Laboratory Report

Anesthesiology
50:350–352, 1979

Porphyrin-inducing Activity of Alfaxolone and Alfadolone Acetate in Chick Embryo Liver Cells

Peter W. F. Fischer, Ph.D., Aida Ferizovic, B.Sc.,† Ian R. Neilson, B.Sc.,† Gerald S. Marks, D.Phil.‡*

The two steroid components of Alfathesin®, alfaxolone and alfadolone acetate, have been tested for porphyrin-inducing activity in chick embryo liver cell culture and for hepatic ALA-synthetase-inducing activity in the 17-day-old chick embryo. In cell culture alfoxolone was shown to have potency comparable to that of thiopental, while alfadolone acetate had low potency. In the 17-day-old chick embryo alfaxolone has a third the potency of thiopental; alfadolone acetate showed low potency. The authors conclude that an induction dose of Alfathesin® would be less likely than a comparable dose of thiopental to increase ALA-synthetase activity in a patient with hereditary hepatic porphyria. (Key words: Anesthetics, intravenous: steriod; thiopental; porphyria.)

* Professional Assistant.
† Graduate Student.
‡ Professor and Head.

Received from the Department of Pharmacology, Queen's University, Kingston, Ontario, Canada. Accepted for publication July 17, 1978. Supported by the Medical Research Council of Canada.

Address reprint requests to Dr. Marks, Department of Pharmacology, Queen's University, Kingston, Canada K7L 3N6.

THE NEED to choose a suitable drug for induction of anesthesia for patients who have hereditary hepatic porphyria sometimes confronts the anesthetist.[1] It was thus of interest to study the porphyrin-inducing activity of the new steroid anesthetic Alfathesin® in two well-known screening procedures, the chick embryo liver

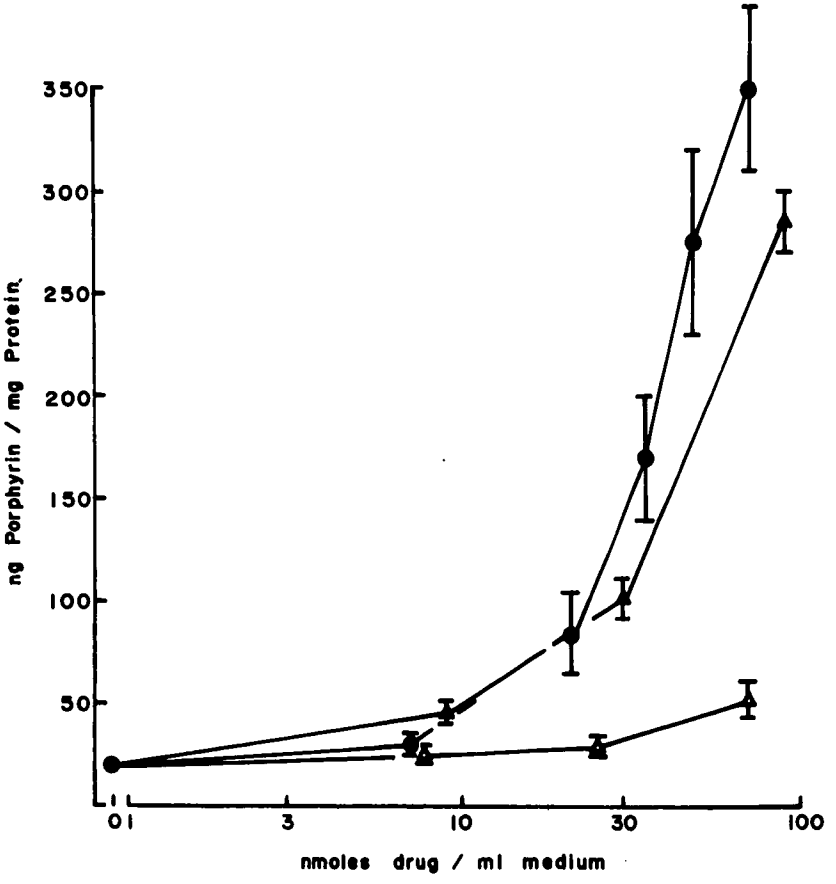

FIG. 1. Porphyrin accumulation in the cells and medium 24 hours after administration of increasing concentrations of AIA (●), alfaxolone (▲), and alfadolone acetate (△). The points represent the means of at least four determinations ± standard error.

