PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 299–301 (1975)

Effect of Hamycin on Plasma Cholesterol of Rat[1,2] (38793)

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(Introduced by George Brecher, M.D.)

Since the observation by Gottlieb *et al.* (1), a number of investigators have studied the interaction between polyene antibiotics and cholesterol. Such an interaction may have a role in the recently observed hypocholesterolemic effect of Amphotericin B on dogs (2, 3), chicks (4), and rats (5). In 1961, Ramchandran (6) clearly demonstrated that interaction with cholesterol was the basis of antifungal activity of Hamycin, a polyene antibiotic. Since Hamycin is an heptaene antibiotic like Amphotericin B, it was of considerable interest to investigate its action on plasma cholesterol of rat by methods analogous to those used for recent studies with Amphotericin B (5). In this report, it is demonstrated that Hamycin has a pronounced hypocholesterolemic effect on rats.

Experimental. Maintenance of animals. Male albino rats, 1.0–1.5 mo old, weighing 100–150 g, were employed. The animals were housed at 22–23° and, unless stated otherwise, were given food and water *ad libitum.*

Hamycin solution. Hamycin, 98.7% pure, was obtained as a gift from the Managing Director of Hindustan Antibiotics, Ltd. (Pimpri, India). For intraperitoneal administration, Hamycin was dissolved in 0.1 *N* NaOH (100 mg Hamycin per 1 ml of NaOH), and diluted with 5% dextrose. This solution then was neutralized with 0.1 *N* HCl to pH 7.0, as described by Parekh *et al.* (5), and was further diluted with 5% dextrose to 10 mg/ml concentration. In view of the rapid deterioration of Hamycin in light (7), precautions were taken to avoid light during the preparation of the solution and its injection into the animals. For oral administration, a suspension of Hamycin (200 mesh powder) was prepared in 0.5% tween-80 containing 5% dextrose, as described by Dave and Kaul (7).

Administration of hamycin. Groups of rats, consisting of five animals each, were randomized and were given Hamycin (10–100 mg/kg), or equal volume of the vehicle alone, by cannula or with food. For administration with food, ground food pellets were mixed with an adequate quantity of Hamycin, and sufficient water was added to form a semi-solid mass. In order to insure complete drug administration when Hamycin was given with food pellets, the rats were fasted overnight, and food was limited to an amount which the rats would eat within 2 hr. Control rats received equal amounts of food with the vehicle. Oral treatment with Hamycin was given daily from Day 0 up to Day 4, whereas intraperitoneal treatment (0.5 or 1.0 mg/kg) was given as a single dose. The rats were anesthetized with ether 24 hr after the last dose, and blood was obtained by heart puncture, taking care to prevent hemolysis.

Plasma cholesterol determination. Total plasma cholesterol in the rat was deter-

[1] This research was carried out in part at the Department of Pathology, State University of New York Upstate Medical Center, Syracuse, New York 13210, and was partly supported by Grant Nos. 11-8433-A and 11-1361-A from the Research Foundation of the State University of New York.

[2] The authors wish to thank Dr. Hans Loken, Mrs. Helen Bent, and Miss Susan Eastwood for their help in review and preparation of this manuscript.

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mined by the semi-micro procedure (5) based on the Parekh–Jung method (8).

Results. Administration of Hamycin resulted in a 0–10% decrease in the initial weight of the rats. This weight reduction was variable, however, and no dose- or time-dependent effects were discerned. These results are consistent with the data of a study reported by Dave *et al.* (9), in which Hamycin was administered with food. With rats to which Hamycin was administered with food, the food consumption was equal in both the control and treated groups. However, in contrast to the drug-treated animals, an increase of about 6% occurred in the body weight of control animals by the end of 5 days.

Oral administration of Hamycin (100 mg/kg/day) with food pellets resulted in a pronounced initial decrease in plasma cholesterol within the first 24 hr, followed by a gradual decrease to about 40% of initial levels after 120 hr (Fig. 1). Similar results were obtained when Hamycin was administered in a 50 mg/kg dose by cannula. The hypocholesterolemic effect of the 50 mg/kg dose administered by cannula, and that of the 100 mg/kg dose administered with food, were of similar magnitude. However, as shown in Fig. 1, the effect obtained with the 100 mg/kg dose by cannula for

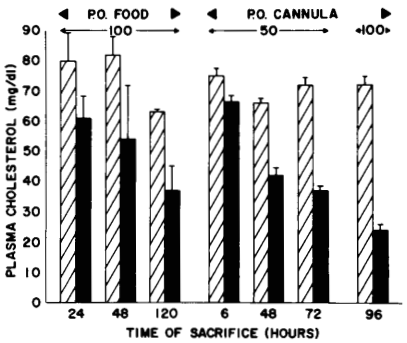

FIG. 1. *Hypocholesterolemic effect of oral hamycin in rats.* Beginning on Day 0, Hamycin suspension was administered with food or by gastric intubation once daily at 50–100 mg/kg/day (solid bars), as shown by the numbers at the top of vertical bars. Rats were sacrificed 24 hr after the last dose as indicated on the abscissa and plasma was collected for cholesterol determination. Control rats (shaded bars) were given the vehicle under identical conditions. Each is an average of 5–6 values + SD.

