

Effect of Candicidin on Plasma Cholesterol and Avian Atherosclerosis¹ (37905)

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Candicidin, a member of the polyene macrolide family, was first shown effective against canine prostatic glandular hyperplasia (1). These observations suggested the possibility that this agent might possess interesting properties relative to lipid and, in particular, to cholesterol utilization. Studies in dogs (2) demonstrated a marked serum cholesterol-lowering effect from candicidin, and studies in this laboratory have shown candicidin as well as other polyene macrolides to be effective in reducing plasma cholesterol in cholesterol-fed chickens (3). The present experiments evaluate the long-term effects of a low level (0.01%) of candicidin on cockerels fed cholesterol-containing or cholesterol-free diets over a period of 18-27 months.

Materials and Methods. Day-old White Leghorn cockerels purchased from a local hatchery were assigned to eight lots of 25 chicks each and maintained in electrically heated battery brooders. Two lots of 25 chicks were fed a control diet that contained 0.8% cholesterol provided by egg powder, and an equal number were fed an identical diet except that 0.01% candicidin was added. Similarly, two lots of 25 chicks were given a cholesterol-free diet, and another two lots received the same diet supplemented with 0.01% candicidin. To avoid loss of activity of candicidin upon prolonged exposure, the candicidin-containing diets

were mixed at weekly intervals. Since candicidin is sensitive to light and deactivated by sunlight, the windows were darkened and the birds raised with artificial light.

The composition of the basic diets is given in Table I. Protein was provided by soybean meal or by a mixture of soybean meal and dried whole egg powder. The dietary fat and cholesterol, when provided, were supplied by whole egg powder. Feed and water were available ad lib. to the chickens throughout the experimental period.

At various intervals (Table II) blood samples were obtained from the cockerels by heart puncture, and plasma cholesterol determined by the extraction procedure of Searcy and Berquist (4) and the colorimetric analysis of Zlatkis *et al.* (5). After 6 months, 19 of the cholesterol-fed controls and 21 of the cholesterol- and candicidin-fed cockerels were killed and the heart, aorta, and testes carefully removed for histological examination; coronary arteries and aortas were graded by methods previously described (6). At 18 months of age, all of the surviving birds that were on the cholesterol-containing diet were killed and hearts and aortas removed and examined as described above. At 27 months of age, the same procedure was followed with all surviving birds that had received the cholesterol-free diet throughout.

Results and Discussion. The very marked plasma cholesterol differential ($P < 0.01$) observed after 3 months on the respective cholesterol-containing diets is primarily due to the marked hypercholesterolemia of the control birds (Table II). By 6 months of

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TABLE I. Composition of Experimental Diets.

Ingredient	Amount	
	Cholesterol-containing %	Cholesterol-free %
Soybean meal (50% protein)	25.0	50.0
Whole egg powder	25.0	—
Dicalcium phosphate	2.0	2.0
Calcium carbonate with trace minerals	1.0	1.0
Sodium chloride	0.5	0.5
D,L-Methionine	0.2	0.2
Vitamin mix ^a	0.2	0.2
Choline chloride (70% concentrate)	0.2	0.2
Corn starch	45.9	45.9
	<u>100.0</u>	<u>100.0</u>

^a For composition see Fisher and Griminger (7).

age, the control birds had apparently adapted to the cholesterol intake: the plasma cholesterol concentrations remained essentially constant between 6 and 18 months of age. The plasma cholesterol-lowering effect of candicidin was again statistically significant ($P < 0.05$) at 12 months of age, but was absent at both 6 and 18 months in the cholesterol-fed cocks. No differences were observed among the birds fed the cholesterol-free diets.

At 6 months there was a significant difference between the two groups on the cholesterol-containing diets in the frequency of coronary arterial involvement, with the candicidin-fed birds having fewer and less severe lesions (Table III). Although both

the mean score and the frequency of severe aortic scores were lower for the candicidin-fed cocks at 6 months, the differences were not statistically significant. After 18 months, however, the frequency of severe aortic scores was significantly ($P < 0.025$) less in the candicidin-fed as compared to the control birds. At this period the differences in mean value and frequency of severe scores for the coronary arteries were not statistically significant.

The candicidin-fed cocks on the cholesterol-free diet had lower aortic and coronary scores after 27 months than the controls. The differences between coronary scores were significant ($P < 0.05$). It should be noted that the candicidin-fed birds, although

TABLE II. Plasma Cholesterol of Cockerels Fed Cholesterol-Containing and Cholesterol-Free Diets With and Without Candicidin

Age of Cockerels (months)	Cholesterol-fed		Cholesterol-free	
	Controls (mg/100 ml)	Candicidin-fed (mg/100 ml)	Controls (mg/100 ml)	Candicidin-fed (mg/100 ml)
3	279 ± 21 ^a	191 ± 8	94 ± 3	96 ± 2
6	199 ± 9	179 ± 15	53 ± 2	62 ± 3
12	189 ± 11	146 ± 6	77 ± 7	79 ± 3
18	177 ± 6	200 ± 19	—	—
27	—	—	80 ± 3	80 ± 6

^a Mean ± SE for the following number of birds at each of the sampling periods: 3 months, 20 each; 6 months, 20 each; 12 months, 17 and 20 for the cholesterol-fed birds with and without candicidin, respectively, and 22 each for the birds on the cholesterol-free diet; 18 months, 14 and 16 for controls and candicidin-fed, respectively; 27 months, 22 and 18 for controls and candicidin-fed, respectively.

