CI-744 anesthesia for ophthalmological examination and surgery in monkeys. PAUL L. KAUFMAN AND RUDOLPH HAHNENBERGER.

CI-744 is a new agent for chemical restraint and surgical anesthesia in animals, consisting of equal parts by weight of the cataleptoid agent tiletamine-HCl and the tranquilizer zolazepam-HCl. We have used it in the intramuscular dose of five to ten milligrams per kilogram to anesthetize 110 cynomolgus and 10 vervet monkeys a total of 1,500 times for various ophthalmological procedures. At these dosages its characteristics of action were rapid onset, thirty to fifty minutes of surgical depth anesthesia during which there was excellent muscular relaxation and an absence of ocular movements, a very gradual emergence, and no adverse after effects. In these monkey species, we judge CI-744 superior to phencyclidine or methohexital as a short-acting general anesthetic for most ophthalmological procedures.

CI-744 (Parke-Davis), a new short-acting agent for chemical restraint and surgical anesthesia in animals, is a 1:1 mixture by weight of tiletamine-HCl (CI-634, an arylocycloalkylamine analogue of phencyclidine and ketamine) and zolazepam-HCl (CI-716 or flupyrazol, a nonphenothiazine pyrazolodiazepinone analogue of diazepam) (Fig. 1). The drug is in the clinical investigational stage and studies thus far indicate that it is effective and safe in many primate and nonprimate species.

We report here our experience with CI-744 for ophthalmic examination, medication, and surgery in monkeys.

Materials and methods. The monkey species used were cynomolgus (Macaca fascicularis) and vervet (Cercopithecus ethiops) of both sexes, weighing 1.3 to 5.8 kilograms. The procedures to be performed were direct ophthalmoscopy, slit lamp examination or photography, gonioscopy or goniphotography, Goldmann applanation tonometry, topical drug application, corneal tissue removal, and total iridectomy. For all procedures except topical treatment and ophthalmoscopy, the anesthetized monkey was to be placed in a head-holder, supine at the operating microscope or prone at the slit lamp. Lid retraction for iridectomy and topical drug treatment was to be by lid speculum, and for the other procedures by finger tips or toothed forceps.

We did not intend to define a precise dose-response relationship for CI-744 anesthesia, but rather to determine adequate and safe dosages for the above procedures. We therefore first conducted two trial series of experiments:

Trial series A. Fifteen vervets and nine cynomolgus were divided into three groups, each consisting of five vervets and three cynomolgus.

CI-744 (5 mg. per milliliter in 0.9 per cent NaCl) was injected intramuscularly, the groups receiving, respectively, 3.2, 4.0, and 5.0 mg. per kilogram. The monkeys could be safely handled without gloves within a few minutes (time not precisely noted) after receiving any of these doses. The monkey was then placed prone in a head-holder and applanation tonometry performed with a slit lamp mounted minified Goldmann tonometer every 90 seconds, alternating right and left eyes, and using topical benoxinate-HCl anesthesia (0.4 per cent Novesine) and digital retraction of the lids. The length of time from injection during which the monkey tolerated tonometry without resistance was noted, up to a maximum of 25 minutes.

Trial series B. Sixteen cynomolgus were divided into four groups of four monkeys each. CI-744 (2.5, 5.0, 10.0, and 20.0 mg. per milliliter in H₂O) was injected intramuscularly, the groups receiving, respectively, 2.5, 5.0, 10.0, and 20.0 mg. per kilogram (1 ml. per kilogram). When the monkeys could be safely handled without gloves, they were placed supine in a head-holder and subjected to the following sequence of stimuli: tonometry (Draeger) with digital retraction of the lids, gonioscopy, pinching the upper lid with a toothed utility forceps, pinching the conjunctiva just temporal to the limbus with a toothed utility forceps, inserting and removing a lid speculum, depressing the globe with a muscle hook, and grasping the superior rectus tendon with a toothed utility forceps. Topical anesthesia was not employed. The sequence was repeated at approximately four-minute intervals, alternating right and left eye, until the monkey no longer tolerated any of the stimuli and objected to being in the head-holder. Spontaneous movements and the state of muscular relaxation were also noted.

