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A Steroidal Analgesic. (31000)

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A new conceptus concerning the structure-pharmacologic activity relationship in the central nervous system has led to the synthesis of a new class of analgesics having poly lower alkoxy estrane structures(1). These compounds, represented by *d* 2,3,4-trimethoxyestra-1,3,5(10)-triene-17β-ol (Fig. 1)*, designated MP-2001, are potent analgesics. At this time we wish to report on the pharmacology and metabolism of MP-2001.

Materials and methods. Pharmacology. All studies were carried out in propylene glycol solutions. The intravenous LD_{50} of MP-2001 was determined in Swiss-Webster mice and Wistar strain rats, utilizing the method of Bliss(2).

The drug was injected at the manually controlled rate of 1 ml/min. The oral LD_{50} was determined in mice fasted for 18 hours prior to administration of the MP-2001. Range finding studies to determine acute toxicity were also carried out in rabbits, guinea pigs, and mongrel dogs. Analgesia was studied in rats utilizing the "rat tail flick" method of D'Amour and Smith(3), as modified by Baeder. The estrogenic activity of MP-2001 was studied according to the method of Allen and Doisy(4). MP-2001 was studied for effects on blood pressure, respiration, and electro-

* M. P. 131.5-132.5°C; $[\alpha]_D^{27}$ + 74 $CHCl_3$; λ_{max}^{MeOH} 280 (E, 1857).

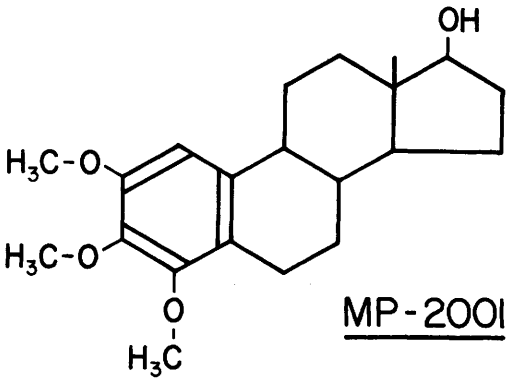

FIG. 1. *d* 2,3,4-trimethoxyestra-1,3,5(10)-triene-17β-ol

cardiogram in anesthetized dogs and unanesthetized rats. The dogs were anesthetized with 35 mg pentobarbital Na/kg intravenously. All recordings were made on a Sanborn model 350. Blood pressure was recorded *via* the cannulated femoral artery. Respiration was recorded from the cannulated trachea. The electrocardiogram was standard Lead II. The compounds were administered by the cannulated femoral vein. In the rat study, the blood pressure, respiration, and electrocardiogram, were monitored with the Physiograph system. All drugs were administered for these tests subcutaneously. The subacute toxicity of MP-2001 was studied in dogs and rats. There were 5 treatment groups: Group I were untreated controls; Group II received a volume of propylene glycol equivalent to the highest dose of drug; Group III were administered 0.5 mg MP-2001/kg/day; Group IV, 1.5 mg/kg/day, and Group V, 4.0 mg/kg/day. The drug was administered for 12 consecutive days. In each group of rats there were 10 males and 10 females; in each group of dogs, 4 and 4. Prior to the start of experiment, and at the end of each treatment period, all animals had a complete hematological examination consisting of a red blood cell count, white blood cell count, 200 cell differential count, hemoglobin determination, and a blood clotting time by the Lee-White technique(5). In addition, determinations were made of the blood urea nitrogen(6), the PSP excretion pattern(7), and routine urinalysis. Liver function tests carried out were BSP retention(8), SGOT, and SGPT levels(9), and serum alkaline phosphatase(10). At the end of the experiment all animals were sacrificed by intravenous administration of pentobarbital sodium and exsanguination. The animals were autopsied and sections of organs obtained for histological examination.

Metabolism. Tritiated DL MP-2001 in the 8, 9 position was prepared in stable form with a specific activity of 229 millicuries per milligram. Three ml of a normal saline solution containing 0.68 curie (3 mg) was injected intravenously into a 12.5 kilo dog. Separate 24-hour urine specimens were collected in a metabolic cage. An aliquot of each urine was dried and counted in a Packard Tricarb Scintillation Spectrometer. The urines were then treated by solvolysis for sulfate conjugates and then Ketodase (β-glucuronidase) for glucosiduronate conjugates. The total lipid-solubles extracted from the urine were chromatographed on Whatman No. 1 filter paper. The solvent system used was methylcyclohexane-1,2 propanediol (diluted 1:1 with methanol v/v). The dried chromatograms were then scanned in an Atomic Accessories Paper Chromatogram Scanner.

Results. The acute intravenous LD_{50} of MP-2001 in mice was found to be 175 mg/kg $\pm$ 28.6 mg/kg. The oral LD_{50} in mice was greater than 500 mg/kg. The intravenous LD_{50} in rats was 193.9 mg/kg $\pm$ 31.4 mg/kg. Range finding studies in rabbits, guinea pigs, and mongrel dogs indicated that the LD_{50} was greater than 50 mg/kg.

Analgesic studies. MP-2001 was compared to other analgesics for effect on the "rat tail flick" test. The analgesic $dose_{50}$ of the comparative drugs is found in Table I. The AD_{50}s of the drugs in mg/kg are higher than that used clinically; however, they do rank in the same potency order as is found in

TABLE I. Effect of Various Analgesics on the "Rat Tail Flick Test".

