>
<tr>
<td>Cyclophosphamide (40 mg/kg)</td>
<td>M</td>
<td>5</td>
<td>0.271 ± 0.04</td>
<td>28.8 ± 3.27*</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>0.357 ± 0.08</td>
<td>27.8 ± 5.82*</td>
</tr>
</tbody>
</table>

Note. Dashes indicate that MPCEs were not evaluated at 48 h in these groups.
5 Five animals evaluated at each time point.
6 An additional five animals were treated as extras to ensure availability of five animals for evaluation.
* $p \leq 0.05$.

---

**FIG. 1.** Body temperature in rats following a single oral dose of 20 or 40 mg/kg oxymorphone. $N = 5$ (control, 20 mg/kg) or 8 (40 mg/kg).
variable than in rats (Fig. 2). A sharp, transient increase in body temperature also occurred in control mice immediately after dosing, suggesting that handling of these animals produced fluctuations in body temperature and making interpretation difficult in this species. Therefore, all subsequent studies were conducted in rats.

Control of Oxymorphone-Induced Hyperthermia With Antipyretic Agents

Studies were conducted to develop a paradigm to prevent oxymorphone-induced hyperthermia in rats by pretreatment with antipyretic agents. Acetaminophen, naproxen sodium, and sodium salicylate were evaluated using doses that were selected based on information from the published literature. Pretreatment with acetaminophen or naproxen sodium was ineffective in controlling body temperature following oxymorphone administration (data not shown).

Sodium salicylate was tested at doses of 100–500 mg/kg and using pretreatment times of 30–120 min before oxymorphone administration. The effects of sodium salicylate pretreatment on oxymorphone-induced hyperthermia were dependent on both dose (Fig. 3) and pretreatment time. Sodium salicylate was not completely effective in preventing the increase in body temperature. However, at doses ≥ 200 mg/kg, peak body temperature was lower and body temperature returned to baseline more rapidly in animals pretreated with sodium salicylate than in those given oxymorphone alone. At a sodium salicylate dose of 300 mg/kg, a pretreatment time of 60 min provided the most robust and least variable control of body temperature following oxymorphone administration, while at 500 mg/kg, a pretreatment time of 30 min was most effective (data not shown).

Tolerability was also dependent on sodium salicylate dose and pretreatment time. Mortality was increased in groups given 300 mg/kg at 120 min prior to oxymorphone (2/6 died) or 500 mg/kg at 90 or 120 min prior to oxymorphone (5/6 and 4/6 died, respectively, vs. 0–1 animals in groups given oxymorphone alone or using other pretreatment times). Doses and pretreatment times were selected for micronucleus evaluation (300 mg/kg, 60 min, and 500 mg/kg, 30 min) to achieve acceptable control of body temperature with limited mortality.

Evaluation of Micronuclei Following Oxymorphone With and Without Sodium Salicylate Pretreatment

This study was conducted to evaluate the micronucleus response to oxymorphone in male rats (40 mg/kg) when body temperature was controlled using sodium salicylate pretreatment. Body temperature results are presented in Figure 4. After administration of oxymorphone alone, mean body temperature was elevated from 0.5 to 8 h after dosing, with peak mean body temperatures of 39.1–38.8°C between 1 and 4 h postdose. Peak body temperature in individual animals reached as high as 39.7°C, and 4/7 animals had sustained body temperatures of ≥ 39.0°C (i.e., in this range for multiple time points). As previously observed, sodium salicylate pretreatment did not completely prevent the increase in body temperature after oxymorphone administration, but peak body temperature was 0.5–1°C lower and body temperature returned to baseline more quickly (4–6 h) than in the group given oxymorphone alone. No animals pretreated with sodium salicylate had body temperatures > 39.0°C at any time after dosing. Body temperature was
unaffected by the administration of sodium salicylate alone at either dose.

Micronuclei results are presented in Table 2. The ratio of PCEs to total erythrocytes was unaffected with any treatment, indicating no bone marrow toxicity. A statistically significant increase \((p \leq 0.05)\) in the number of MPCEs occurred following administration of oxymorphone alone \((-9\)-fold the vehicle control). The number of MPCEs following oxymorphone administration with sodium salicylate pretreatment at both doses was similar to the vehicle control. The number of MPCEs following sodium salicylate alone was also similar to the vehicle control.

Toxicokinetic parameters are summarized in Table 3. Sodium salicylate pretreatment had no effect on the maximal plasma oxymorphone concentration \((C_{\text{max}})\) or time to maximal plasma concentration \((T_{\text{max}})\). The area-under-the-plasma concentration curve \((\text{AUC}_{0-24\ h})\) was also unaffected by sodium salicylate pretreatment at 300 mg/kg but was \(-20\%\) lower in animals at 500 mg/kg. Plasma concentrations in all groups were within the range found at doses associated with micronuclei in the initial study.

