Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 104 AUGUST-SEPTEMBER, 1960 No. 4

SECTION MEETINGS

DISTRICT OF COLUMBIA	
Naval Medical Center	June 2, 1960
IOWA	
State University of Iowa	May 24, 1960
MARYLAND	
Baltimore City Hospitals	May 27, 1960
MINNESOTA	
University of Minnesota	June 2, 1960

Hypocholesteremic Effect of β-Diethylaminoethyl Diphenylpropylacetate Hydrochloride (SKF No. 525-A) in the Dog. (25896)

EDNA C. DICK, SAMUEL M. GREENBERG, JOHN F. HERNDON, MILDRED JONES AND E. J. VAN LOON

Smith Kline & French Labs., Philadelphia, Pa.

In view of general acceptance of relationship between hypercholesteremia and incidence of atherosclerosis, agents capable of reducing plasma cholesterol levels are of considerable current interest. In this laboratory β - diethylaminoethyl diphenylpropylacetate hydrochloride (SKF No. 525-A) was a potent hypocholesteremic agent. This report describes results of *in vivo* tests with this compound.

Methods. Adult mongrel dogs with naturally-high plasma total cholesterol values (greater than 150 mg%) were used. Dogs of both sexes were distributed evenly throughout the test. A commercial dog food and enriched whole milk diet sufficient to maintain body weight(1) was fed once a day (morning), and water supplied *ad lib.;* food not consumed within 3 hours was removed. Animals were housed individually in raised, wide-mesh screen cages in room at constant temperature and humidity. Medication was given orally in gelatin capsules, twice a day on weekdays (following 11:00 a.m. feeding and at 4:00 p.m.), and once a day on Saturday and Sunday after 11:00 a.m. feeding, so that on weekends animals received half usual daily dose of test material. Control dogs were administered an equal number of empty capsules when compound was given to test dogs. Blood samples were obtained from brachiocephalic vein of test dogs in fasting condition (before morning feeding). In Exp. I, 5 dogs received SKF No. 525-A in 20 mg/kg body weight/day for 2½ weeks, while 5 comparable dogs were given placebo treatment. In Exp. II, 3 dogs received the compound at 10 mg/kg/day for 19½ weeks, and 5 dogs again served as controls. Plasma total cholesterol was studied at intervals; plasma total lipids, phospholipids,

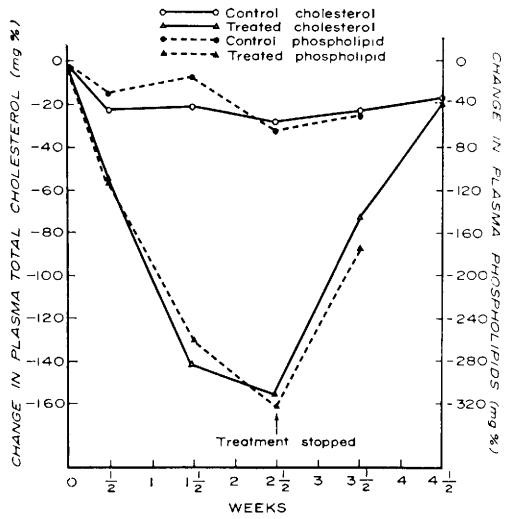

FIG. 1. Changes in plasma total cholesterol and plasma phospholipid in dogs treated with 20 mg SKF No. 525-A/kg/day (Exp. I). At start of experiment (0 time) absolute plasma base values in mg % were: Control cholesterol, 238; SKF No. 525-A treated cholesterol, 186; control phospholipid, 466; SKF No. 525-A treated phospholipid, 403.

esterified cholesterol and free cholesterol were studied at or near termination of experiment. A thyroid function test (protein-bound iodine) and liver function tests (serum alkaline phosphatase, bromsulfalein clearance and serum bilirubin) were determined after 17 weeks of drug administration. Other tests included glucose tolerance, complete blood study, urinary sodium and potassium, and urinary creatine/creatinine. Dogs were then autopsied for gross examination of internal organs, histological studies, and lipid analyses of livers and aortas. Cholesterol analyses were done by modification of method of Anderson and Keys(2). Other determinations were done by standard procedures(3-6).

Results. In Exp. I, 20 mg of the compound/kg/day administered orally to dogs resulted in an 84% reduction of plasma total cholesterol in 2½ weeks. Upon withdrawal of the compound these values returned to pretreatment levels (Fig. 1). Plasma phospholipids followed a pattern similar to that of the plasma total cholesterol (Fig. 1). Results of Exp. II (Table I) show similar reduction of plasma total cholesterol, plasma total lipids, and plasma phospholipids in dogs treated with a lower level of test material (10 mg/kg/day). Although studies during fifth month of treatment (Exp. II) showed that serum bilirubin, bromsulfalein retention and per cent esterified cholesterol were not significantly altered in treated dogs, serum alkaline phosphatases were significantly higher than for untreated dogs (Table I), indicating altered

TABLE I. Dog Exp. II. Effect of Oral Treatment of Dogs with 10 mg SKF No. 525-A/kg/Day for 19½ Weeks on Plasma Lipids, Liver and Thyroid Functions, and Body Weights.