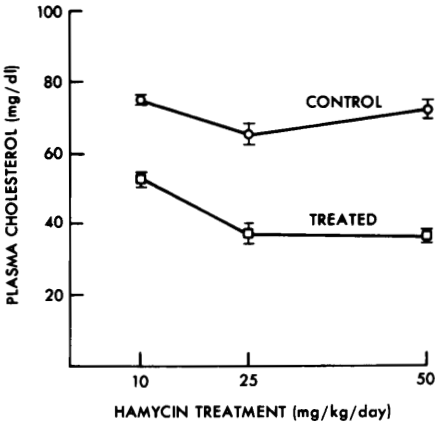

FIG. 2. *Effect of varying doses of hamycin on the plasma cholesterol of rats.* Hamycin suspension was administered daily on Days 0–2 at 24 hr intervals, and rats were sacrificed at 72 hr. Control rats received vehicle alone. Plasma cholesterol was determined as described in the text. Each value is an average from six animals. Vertical bar represents SD.

Day 0 through Day 3 (rats sacrificed at 96 hr) was significantly greater than that obtained in the corresponding dose-group receiving Hamycin with food (rats sacrificed at 72 hr or 120 hr). When Hamycin was administered by cannula rather than with food, the drug effect was more uniform. For this reason, oral administration of Hamycin was carried out by gastric intubation in subsequent experiments.

The hypocholesterolemic action of different doses of Hamycin shows an asymptotic decrease of plasma cholesterol with increasing doses of the drug (Fig. 2), reaching a maximum decrease of about 50% in plasma cholesterol with doses between 25–50 mg/kg.

The hypocholesterolemia by oral Hamycin could have been produced by inhibition of the transport of cholesterol or its precursors through the intestinal mucosa, due to complexation with the drug in the intestinal cavity. However, this possibility seems to be ruled out by a similar hypocholesterolemic action of the drug within 3 hr after intraperitoneal administration (Fig. 3).

Discussion. A pronounced decrease in the plasma cholesterol level of rats was observed upon oral administration of Hamycin. The decrease was more rapid in onset, and of

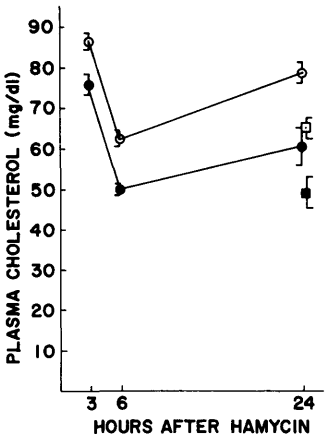

FIG. 3. *Hypocholesterolemic effect of hamycin administered intraperitoneally in rats.* Groups of rats (5–6 per group) were administered 0.5 (●) or 1.0 (■) mg Hamycin solution per kg body weight at time zero. Control rats received equal volume of the vehicle (○ or □).

greater magnitude, than that observed with Amphotericin B administered intravenously to rats (5) and orally to dogs (2, 3). No such hypocholesterolemic effect has been demonstrated with orally administered Amphotericin B in either man (10) or rats (11). The possibility that the hypocholesterolemic action of orally administered Hamycin may be due to the interference with cholesterol absorption (4) resulting from drug-sterol interaction is not consistent with the effect demonstrated with much lower doses of the drug when administered intraperitoneally.

The lack of clear dose-response curves (Figs. 2, 3) could be because the lowest dose employed in these studies may be close to the dose that will achieve a maximal response to the drug. However, it possibly may be due to other factors that cannot be evaluated from the data obtained in this study. For example, with respect to oral doses (Fig. 2), the flattening of the curve at 25 mg/kg could very well be due to a limited absorption of Hamycin from the gastrointestinal tract—a possibility that is consistent with the observation of Dave and Kaul (7).

No attempts were made to clarify the drug effect on total body weight. However, it did not seem to beu de to a reduced intake of the total food.

Regardless of the mechanism of action, the hypocholesterolemia produced by polyene antibiotics, particularly Hamycin, is of considerable interest, in view of its potential clinical usefulness, and the possibility that these drugs may provide a suitable tool to manipulate and understand cholesterol metabolism under physiological and pathological conditions.

Summary. Hamycin, a polyene antibiotic, when administered orally with food or by a cannula or administered intraperitoneally produced a dose-dependent hypocholesterolemic effect on normal albino rats. A 50% decrease occurred in serum cholesterol level at 25–50 mg/kg oral dose of hamycin. No further decrease occurred in serum cholesterol level with increase in the oral dosage. Decrease in the serum cholesterol occurring with 0.5–1.0 mg/kg, ip dose of the antibiotic was similar to that observed with oral hamycin and suggest that the effect was possibly mediated through mechanisms other than those involving prevention of absorption of cholesterol through the gastrointestinal tract.

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Received October 21 1974. P.S.E.B.M. 1975, Vol. 149.