Anesthetic-Induced Corneal Lesions in Developmentally Sensitive Rats

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Developmental critical periods for the induction of abnormalities by exposure to exogenous substances need not be confined to the early embryonic stage of organogenesis. The combination of ketamine hydrochloride and xylazine, two commonly used anesthetic agents, resulted in a corneal epithelial calcium deposition in 84% of rat pups whose exposure was limited to a single injection during the third postnatal week only. Concurrent exposure to ketamine hydrochloride, xylazine, and yohimbine, an alpha2 adrenergic receptor antagonist, resulted in corneal lesions in only 6% of rat pups so exposed. The etiology is presently not understood but may involve interference with neurally directed corneal development. Corneal desiccation may also play a role. Altered drug metabolism, and toxic interactions resulting from a changing oxygen or light milieu are less likely etiologic mechanisms. Aspects of corneal development and mechanisms by which drugs can interact with and disturb normal maturational sequences can now be approached. Invest Ophthalmol Vis Sci 29:949-954, 1988

Developing organisms can be highly sensitive to drugs, environmental chemicals and viruses during very limited periods of development. Deleterious effects result from exposure during that precise developmental period. One classical example of a drug that can induce abnormalities during a limited exposure is the tranquilizing drug thalidomide. Exposure to thalidomide from 21 to 36 days of gestation can lead to phocomelia in human offspring. Most critical periods for drug-induced structural abnormalities in an organism relate to the period of organogenesis. We have identified, however, a much later developmental critical period for the induction of corneal pathology in the rat from combined exposure to ketamine hydrochloride and xylazine.

Ketamine hydrochloride (2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride) is an anesthetic agent that is widely used in veterinary and clinical medicine. The combination of ketamine and the sedative analgesic drug xylazine (2(2,6-dimethylphenylamino)-4OH-5,6-dihydro-1,3-thiazine hydrochloride) is commonly used in laboratory research and veterinary medicine. Most reports on the combined use of the drugs are based on studies in adult organisms. This report presents evidence for a differential effect of ketamine plus xylazine in juvenile vs. adult rats.

Materials and Methods

Animals

Rats (Long Evans, Blue Spruce Farms, Altamont, NY) were housed in supervised quarters with constant temperature (72 ± 2°F) and humidity (60 ± 5%), in a room having a complete air change every 20 min, and with a 12 hr light/dark cycle (lights on at 0600). They were housed in plastic cages with Pine-DRI bedding and received Purina rat chow (5001) and tap water ad libitum. The day of birth was designated as day 0. Litters were culled to 10–12 pups on day 1 and remained with the lactating female until weaning on day 20–21.

Procedures

On the day of injection, pups selected from a minimum of three different litters were injected with a solution of ketamine hydrochloride (12.5 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.) at a final volume of 0.1–0.2 cc/100 g. Following recovery (spontaneous locomotion), they were returned to their home cages. Ketamine hydrochloride (Ketalar) was obtained from Parke-Davis through the hospital pharmacy and xylazine (Rompun) was purchased from Miles Laboratories through Butler Labs (Rochester, NY).

The drugs were administered concurrently to rat pups on postnatal days 13–14 (n = 16), 16–17 (n = 11), 20–21 (n = 29), 23–24 (n = 12), 27–28 (n = 12), or 60–90 (n = 24). Of the 16 rats studied at
13–14 days of age, ten had unopened eyelids, one had eyelids that had opened spontaneously, and five had eyelids that were manually opened 3–5 min after administration of ketamine hydrochloride and xylazine and were deemed sufficiently anesthetized. To evaluate the role that dessication may play in producing the lesion, additional rats at 20–21 days of age (n = 10) also received an ocular lubricant ointment (Duratears; Alcon Laboratories, Inc., Fort Worth, TX) bilaterally throughout the period of combined anesthesia. To assist in defining the etiology of the lesion, several pharmacological studies were conducted, including: ketamine hydrochloride alone (12.5 mg/kg, n = 7; 25 mg/kg, n = 5; or 50 mg/kg, n = 5), xylazine alone (15 mg/kg, n = 6; 30 mg/kg, n = 5; or 60 mg/kg, n = 3), nembutal (25–30 mg/kg, i.p.) (n = 10), or chloropent (3 cc/kg, i.p.; Fort Dodge Laboratories, Inc., Fort Dodge, IA; each cc contains: chloral hydrate 42.5 mg, magnesium sulfate 21.2 mg, pentobarbital 8.86 mg, ethyl alcohol 14.25%, propylene glycol 33.8%, and purified water q.s.) (n = 20). Another cohort of rats 17–19 days old (n = 18) received yohimbine, an alpha2 adrenergic receptor antagonist (2 mg/kg, i.p.), concurrent with or 30 min after ketamine hydrochloride (12.5 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.). All experimental groups included animals from a minimum of three litters and both sexes were equally represented. The eyes of all animals were visualized unmagnified or at X10 magnification three to five times weekly from the day of injection for up to 7 weeks.