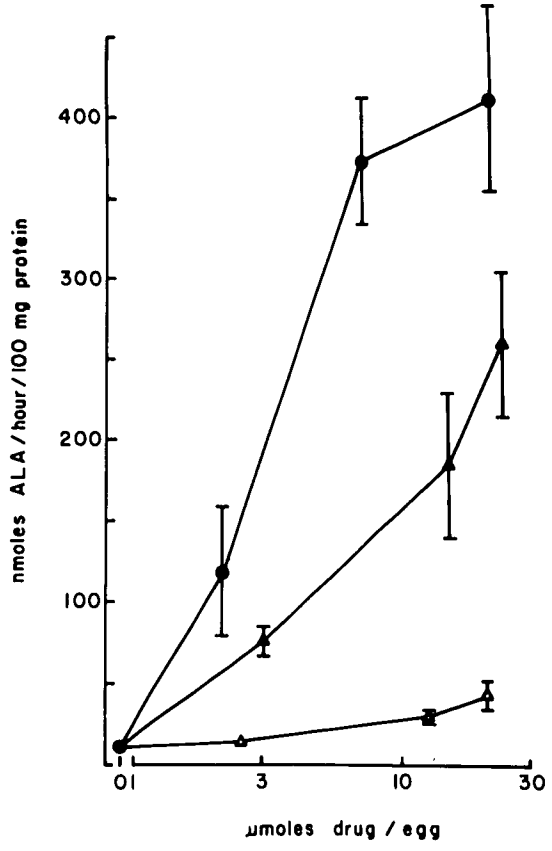

FIG. 2. δ-Aminolevulinic acid synthetase activity in 18-day-old chick embryo livers six hours after injection of increasing concentrations of AIA (●), alfaxolone (▲), and alfadolone acetate (△). The points represent the means of at least five determinations ± standard error.

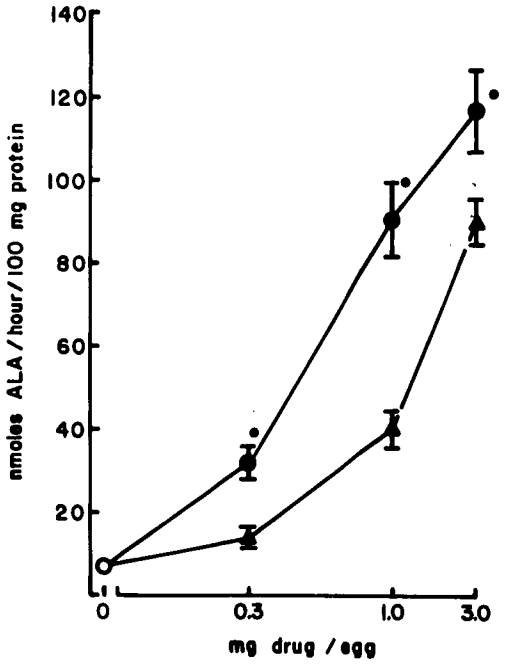

FIG. 3. δ-Aminolevulinic acid synthetase activity in 18-day-old chick embryo livers six hours after injection of increasing concentrations of thiopental (●) and alfaxolone (▲). The dots indicate significant differences ($P \leq 0.05$) between thiopental and alfaxolone. The points represent the means of at least seven determinations ± standard error.

cell culture[2] and the 17-day-old chick embryo.[3] Alfathesin consists of two steroids, alfaxolone and alfadolone acetate, in a 3:1 ratio, dissolved in polyoxyethylated castor oil (Cremophor EL®).[4] Alfaxolone is the principal anesthetic agent, and alfadolone acetate, which has half the anesthetic potency of alfaxolone, is added to enhance the solubility of alfaxolone. Each steroid component was tested separately.