Fig. 1. Components of CI-744. CI-744 is a 1:1 mixture by weight of tiletamine-HCl (CI-634) and zolazepam-HCl (CI-716).
Table I. Dose-response relationship for CI-744 anesthesia for ocular manipulations

| CI-744 dose (mg./Kg. IM) | No. monkeys | Head-holder | Tonometry | Gonioscopy | Lid pinch | Conj. pinch | Lid speculum | Globe depression
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<td></td>
<td></td>
<td>Minutes (mean ± S.D.) procedure was tolerated after CI-744 injection</td>
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<td></td>
<td>rectus pinch</td>
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<tr>
<td>2.5</td>
<td>4</td>
<td>25.3 ± 7.0</td>
<td>20.0 ± 7.4</td>
<td>20.0 ± 7.4</td>
<td>13.5 ± 9.2</td>
<td>13.0 ± 8.9</td>
<td>12.5 ± 8.7</td>
<td>11.8 ± 8.4</td>
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<tr>
<td>5.0</td>
<td>4</td>
<td>42.8 ± 18.0</td>
<td>40.0 ± 12.8</td>
<td>37.0 ± 9.3</td>
<td>31.8 ± 7.5</td>
<td>34.3 ± 10.1</td>
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<tr>
<td>10.0</td>
<td>4</td>
<td>87.3 ± 9.0</td>
<td>86.0 ± 9.6</td>
<td>78.0 ± 18.3</td>
<td>70.0 ± 18.1</td>
<td>72.3 ± 19.8</td>
<td>74.8 ± 15.6</td>
<td>73.5 ± 17.6</td>
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<tr>
<td>20.0</td>
<td>4</td>
<td>136.8 ± 13.4</td>
<td>136.8 ± 13.4</td>
<td>132.5 ± 8.6</td>
<td>125.8 ± 4.5</td>
<td>128.8 ± 5.9</td>
<td>127.3 ± 6.8</td>
<td>133.4 ± 12.2</td>
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Sequence of stimuli repeated approximately every four minutes on each animal. End-point for each stimulus taken as... spontaneous movements of the limbs which occurred as an early sign of emergence from the influence of the drug (see text).

Main series. Based on these experiments, approximately 110 cynomolgus and 10 vervets were anesthetized with CI-744 a total of approximately 1,500 times for one or more of the procedures initially described. Seven cynomolgus were anesthetized four times within 24 hours, 40 cynomolgus were anesthetized daily for three to four weeks, and ten cynomolgus four to six weeks weekly for six weeks. Most of the 120 monkeys were anesthetized with CI-744 a total of approximately 1,500 times for one or more of the procedures not precise.

Results. Trial series A. At the 4 and 5 mg. per kilogram doses, all the monkeys tolerated the head-holder and tonometry for the full 25 minutes after intramuscular administration of CI-744. At the 3.2 mg. per kilogram dose, two monkeys tolerated the procedure for 25 minutes, two for 19.5 minutes, and four for 16.5 minutes. There were no obvious differences in response between the vervets and the cynomolgus.

Trial series B. The earliest time after injection when the monkeys could be handled without gloves (i.e., they made no attempt to bite or scratch, although they were not completely immobile), was 7.9 ± 2.6 minutes (mean ± S.D.) for the 2.5 mg. per kilogram dose, 3.5 ± 0.6 minutes for the 5 mg. per kilogram dose, and less than 2.5 minutes for the 10 and 20 mg. per kilogram doses (times not recorded exactly for the two larger doses, but all animals receiving these doses could be safely handled within 2.5 minutes). Table I gives the length of time after CI-744 injection that the monkeys tolerated each of the procedures. The duration of anesthesia, as measured by these criteria, increased with increasing dosage. Good muscular relaxation was not achieved with the 2.5 mg. per kilogram dose in any monkey. With 5, 10, and 20 mg. per kilogram, all monkeys were completely flaccid and immobile. Muscular relaxation and immobility did not last as long as tolerance to the various ocular stimuli.

No monkey in Trial series A or B showed any obvious ill effects from CI-744.