Measurement	Control dogs (5)	Treated dogs (3)	"P"*
Plasma lipids, mg %			
Total cholesterol			
Initial	231 ± 76	216 ± 53	.8
After 3½ weeks' treatment	215 ± 56	88 ± 47	.02
" 19½ " "	245 ± 66	92 ± 16	.005
Free cholesterol, 17½ wk	66 ± 20	17 ± 8	.02
Esterified cholesterol, 17½ wk	200 ± 63	73 ± 10	.05
% esterified (of total), 17½ wk	76 ± 1	81 ± 5	.5
Total lipids, 19½ wk	1097 ± 89	770 ± 73	.001
Phospholipids, 19½ wk	406 ± 63	183 ± 28	.005
Cholesterol/phospholipid ratio, 19½ wk	.55 ± .05	.50 ± .04	.5
Liver function tests (17 wk)			
Serum alkaline phosphatase, Bodansky units	1.3 ± .9	7.9 ± 2.1	.001
Bromsulfalein, % retention	10.9 ± 6.0	12.9 ± 7.9	.5
Serum bilirubin, mg %	.10 ± .08	.15 ± .05	.5
Thyroid function test (16½ wk)			
Protein-bound iodine, μg %	2.1 ± .6	1.2 ± .1	.05
Body wt, kg			
Initial	12.5	11.0	
Final	12.3	11.1	

* "P" value determined by Student 't' test comparing values obtained with treated dogs to those with control dogs at times indicated.

TABLE II. Dog Exp. II. Lipid Analyses of Livers and Aortas of Dogs Treated with 10 mg SKF No. 525-A/kg/Day for 19½ Weeks.

Dog No.	Treatment	Livers			Aortas,
		% dry wt	% fat (dry wt)	mg chol/g dry wt	mg chol/g dry wt
165	None	32.0	4.7	7.8	5.8
208	"	31.0	4.1	7.2	5.8
	Avg	31.5	4.4	7.5	5.8
171	SKF No. 525-A	33.2	22.5	9.0	4.0
213	*Idem*	32.4	19.5	9.3	4.7
	Avg	32.8	21.0	9.2	4.4
54	SKF No. 525-A 19½ wk, removed from treatment for following 3 wk	30.4	5.4	7.2	4.9

liver function(7). Protein-bound iodine levels of test animals were significantly lower than were those of control dogs. The following tests gave normal values in treated dogs: glucose tolerance (19½ wks), urinary creatine and creatinine (6 wks), urinary sodium and potassium (6 wks), blood count and white cell differential (16½ wks), blood volumes (18 wks), and albumin/globulin ratios (15 wks). There were no apparent changes in appetite nor did body weights change during experimental periods.

Fatty infiltration of livers of treated dogs brought to autopsy was observed grossly and was confirmed by chemical analyses (Table II). Liver cholesterol of treated animals was 23% higher than that of controls, whereas aortic cholesterol was 24% lower. By histopathologic examination no abnormalities were observed in organs (including the thyroid) other than the liver in treated dogs.

One dog, which received SKF No. 525-A for 19½ weeks of Exp. II, was removed from treatment for 3 weeks before autopsy (Dog No. 54). In this dog plasma alkaline phosphatase level returned to normal and plasma total cholesterol increased from 35 mg% to 194 mg% (pretreatment value, 196 mg%); the liver appeared on autopsy both chemically (total lipids) and histologically normal. Although fat and cholesterol contents of this dog's liver had returned to levels similar to those of control dogs, aortic cholesterol was still depressed below control level.

Discussion. SKF No. 525-A has previously been known as potentiator of various drugs including hypnotics, analgesics, spinal cord depressants and CNS stimulants(8). The mechanism of action of SKF No. 525-A as a potentiator is related to its activity as inhibitor of side-chain oxidation, hydroxylation, ether cleavage, dealkylation, deamination(8), acetylation and cholinesterase activity(9). *In vitro* studies on rat liver homogenates, performed by Dr. W. L. Holmes, showed that SKF No. 525-A also inhibits synthesis of cholesterol from mevalonate. SKF No. 525-A, therefore, appears similar to other recently-announced hypocholesteremic agents which inhibit cholesterol synthesis from mevalonate (10,11).