Methods

The method used for investigating drug-induced porphyrin accumulation in chick embryo liver cells[5] is a modification of the procedure of Granick.[2] The steroids (1.5–100 μg) were dissolved in 10 μl of 95 per cent ethanol for addition to the cell culture medium (5 ml). Porphyrin accumulation was determined 24 hr after the addition of steroids by the procedure of Granick,[2] and was expressed as ng porphyrin formed per mg cellular protein. The procedure used for determining hepatic δ-aminolevulinic acid (ALA) synthetase activity in 17-day-old chick embryos was that of Racz and

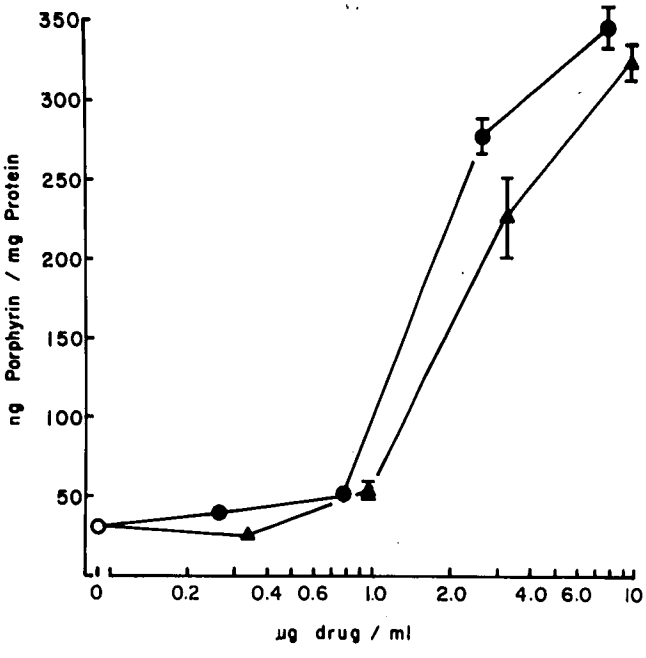

FIG. 4. Porphyrin accumulation in the cells and medium 24 hours after administration of increasing concentrations of thiopental (●) and alfaxolone (▲). The differences between the two drugs were not significant ($P \leq 0.05$) at any concentration. The points represent the means of at least four determinations ± standard error.

Marks.[6] Steroids (0.3–7 mg) were dissolved in 0.1 ml of dimethylsulfoxide, and injected through the chorioallantois into the fluids surrounding the embryo; hepatic ALA-synthetase activity was determined six hours later. This activity was expressed as nmol ALA formed per hour per 100 mg liver protein. Liver and cellular proteins were determined by the method of Lowry *et al.*[7] Neither the 95 per cent ethanol nor the dimethylsulfoxide had any effect on porphyrin biosynthesis or on ALA-synthetase activity.

Results and Discussion

Alfadolone acetate has low potency as a porphyrin-inducing agent in chick embryo liver cells when compared with a standard porphyrin-inducing chemical, allylisopropylacetamide (AIA), while alfaxolone has a potency comparable to that of AIA (fig. 1). The hepatic ALA-synthetase-inducing activities of alfaxolone, alfadolone acetate, and AIA in the 17-day-old chick embryo differ (fig. 2). Alfadolone acetate has very low potency, while alfaxolone has a potency intermediate between those of AIA and alfadolone acetate. Parikh and Moore[8] injected Alfathesin, 12 mg/kg, intraperitoneally daily into rats for four consecutive days and demonstrated a 2.5-fold increase in levels of hepatic ALA-sythetase. It was unclear from their study which of the steroids was responsible for the activity. Our study indicates that it is alfaxolone. Until recently it was believed that 5β-steroids are potent porphyrin-inducing agents while 5α-steroids have a much lower potency.[9] Recent studies have failed to substantiate this presumed difference.[10] That alfaxolone, a steroid with a 5α-configuration, is a potent porphyrin-inducing drug is in agreement with these recent findings.