Main series. Induction was smooth and the monkeys could be safely handled within 1.5 to 4 minutes after intramuscular injection of 5 to 10 mg. per kilogram of CI-744. In only a few instances were more than four minutes required. Surgical depth anesthesia (defined here as anesthesia adequate for all the procedures described without the monkey objecting) was reached within three to six minutes after injection, and lasted between 30 and 50 minutes. The monkey then began very gradually to awaken, but, if necessary a second intramuscular injection, usually given arbitrarily as approximately 4 to 5 mg. per kilogram (dosage not precise), quickly restored surgical-depth anesthesia for at least another 30 minutes. Reinjection when the animal first showed signs of emerging could be repeated several times; the emergence form anesthesia was so gradual that whatever procedure was being performed was not disrupted and did not need to be interrupted. In some cases where we knew that one to two hours of anesthesia would be needed, we arbitrarily gave approximately 4 to 5 mg. per kilogram every 30 minutes even though the monkey was still deeply anesthetized, and avoided incipient emergence completely.

Muscular relaxation was excellent; the animals were completely limp and there were no movements of the extremities, trunk, or head. Mild salivation always occurred, but, since the swallowing reflex was present, posed no problems even with the monkey supine. The laryngeal reflex was present, but coughing or choking never occurred and it was never necessary to suction the pharynx or administer atropine.

Emergence from anesthesia was gradual and the monkeys appeared completely normal (normal meaning that the monkey was fully alert and active in its cage and one could not tell it had been anesthetized) within about four hours after...
drug injection. There were no anesthetic-related adverse episodes or deaths, and no clinical evidence of injury or infection at the injection sites.

During surgical-depth anesthesia, the eyes were sometimes open and the spontaneous, cornental, and palpebral blink reflexes were sometimes present (even though the monkeys showed no movements or tension of muscles during the ocular manipulations or during vigorous pinching with toothed forceps of the tongue, nipples, or skin of the face, fingers, toes, chest, or abdomen). This was more common at lower dosages and late after injection, suggesting a lighter level of surgical anesthesia. However, blinking was easily prevented by the lid speculum, gonioscopy lens, or forceps or digital retraction of the lids, manipulations which were necessary anyway to perform the procedure at hand. Tonometry and gonioscopy were usually performed as easily without topical anesthesia as with it. The eyes usually remained stationary in the primary position, although conjugate horizontal and/or vertical deviations occurred transiently in a few animals. Conjugate vertical nystagmus nearly always occurred during induction, but never during surgical-depth anesthesia. Pupillary diameter was 5 to 6 mm. in dim light, pupils reacted grossly normally to light.

We noted no obvious differences between the cynomolgus and the vervets in response to CI-744 in doses of 5 to 10 mg. per kilogram, although of course we had relatively few vervets.

The ten cynomolgus anesthetized four to six times weekly for six weeks permitted evaluation of the development of tolerance to the drug. These monkeys, all of which weighed around 2 kilograms, were all subjected to identical stimuli during each anesthetization; insertion of lid specula and topical drop application bilaterally simultaneously. A head-holder was not employed. The parameter noted was whether the monkeys would tolerate this without protesting. Initially, the monkeys received precisely 5.0 mg. per kilogram. Over succeeding weeks, this dosage became insufficient in all the monkeys, and each monkey had its dosage increased by approximately 1.25 mg. per kilogram when it objected to the manipulations. Successive 1.25 mg. per kilogram increments were required in all monkeys over the ensuing weeks, so that by the end of six weeks, all the monkeys required 10 to 12.5 mg. per kilogram. The weights of the monkeys decreased by about 10 per cent during this period; whether this was related to the repeated CI-744 administration or to systemic effects of the topical echothiophate iodide they were receiving we cannot say.

The drug powder was stored at room temperature in a brown glass bottle for over a year, and the 1 per cent aqueous solutions were kept at room temperature in brown glass or translucent polyethylene bottles for several weeks. No loss of potency was evident.