**Histologic Studies**

Histologic examination was performed at 10–14 or 50 days after drug exposure. Necropsies were performed in a separate laboratory. A complete necropsy examination included gross dissection and histologic examination of the following tissues: eye and adnexal structures, brain (including pituitary), heart, lung, liver, spleen, gut (stomach, duodenum, jejunum, cecum, colon), pancreas, adrenal, kidney, urinary bladder, mammary gland, thyroid, skeletal muscles, and skin. Tissues were fixed (within 1 min of euthanasia for eyes, within 20 min of euthanasia for other tissues) in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. Additional stains, including Periodic Acid Schiff, Oil Red O, alizarin red-S, and gold chloride (of whole cornea), were used as indicated. Serologic ELISA studies (Microbiological Associates, Inc., Bethesda, MD) for rat coronavirus and sialodacryoadenitis virus were also done on five severely affected animals 50 days after exposure.

An estimate of the partial pressure of oxygen reaching the corneal epithelial cells was made assuming that the oxygen reaching these cells did so solely by diffusion from the limbic blood vessels prior to eye opening. PaO2 in the arterioles approximates 90–100 mm Hg and thus the PaO2 at the cell must be less than 90–100 mm Hg. Once the lids opened, however, oxygen could diffuse from the atmosphere (PO2 approximately 150–170 mm Hg) to the epithelium, providing as much as 170 mm Hg O2.

All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

**Results**

Administration of the combination of ketamine hydrochloride (12.5 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.) to rat pups 13 days of age and older resulted in rapid onset of anesthesia (within 3–5 min). Absence of response to tail pinch lasted 60–90 min in 13–14-day-old pups and 30–40 min in pups 21 days and older. Spontaneous locomotion resumed within 2 hr in the youngest pups and within 30–50 min in the older rats. Ketamine hydrochloride given alone at 12.5 mg/kg resulted in hyperactivity; at 25–50 mg/kg it induced rigidity and tremors. Xylazine given alone at 15–30 mg/kg produced sedation; at 60 mg/kg irreversible respiratory depression occurred. The combination of ketamine hydrochloride (12.5 mg/kg), xylazine (15 mg/kg), and yohimbine (2 mg/kg) (whether yohimbine was given simultaneously with or 30 min following administration of ketamine and xylazine) produced anesthesia similar to that seen when ketamine hydrochloride and xylazine were given without yohimbine. Both nembutal and chloropent resulted in onset of anesthesia within 10–15 min and duration of anesthesia was approximately 3–4 hr.

A corneal epithelial and superficial stromal calcium deposition (Figs. 1, 2) appeared within 7 days of
Fig. 2. Photomicrographs of the cornea in control and ketamine/xylazine-exposed rats. Posterior endothelium (p), corneal stroma (c), and corneal epithelium (a). Bar = 0.05 mm. (A) Normal control cornea devoid of lesions. (B) Mildly affected cornea with numerous clusters of large abundantly vacuolated cells in the corneal epithelium (arrow). (C) Moderately affected cornea with occasional inflammatory cells in the outer portion of the stroma. The overlying corneal epithelium is focally mildly eroded with numerous necrotic cells in the affected areas. There is moderate necrotic debris (n) in the outer portion of the corneal stroma. (D) Severely affected cornea with numerous polymorphonuclear leukocytes and abundant necrotic debris (n) in clefts within the stroma, separating the overlying corneal epithelium. The epithelium, most prominently over the central area, is thickened (small arrows). Same animal as shown in Figure 1.

exposure to the combination of ketamine and xylazine in 84% of pups 20–21 days old at the time of injection, with or without eye lubrication. The lesion did not occur following exposure to either drug alone, when yohimbine was administered either concurrently or 30 min later, nor following either nembutal or chloropent (Table 1). The severity of the lesion ranged from mild (involving central cornea with vacuole formation in the basal cells only, Fig. 2B) to moderate (involving central cornea and extending throughout most of the cornea and a moderate amount of necrosis with inflammatory cells, Fig. 2C) to severe (involving the entire cornea and a severe degree of necrosis with epithelial thickening and the presence of blood vessels, Figs. 1, 2D). The epithelium was primarily affected with only secondary stromal changes in the corneas that were more severely affected. No histologic changes were seen in any non-affected rats. In moderate and severe lesions, alizarin red-S staining revealed calcium deposition in the areas of necrosis, as is seen in band keratopathy type lesions. Periodic Acid Schiff staining was negative, indicating that vacuolization was not due to abnormal glycogen deposition. Gold chloride staining (Fig. 3) of whole cornea revealed neovascularization (low power view, Fig. 3A) in affected rats. High power views (Fig. 3B) revealed similar patterns of corneal innervation in control and affected rats.