From a clinical point of view it is important to compare the effects of alfaxolone on the heme biosynthetic pathway with those of thiopental. This follows from the fact that these two drugs are used for comparable anesthetic purposes and thiopental is known to precipitate attacks of hepatic porphyria in patients who have the latent genetic disease.[1] Thiopental, 0.3 mg/egg, injected into the 18-day-old chick embryo produces an increase in ALA-synthetase activity comparable to that produced by alfaxolone, 1 mg/egg (fig. 3). Similarly, thiopental, 1 mg/egg, produces an effect comparable to that produced by alfaxolone, 3 mg/egg. On this basis, alfaxolone can be judged to have approximately a third the potency of thiopental. Alfaxolone has a potency comparable to that of thiopental in chick embryo liver cell culture (fig. 4). Since the dosage of alfaxolone used as an induction anesthetic in man (47.3 mg/70 kg) is considerably less than that of thiopental (350 mg/70 kg), the data indicate that alfaxolone in therapeutic doses is less likely than comparable anesthetic doses of thiopental to increase ALA-synthetase activity in a patient who has hereditary hepatic porphyria.

An important question that remains to be answered concerns the relevance of the chick embryo data to patients who have hereditary hepatic porphyria. In a recent study, Marks[11] compared the chick embryo data obtained with 29 drugs with clinical experiences with these drugs in hereditary hepatic porphyria. The results of the chick embryo data were in accord with clinical experience for 23 drugs; for the remaining six drugs the results were not definitive. It was concluded that tests in the chick embryo have considerable predictive value for hereditary hepatic porphyria.

The steroids were a gift from Glaxo, Toronto, Canada.

References

1. Eales L: The acute porphyria attack. III. Acute porphyria: The precipitating and aggravating factors. S Afr J Lab Clin Med 17:120–125, 1971
2. Granick S: The induction *in vitro* of the synthesis of δ-aminolevulinic acid synthetase in chemical porphyria: A response to certain drugs, sex hormones, and foreign chemicals. J Biol Chem 241:1359–1375, 1966
3. Racz WJ, Marks GS: Drug-induced porphyrin biosynthesis—II. Simple procedure for screening drugs for porphyria-inducing activity. Biochem Pharmacol 18:2009–2018, 1969
4. Davis B, Pearce DR: An introduction to althesin (CT 1341). Postgrad Med J 48 suppl 2:13–17, 1972
5. Morgan RO, Fischer PWF, Stephens JK, et al: Thyroid hormone enhancement of drug-induced porphyrin biosynthesis in chick embryo liver cells maintained in serum-free Waymouth medium. Biochem Pharmacol 25:2609–2612, 1976
6. Racz WJ, Marks GS: Drug-induced porphyrin biosynthesis—IV. Investigation of the differences in response of isolated liver cells and the liver of the intact chick embryo to porphyria-inducing drugs. Biochem Pharmacol 21:143–151, 1972
7. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951
8. Parikh RK, Moore MR: Anaesthetics in porphyria: Intravenous induction agents. Br J Anaesth 47:907, 1975
9. Granick S, Kappas A: Steroid induction of porphyrin synthesis in liver cell culture. I. Structural basis and possible physiological role in the control of heme formation. J Biol Chem 242:4587–4593, 1967
10. Stephens JK, Fischer PWF, Marks GS: Porphyrin induction: Equivalent effects of 5αH and 5βH steroids in chick embryo liver cells. Science 197:659–660, 1977
11. Marks GS: Handbook of Experimental Pharmacology. Berlin, Springer Verlag, 1978, pp 201–237