**TABLE 2**

<table>
<thead>
<tr>
<th>Oxy morphine (mg/kg)</th>
<th>Sodium salicylate (mg/kg)</th>
<th>PCE/total erythrocytes (mean ± SD)</th>
<th>MPCE per 1000 PCEs (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.624 ± 0.05 0.4 ± 0.35</td>
<td>0.642 ± 0.04 3.6 ± 1.65*</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>0.625 ± 0.05 0.5 ± 0.45</td>
<td>0.638 ± 0.04 0.4 ± 0.49</td>
</tr>
<tr>
<td>40</td>
<td>300</td>
<td>0.623 ± 0.02 0.4 ± 0.35</td>
<td>0.637 ± 0.03 0.5 ± 0.29</td>
</tr>
<tr>
<td>0</td>
<td>300</td>
<td>0.623 ± 0.02 0.4 ± 0.35</td>
<td>0.637 ± 0.03 0.5 ± 0.29</td>
</tr>
<tr>
<td>0</td>
<td>500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\text{AUC}_{0-24\ h}\) 21.0 33.4 32.0 41.4 33.9

\(T_{\text{max}}\) 1.0 1.0 0.5–1.0\(^a\) 0.5–1.0\(^b\) 0.5–1.0\(^b\)

\(T_{\text{max}}\) 192 343 336 361 269

\(\text{AUC}_{0-24\ h}\) "One of seven animals died in this group.

\(^a\)One of seven animals died in this group.

\(^p \leq 0.05.\)

**DISCUSSION**

The studies reported here show that doses of oxymorphone associated with increased MPCEs in the bone marrow of rats also produce a rapid increase in body temperature and that reduction in the hyperthermic response to oxymorphone by pretreatment of animals with sodium salicylate prevents the increase in MPCEs. These results suggest that the increase in MPCEs following oxymorphone administration occurs secondarily to the increase in body temperature. Body temperature was also elevated in male mice after administration of oxymorphone at doses associated with increased MPCEs, although the effect was more variable than in rats.

Animals were group housed, with bedding, for all studies, which could conceivably affect regulation of body temperature. However, since housing conditions were the same across all dose groups and studies, this was not considered to be a confounding factor in the interpretation of these studies. If anything, it might be expected that group housing would exacerbate the hyperthermic effects of treatment and potentially diminish the effectiveness of antipyretics.

Since a single time point for evaluation of MPCEs was used in these studies \((24\ h\ postdose)\), it is conceivable that sodium salicylate pretreatment may have produced a shift in the kinetics of MPCE formation that went undetected, rather than prevention of MPCE formation. However, this possibility is considered unlikely. The kinetics of MPCE formation \(\text{in vivo}\) have been described for a number of clastogens and are determined primarily by compound pharmacokinetics, bone marrow cytotoxicity \(\text{i.e.},\) effects on cell cycle/proliferation, and mechanism of clastogenicity \(\text{Abramsson-Zetterberg et al., 1996; Morales-Ramirez et al., 1997; Vallarino-Kelly and Morales-Ramirez, 2001.}\) Sodium salicylate pretreatment did not significantly impact the pharmacokinetics of oxymorphone, and there was no evidence of bone marrow toxicity in these studies.

To our knowledge, this is the first demonstration of a link between drug- or chemical-induced hyperthermia and increased MPCEs, as well as the first investigation of high body temperature and micronuclei in rats. The association between environmentally induced hyperthermia and micronuclei in mice has been described in a number of studies. The clastogenic effects of hyperthermia in mice appear to be dependent on both the magnitude and the duration of increased body temperature. Asanami and Shimono \(\text{(1997b)}\) found that a body temperature of \(\geq 39.5^\circ\text{C}\) for at least 30 min was required to produce an increase in MPCEs in mice. In another study, increased MPCEs were found in mice maintained at an ambient temperature.

**TABLE 3**

<table>
<thead>
<tr>
<th>Oxy morphone Toxicokinetic Parameters in Rat Micronucleus Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial micronucleus study</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Oxy morphone (mg/kg)</td>
</tr>
<tr>
<td>Sodium salicylate (mg/kg)</td>
</tr>
<tr>
<td>(C_{\text{max}}) (ng/ml)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
</tr>
<tr>
<td>(\text{AUC}_{0-24\ h}) (ng-h/ml)</td>
</tr>
</tbody>
</table>

\(\text{AUC}_{0-24\ h}\) "The time of maximal mean plasma concentration was 1.0 h in this group. However, there was overlap in individual animal values at 0.5 and 1.0 h. Therefore, \(T_{\text{max}}\) is reported as a range.