Developmentally the rat pup cornea was most sensitive to effects of the drug combination between days 16 and 21 (Table 2). Lesions were detected either grossly or histologically in only 1/48 rats 23 days or older. No 13–14-day-old rat with unopened eyes had corneal lesions; whereas 1/1 rat whose lids opened spontaneously on day 14 and 4/5 rats whose eyelids were manually opened on day 14 developed lesions. These lesions, however, were all characterized as "mild." The range of severity of corneal involvement...
Table 1. Corneal lesions in animals injected at 20-21 days

<table>
<thead>
<tr>
<th>Drug regimen (n)</th>
<th>Unaffected (n)</th>
<th>Unilateral lesions (n)</th>
<th>Bilateral lesions (n)</th>
<th>% Animals affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine + Xylazine (29)</td>
<td>4</td>
<td>4</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>Ketamine + Xylazine + Lubricant</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>Ketamine 12.5 mg/kg (7)</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ketamine 25 mg/kg (5)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ketamine 50 mg/kg (5)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Xylazine 30 mg/kg (5)*</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Xylazine 60 mg/kg (3)†</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ketamine + Xylazine + Yohimbine</td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Nembutal (10)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloropent (20)</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* One died—irreversible respiratory depression; † all three died—irreversible respiratory depression.

was not age-dependent from 16 to 21 days of age. The lesions that developed in animals 13-14 days of age, however, were all mild. The latency to appearance of the lesion was shorter in the 16-17-day-old pups (as early as 2 days following injection) than in the 20-21-day-old pups (lesions first noted 5-7 days following injection). All lesions noted remained stable (ie, did not change in severity) and did not regress during the study period of up to 7 weeks.

No evidence of rat coronavirus or sialodacryadenitis virus was obtained.

Discussion

It is evident from these observations that exposure to the combination of ketamine hydrochloride and xylazine (but to neither alone at dosages up to two to four times those used in combination) during a particular developmental period has a high likelihood of resulting in lasting lesions in the visual axis of the juvenile rat. The corneal lesions were induced at doses considerably lower than those generally used as anesthetic doses in laboratory animals.

The production of corneal lesions by drugs is not unique to exposure to the combination of ketamine and xylazine. Corneal opacities in rodents have been reported to result from exposure to antidepressants, capsaicin, morphine sulfate, and 1-α-acetylmethadol. The described lesions differ histologically to some degree from each other and from the lesion reported here, suggesting that the cornea is a common target organ with a multiplicity of influences on it. All of the drugs reported to induce corneal lesions, however, act on the central nervous system: ketamine, xylazine, and the antidepressants interact with noradrenergic neurons; ketamine may also act at glutamate receptors; capsaicin causes damage to primary sensory afferent neurons whose neurotrans---

Fig. 3. Photomicrographs of a moderately affected cornea stained with gold chloride. (A) Low magnification view demonstrating neovascularization (arrows) extending from limbus to lesion (L). (B) High magnification view demonstrating innervation (arrows). There was no qualitative difference in innervation between control and exposed corneas.
Corneal lesions in animals injected with ketamine and xylazine as a function of age at injection

The observation that, in the present study, lesions ties in animals treated with capsaicin. Second, histo-
during anesthesia in the present study failed to pre-
could be produced in 13-14-day-old pups with
corneal lesions, as suggested by Fabian et al9 and by
vent development of the lesion in 20-21-day-old
pups. Furthermore, despite absence of lubricant in
expression; however, application of an ocular lubricant
may be one factor contributing to the production of
corneal lesions, as suggested by Fabian et al9 and by
the observation that, in the present study, lesions
could be produced in 13-14-day-old pups with
opened eyelids and not in pups whose eyelids
remained closed, it is not the sole etiologic factor. Fa-
bian et al9 could reduce the incidence of corneal opa-
cities by application of warm saline during deep de-
pression; however, application of an ocular lubricant
during anesthesia in the present study failed to pre-
vent development of the lesion in 20-21-day-old
pups. Furthermore, despite absence of lubricant in
rats 23 days of age or older, no lesions developed.
Similarly, Shimizu et al8 demonstrated that tarsorr-
haphy did not prevent development of corneal opac-
ties in animals treated with capsaicin. Second, histo-
logic examination of mildly affected corneas showed
involvement of the epithelial basal cells but not the
outer layer of squamous cells, as would be expected if
desiccation were a critical factor. Third, neither nem-
butal nor chloropent exposure, which resulted in a
longer duration of anesthesia and hence a greater
likelihood of drying of the cornea, resulted in corneal
lesions. Additionally, when yohimbine was given ei-
er simultaneously with or 30 min after the combi-
nation of ketamine and xylazine, corneal lesions did
not develop. Lesions were prevented despite no ap-
parent change in degree or duration of anesthesia.