Rapidity of action of SKF No. 525-A is exhibited in Exp. I where 30% reduction in plasma total cholesterol levels was produced in less than a week of treatment. The second experiment reveals that the hypocholesteremic effect is sustainable over a long period without overt signs of toxicity.

It has recently been reported that in rats long-term administration of SKF No. 525-A at high levels (25-50 mg/kg/day for 2 months) resulted in fatty infiltration of the liver(12). Doses of 10 mg/kg/day over the 2-month test caused no liver changes in rats (perhaps a longer dosage administration of lower level of the compound would have resulted also in liver changes).

Under our conditions with dogs, the alkaline phosphatase levels were more sensitive to liver changes than were bromsulfalein clearance times or serum bilirubin levels. The change in alkaline phosphatase was the only indication of hepatic toxicity, which suggests that liver damage with a drug of this type

might be overlooked if reliance were placed only on such standard liver-function tests as per cent esterified cholesterol, bilirubin levels or BSP clearance.

Tests on the dog when treatment was stopped after 5 months, indicate ready reversibility of fatty infiltration of the liver. It is therefore possible that intermittent use of the drug might lower serum cholesterol without causing excessive fatty livers. The prompt return of plasma cholesterol to pretreatment levels, observed in Exp. I and II upon cessation of treatment, indicates lack of permanent alteration of cholesterol-synthesizing mechanisms.

Additional work in this laboratory with species other than the dog showed that SKF No. 525-A produces hypocholesteremic effects in rats(13), mice and monkeys. In both rats and mice treated with SKF No. 525-A, we observed fatty infiltration of liver; monkeys were not autopsied for hepatic examination.

Summary. β-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF No. 525-A) reduced significantly plasma total cholesterol and aortic cholesterol in dogs. Chronic daily administration of the SKF No. 525-A to dogs for 5 months resulted in marked fatty infiltration of the liver, which was rapidly reversible upon withdrawal of the compound.

The authors wish to thank Dr. C. A. Leonard for drug studies in monkeys and Dr. L. Z. Saunders for histopathological studies.

1. Cowgill, G. R., Drabkin, D. L., *Am. J. Physiol.,* 1927, vol. 36.

2. Anderson, J. T., Keys, A., *Clin. Chem.,* 1956, v2, 145.

3. Zilversmit, D. B., Davis, A. K., *J. Lab. Clin. Med.,* 1950, v35, 155.

4. Kunkel, H. G., Ahrens, E. H., Jr., Eisenmenger, W. J., *Gastroenterology,* 1948, v11, 499.

5. Brown, H., Reingold, A. M., Samson, M., *J. Clin. Endocr. Metab.,* 1953, v13, 444.

6. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry,* 13th ed., Blakiston Co., New York, 1954.

7. Popper, H., Schaffner, F., *Liver: Structure and Function,* McGraw-Hill, New York, 1957, pp48-49.

8. Cooper, J. R., Axelrod, J., Brodie, B. B., *J. Pharm. and Exp. Therap.,* 1954, v112, 55.

9. Kuriaki, K., Marumo, H., *Jap. J. Pharmacol.,* 1959, v8, 96.

10. Blohm, T. R., Mackenzie, R. D., *Arch. Biochem. and Biophysics,* 1959, v85, 245.

11. Huff, J. W., Gilfillan, J. L., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 41.

12. Lena, E. T., *Boll. chim. farm.,* 1959, v98, 201.

13. Rice, E., Greenberg, S. M., *Fed. Proc.,* 1960, v19, 15.

Received November 18, 1959. P.S.E.B.M., 1960, v104.

Further Evidence of Immunologic Dissimilarity of Distemper (CD) and Measles (M) Viruses.* (25897)

V. J. CABASSO, J. E. AVAMPATO, K. H. KISER AND M. R. STEBBINS
(Introduced by H. R. Cox)
Viral and Rickettsial Research Section, Lederle Labs., Amer. Cyanamid Co., Pearl River, N. Y.

Although a possible relationship between canine distemper (CDV) and measles (MV) viruses has been proposed by various investigators(1-5), others find experimental evidence against such a theory(6,7). Inasmuch as modified canine distemper virus has been suggested for use in immunizing children against measles(8), further investigation of the relationship of these 2 viruses is required. Additional experimental evidence of their dissimilarity is presented here.

Materials and methods. Virus strains used: Edmonston strain† of MV adapted to chick embryo tissue culture(9); HeLa-grown MV with titers of $10^{4.5-5.5}$ TCD_{50} for inoculation of monkeys and ferrets and for neutralization

* Presented in part at SAB meeting in Philadelphia, May 1, 1960.

† Courtesy of Dr. J. F. Enders, Children's Hosp., Boston.