\(^a\)The time of maximal mean plasma concentration was 0.5 h in this group. However, there was overlap in individual animal values at 0.5 and 1.0 h. Therefore, \(T_{\text{max}}\) is reported as a range.

\(^b\)The time of maximal mean plasma concentration was 1.0 h in this group. However, there was overlap in individual animal values at 0.5 and 1.0 h. Therefore, \(T_{\text{max}}\) is reported as a range.
temperature of 35–36°C for ≥ 20 h but not for shorter durations (King and Wild, 1983); these conditions produced average increases in body temperature of 1.4°C in females and 1.9°C in males. Lipopolysaccharide induced hyperthermia did not increase MPCEs in mice, which may be attributable to the short duration of the effect, although a high level of bone marrow toxicity was observed with this treatment, which may confound interpretation (King and Wild, 1983). Results of the current studies are consistent with the existence of a threshold hyperthermic response required for increased MPCEs in rats. Pretreatment of animals with sodium salicylate did not completely prevent the hyperthermic response to oxymorphone but reduced both the peak body temperature and duration of hyperthermia, which was sufficient to prevent the increase in MPCEs.

Pretreatment of animals with sodium salicylate increased mortality following oxymorphone administration; this effect was dependent on both dose and pretreatment time. The basis for this is unknown. Interestingly, a similar observation was made in mice provided thermoregulatory support to prevent hypothermia associated with phenol (Spencer et al., 2004). In this case it was hypothesized that hypothermia and hypometabolism are adaptive responses to a toxic insult to reduce lethality (Watkinson and Gordon, 1993), and prevention of this protective mechanism thereby increases toxicity. The relevance of these findings to the increased mortality following prevention of a hyperthermic response is unclear.

Aspirin (sodium acetylsalicylate) has been previously shown to suppress chromosomal aberrations induced by mitomycin C (MMC) in mice (Nikawa et al., 2001). Aspirin was most effective when administered 24 h after MMC, and it was concluded that the prevention of chromosome aberrations by aspirin was related to its ability to scavenge oxygen radicals produced by reactive metabolites of MMC. Oxymorphone is not reactive and is not metabolized to reactive species (Cone et al., 1983), indicating that the preventive effects of sodium salicylate in the studies presented here are likely related to an effect of oxymorphone itself, not its metabolites.

Morphine also increases MPCEs in mice but is not clastogenic in in vitro systems. The effects of morphine in vivo were diminished following daily administration for 7 days (Swain et al., 1980), suggesting the development of tolerance. Morphine-induced increases in MPCEs are partially blocked by naloxone (an opioid antagonist). Morphine does not increase MPCEs in adrenalectomized mice, and the clastogenic effects of plasma from morphine-treated animals in vitro are partially blocked by RU486 (a steroid antagonist) (Sawant et al., 2001). These results suggest that morphine-induced increases in the incidence of MPCEs involve opioid-mediated effects on circulating adrenal corticosteroids. Body temperature was not monitored in these studies, although effects of morphine on body temperature in rats have been described extensively (reviewed in Clark, 1979). Interestingly, the effects of opioids on thermoregulation also involve centrally mediated effects on the hypothalamic-pituitary-adrenal axis, although this effect is likely more dependent on medullary catecholamines than on corticosteroids (Lansberg et al., 1984; Zeisberger, 1998). Adrenalectomy or adrenal demedullation prevent morphine-induced changes in body temperature in rats (Wallenstein, 1982). Thus, the prevention of the morphine-induced increase in MPCEs by adrenalectomy could, in part, be related to prevention of morphine-induced hyperthermia.

In conclusion, the studies presented here provide evidence that increased MPCEs following oxymorphone administration to rats occurs secondary to increased body temperature. Two-year carcinogenicity studies of oxymorphone in Crl:CD (SD) IGS BR rats and CD1 mice have recently been completed, with no evidence of a treatment-related increase in the incidence of any tumors in either species given oxymorphone (Opana Package Insert; manuscript submitted). These results confirm that oxymorphone-related increases in MPCEs following acute administration do not present a risk for carcinogenicity from chronic use.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

ACKNOWLEDGMENTS

The studies presented in this article were sponsored and paid for by Endo Pharmaceuticals Inc. D.L.S. and R.J.G. are employed by Endo Pharmaceuticals Inc.

REFERENCES