Direct toxicity to the corneal epithelial cells by the
combination of ketamine and xylazine or their me-
tabolites remains a possibility. At the same dosage on
a per weight basis, ketamine plus xylazine was toxic
(causing irreversible respiratory depression) to a ma-
Jority of rat pups treated at 7 days of age (five of six).
Duration of anesthesia was prolonged in 13-14- vs.
20-21-day-old rats (average 80 min vs. 40 min). If
simply a slower, less efficient rate of metabolism of
the drugs were responsible for the toxicity, a more
severe lesion would have been expected in younger
animals. This was not the case, as 13-14-day-old
pups developed only mild lesions, whereas 16-17- or
20-21-day-old pups developed lesions ranging from
mild to severe.

The limitation of the toxicity to rats 21 days of age
or younger suggests that the cornea itself or its milieu
may be different prior to 23 days. Since the cornea is
an avascular structure, all nutrients, including oxy-
gen, reach the cells by diffusion from the limbic blood
vessels or surrounding environment. The partial
pressure of oxygen reaching the corneal epithelial
cells was estimated to change from a maximum of
approximately 100 mm Hg prior to eye opening to
170 mm Hg following eye opening. Eye opening also
results in a change in the intensity of light to which
the cornea is exposed. In the presence of ketamine
and xylazine (and/or their metabolites), the increased
light intensity may adversely affect the basal epithe-

cular cell layer. However, preliminary studies in which
18-day-old animals were kept in constant darkness

Table 2. Corneal lesions in animals injected with ketamine and xylazine as a function of age at injection

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Total studied (n)</th>
<th>Unaffected (n)</th>
<th>Unilateral lesion (n)</th>
<th>Bilateral lesion (n)</th>
<th>% Animals affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-14 (lids closed)</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(lids open)</td>
<td>6*</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>83</td>
</tr>
<tr>
<td>16-17</td>
<td>19</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>79</td>
</tr>
<tr>
<td>20-21</td>
<td>29</td>
<td>4</td>
<td>4</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>23-24</td>
<td>12</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>27-28</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60-90</td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* One opened spontaneously, five opened manually.
from the time of injection until the time of examination, 6 days later, suggest that this is not the case. All of the injected pups developed moderate to severe bilateral corneal lesions.

How the induction of the corneal lesion is related to mechanisms mediating the anesthetic and analgesic actions of the drugs remains to be determined. Although both ketamine hydrochloride and xylazine interact with monoaminergic neurons, they may do so at different sites. It appears that both drugs are required for production of the corneal lesion since neither drug alone, even at two to four times the initial concentrations, was effective. Yohimbine, an alpha2 adrenergic receptor antagonist, can antagonize the antinociceptive effect of xylazine as well as alter the pharmacologic effects of ketamine. In the present study, although yohimbine, at the dosage chosen, did not appear to significantly alter the anesthetic properties of ketamine hydrochloride plus xylazine, it did prevent the induction of corneal lesions in all but one rat. The drug (ketamine hydrochloride or xylazine)-receptor interaction blocked by yohimbine remains to be elucidated. The limited time period during which single injections of centrally acting drugs can cause a high incidence of corneal lesions in rodents suggests a developmental critical period for the induction of abnormalities. The functional innervation of rodent cornea may be at a crucial stage during the second or third week of postnatal life. Although substance P-like immunoreactive (SPI) fibers are present in the same degree by postnatal day 3 as in adult rats, their function and functional status have not been elucidated. The possible importance of monoaminergic neurons to the development of corneal abnormalities by exposure to exogenous substances need not be confined to the early embryonic stage of organogenesis. Determining the mechanisms underlying the anesthetic-induced pathology will provide valuable information about corneal development that can be applied to other species.

**Key words:** ketamine, xylazine, band keratopathy, neural development, corneal development

**Acknowledgments**

The expert advice of S. Searl and the technical assistance of M. Morgenstern, K. Steinmetz, and L. Johnstone were greatly appreciated.

**References**