Compound	Route of admin	AD_{50} (mg/kg)
Aspirin	P.O.	350
Codeine sulfate	I.P.	32
Ethoheptazine HCl	I.P.	34
Meperidine HCl	I.P.	16
Morphine sulfate	I.P.	4.0
MP-2001	I.P.	.1

P.O. = oral.
I.P. = intraperitoneal.

clinical usage. Doses of 3-5 mg MP-2001/kg were administered intravenously to cats and dogs. The animals did not exhibit any marked sedation or ataxia. In particular, it should be noted the cats did not exhibit any maniacal behavior. Following administration of 3-5 mg MP-2001 to the cats and dogs it was possible to perform abdominal surgery without further medication. Upon completion of the operation the animals were released and had an uneventful recovery.

Estrogenic activity. In the study for estrogenic activity MP-2001 was compared to estrone and estradiol-17β. The effective dose$_{50}$ for estrone was 4.9×10^{-2} μg/mouse; for estradiol, 2.4×10^{-2} μg/mouse. MP-2001 in doses up to 50×10^{-2} μg/mouse did not produce any detectable estrogenic effects.

Effects of MP-2001 on blood pressure, respiration, and electrocardiogram. In dogs that were administered MP-2001 in doses from 1 to 10 mg/kg intravenously, no demonstrable effect on blood pressure or EKG was noticed. Neither rate nor volume of respiration in the anesthetized dogs was affected by the administration of the doses of drugs used. MP-2001 in the doses administered did not block the response to epinephrine, norepinephrine, or acetylcholine. MP-2001 at doses of 5 mg/kg and higher potentiated slightly the pressor response to norepinephrine. At doses of 0.5-1.5 mg/kg, MP-2001 did not have any effect on the vital signs during 3 hours of study. At doses of 2-5 mg/kg, the MP-2001 produced a gradual 1½- to 3-hour reduction in blood pressure. Systolic pressure was as low as 90 mm Hg. Pulse pressure was not diminished, cardiac rate was unchanged, nor was there a change in rate and volume of respiration. In studies in human volunteers receiving up to 1 mg/kg intravenously, Dodd reported no effect on the blood pressure of the patients(11).

Subacute toxicity. Gross and histopathological examination of heart, lung, liver, kidney, spleen, digestive glands, and the lymphoid tissues, showed no evidence of injury which could be attributed to MP-2001. Liver sections showed similar amounts of cell vacuolization and granularity in treated and control animals. Kidney sections from some rats that received MP-2001, and from vehicle control rats receiving only propylene glycol, show an alteration of the distal nephron. This consisted of vacuolization of epithelial cytoplasm and pyknosis of nuclei in the segment of the distal convolution just proximal to the collecting tubicle, and was noted in the sections just above the base of the papillae.

TABLE II. Epithelial Changes in Distal Nephron.

Treatment	Involved/total
None	0/8
Vehicle	8/9
MP-2001 0.5 mg/kg	1/10*
MP-2001 1.5 "	0/10
MP-2001 4.0 "	4/10

* Questionable.

The distribution of renal pathology described is shown in Table II. The kidneys of the dogs did not show this alteration. The adrenal glands, thyroid, and pituitary sections showed no effect of treatment. There was no evidence of thyroid atrophy in any group.

Bone marrow sections of the rats and dogs revealed erythropoiesis and myelopoiesis to be active to somewhat hyperactive in a few animals in the high dose range and vehicle control animals. There was no evidence of marrow aplasia or hypoplasia, or of leukemic change, in any of the animals. The mean activities were regarded as within the normal range.

Hematological examination of the rats and dogs revealed no effect from the doses of the drugs administered. Bromsulphalein excretion was normal for all dogs before and after treatment. The glutamic oxalacetic transaminase levels of all the dogs, both control and treatment, were elevated at the end of the tests. At autopsy the dogs were shown to have "heart worms" which could possibly account for the elevated transaminase.

Excretion. In the 72 hours of urine collection, 0.6 of a curie was found representing 88.2% of the injected dose $\pm$ 5% due to the high specific activity of the materials, and small losses of urine during various manipulations. Of the activity found in the urine during the first 72 hours, 97.4% was found in the first 24 hours, 1.9% in the second 24

hours, and 0.7% in the last 24 hours. Since the dog is also known to excrete steroidal compounds by means of entero-hepatic route (feces) at about 20% of an injected dose of drug, it is probable that within the first 72 hours over 95% of the injected dose is excreted, and of that percentage, over 98% is excreted during the first 24 hours.

The chromatographic analysis shows that MP-2001 is metabolized to at least 5 general categories, all of the same general structure as MP-2001, and all maintaining the trimethoxy group.

Discussion. The effects of steroids on the central nervous system were first reported by Selye(12). He later reported(13) that varieties of steroidal structures possess anesthetic properties in the fish and rat. Both Cortisone and ACTH have been reported to produce euphoria, alertness, and increased activity, and withdrawal produced depression(14). MP-2001 produces an analgesic state without sedation, or ataxia, and in this regard is markedly different from the anesthetic effects produced by other steroids. MP-2001 had been administered to human patients either for the control of post-operative pain or chronic pain due to malignancy. In doses up to 0.5 mg/kg intravenously, the drug produced a level of analgesia equivalent to 50-75 mg Meperidine. The drug produced no respiratory depression, sedation, or signs of toxicity(11,15). The hypotension produced in the rats was not detected in man. In the subacute toxicity study, MP-2001 was without toxicity. This was not completely unexpected as indicated in the structure and metabolism studies the 4 position was already blocked out with the methoxy group. The type of metabolites of MP-2001 is not known to produce toxicity. The effect of the propylene glycol in the rat is in agreement with the earlier literature(16). The data do not indicate the MP-2001 exerted a protective effect against the kidney lesion.

Summary. MP-2001, a lower poly alkoxy derivative of estradiol, exhibits analgesic activity in a variety of animal species, including man. MP-2001 does not possess estrogenic activity. It is rapidly excreted from the body without radical change of the basic steroidal nucleus.

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