Discussion. It is not the purpose of this report to provide a precise, quantitative description of CI-744 anesthetics in monkeys in terms of dose-response relationships or tolerance. Nor do we wish to explore the question, arising in regard to cataleptoid agents of the phencyclidine family, of whether and at what dosage this drug produces complete analgesia. These issues are under consideration by other investigators. We merely wish to bring to the attention of vision researchers a short-acting anesthetic agent we have found to be safe and effective for ophthalmological examination, medical treatment, and surgery in two monkey species, and which we believe has advantages over other short-acting anesthetics commonly used for these purposes. We subjectively judge CI-744 superior to both intramuscular phencyclidine-HCl, 1 to 2 mg. per kilogram, and methohexital sodium (Brietal, Lilly; a short-acting barbiturate), 15 to 20 mg. per kilogram, for most of these manipulations, having had extensive experience with all three drugs. The muscular rigidity, the head, trunk, and limb movements, and the staccato vertical and horizontal eye movements which occur during surgical depth phencyclidine catalepsy are absent with CI-744. Pupillary diameter fluctuates less under CI-744. Induction is more rapid with CI-744 than with phencyclidine, and the duration of surgical depth anesthesia following a single intramuscular dose (5 to 10 mg. per kilogram CI-744 vs. 1 to 2 mg. per kilogram phencyclidine) is comparable. Should the animal begin to emerge during a procedure, deep anesthesia can be restored by a second intramuscular injection much more rapidly with CI-744 than with phencyclidine. We have had only limited experience with ketamine in these monkey species, but compared to CI-744 it would seem to have drawbacks similar to phencyclidine.

With methohexital, induction is as rapid as with CI-744, but not nearly so smooth. The monkeys often twitch, convulse, or thrash about, and coughing or vomiting are not unusual. Duration of surgical-depth anesthesia is about the same for both agents at the intramuscular doses indicated (CI-744, 5 to 10 mg. per kilogram; methohexital, 15 to 20 mg. per kilogram) but emergence is much more rapid under methohexital and we have had procedures under methohexital disrupted because of this. Moreover, methohexital has the major drawback of causing frequent conjugate horizontal and vertical ocular deviations of large amplitude, which can make ophthalmoscopy, biomicroscopy, gonioscopy, tonometry, corneal suture removal, and anterior segment photography very frustrating experiences. This problem rarely occurs with CI-744.

For the purposes indicated, CI-744 in an intra-
muscular dose of 5 to 10 mg per kilogram has proved excellent and safe. Our current practice, for procedures requiring more than just a few minutes of chemical restraint, is to give 10 mg per kilogram intramuscularly, and supplement it as needed with 5 mg per kilogram intramuscularly. Larger doses provide longer anesthesia, but we have had enough experience with intramuscular doses greater than 10 mg per kilogram to properly evaluate their safety. The drug can also be given intravenously1 and, by either route, may prove suitable for ophthalmologic work requiring several hours of surgical-depth anesthesia.

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REFERENCES

Electron microscopic examinations of a conjunctival squamous cell carcinoma before and during beta-irradiation. H. Klug and P. Lommatzsch. With the technical assistance of B. Macer

An electron microscopic study was performed on a conjunctival squamous cell carcinoma in an 82-year-old man. Biopsies were taken before and during beta-irradiation and the morphologic features of nonirradiated tumor cells were compared to those after application of 10,000 rad. There occur striking changes in the nucleus and the cytoplasm after irradiation. The nuclei show hypertrophic and electron-dense nucleoli as well as altered chromatin structures. The cytoplasm of many cells appears destroyed and contains numerous vacuole-like structures, as well as swollen mitochondria. It is of interest that numerous normal lymphoid cells were present within the irradiated tumor.

Although the ultrastructural features of many malignant tumors have been investigated, little is known about the rare squamous cell carcinoma of the conjunctiva, and electron microscopic examinations have not yet been performed. Furthermore, the rapid destruction of the tumor after irradiation with high doses of beta-rays should be of interest because the morphologic changes may explain the tumor-destroying effect of beta-rays.

We have examined the submicroscopic features of both, nonirradiated tumor cells and irradiated ones from a patient with a conjunctival squamous cell carcinoma.

Case report. The patient was an 82-year-old male who had an inflammatory disease of his right eye for half a year. He was sent to our hospital because of an enlarged red, glassy tumor which involved the temporal side of the bulbar conjunctiva, and partly covered the cornea (Fig. 1, A). The tumor was supplied by large vessels from the temporal conjunctiva. The visual acuity was 6/6 in both eyes with correction. Aside from an incipient senile cataract in both eyes, no other ocular abnormality was found. A malignant neoplasm of the conjunctiva had to be considered. We used radiotherapy for treatment in this elderly patient. Before beta-irradiation the clinical diagnosis was proved by biopsy.

Radiation source and exposure data. The tumor