TABLE III. Effect of Candicidin on Coronary and Aortic Lesions After 6 and 18 Months on a Cholesterol-Containing Diet and After 27 Months on a Cholesterol-Free Diet.

Measurement	Controls	Candicidin-fed
Cholesterol-fed cocks (6 Months)		
Body weight (g)	2035 ± 65 ^a	1909 ± 56 ^a
Histological grading ^b		
Aorta score (mean ± SE)	4.7 ± 0.5	4.1 ± 0.5
Frequency of severe scores ^c	6/19	4/21
Coronary arteries score (mean)	0.7	0.1
Frequency of lesions ^d	8/19	3/21
Cholesterol-fed cocks (18 Months)		
Body weight (g)	2318 ± 55 ^e	2449 ± 77 ^e
Histological grading ^b		
Aorta score (mean ± SE)	7.8 ± 1.2	5.9 ± 0.6
Frequency of severe scores ^f	7/14 ^g	3/16 ^g
Coronary arteries score (mean ± SE)	1.7 ± 0.5	1.6 ± 0.4
Frequency of lesions ^h	3/14	1/16
Cocks on cholesterol-free diet (27 Months)		
Body weight (g)	2424 ± 77 ⁱ	2231 ± 65 ⁱ
Histological grading ^b		
Aorta score (mean ± SE)	6.7 ± 0.5	6.0 ± 0.6
Frequency of severe scores ^f	9/23	6/17
Coronary arteries score (mean ± SE) [†]	1.6 ± 0.3	0.8 ± 0.2
Frequency of lesions ^h	4/23	0

^a Mean ± SE for 40 and 46 birds for the control and candicidin-fed groups, respectively.

^b The histological score for aortic lesions was based on (1) the degree of intimal proliferation, graded 1-9, (2) the severity of the atheromatous plaques, graded 1-11, and (3) the severity of medial involvement, graded 1-9; the scores were then added together. Coronary lesions were graded according to their distribution among intramural branches of the coronary artery and their size (i.e., the extent to which they obliterated the lumen).

^c Scores of 6 or above were considered severe for this age group.

^d Difference between groups significant at $P < 0.025$.

^e Mean ± SE for 14 and 16 birds for the control and candicidin-fed groups, respectively.

^f Scores of 8 or above were considered severe for this age group.

^g Difference between groups significant at $P < 0.025$.

^h Graded 4 or above.

[†] Difference between groups significant at $P < 0.05$.

ⁱ Mean ± SE for 23 and 18 birds for the control and candicidin-fed groups, respectively.

of different age (18 and 27 months), had similar aortic scores, regardless of the presence or absence of dietary cholesterol, suggesting that the drug prevented much of the atherogenesis observed in both of the control groups. In the case of the coronary scores, however, the candicidin-fed birds on the cholesterol-free diet were significantly lower ($P < 0.05$) than their cholesterol-fed counterpart.

No gross or histological changes were evident in the testes as a result of candicidin treatment.

General Comments. Candicidin has a marked hypocholesterolemic effect in cholesterol-fed chickens during short-term feeding trials (3). In the present study, the plasma cholesterol results were erratic, perhaps a reflection of adaptation to dietary cholesterol intake over a prolonged time interval. Nevertheless, it is clear that as low a level as 0.01% candicidin is remarkably active. Retarded atherogenesis in the candicidin-treated birds occurred not only in those fed cholesterol, but interestingly enough also in those on a cholesterol-free

diet without any evidence of an effect on the plasma cholesterol level.

No evidence of side effects in terms of gross abnormalities was observed in the candicidin-fed animals at time of autopsy. This was of particular interest relative to the testes, since the possibility existed that testosterone, a compound chemically related to cholesterol, might be affected by candicidin.

The mechanism of action of candicidin has been the subject of extensive studies in our laboratories. It would appear that its action is mediated through impaired bile acid and cholesterol absorption (8). Support for this thesis was obtained in the present study when bile acid excretion was measured after the cockerels had been on their respective diets for approximately 1 year. On the cholesterol-free diet the candicidin-fed birds excreted 41% more bile acids (determined by the method of Haust and Beveridge (9)) than the controls, and on the cholesterol-containing diet the candicidin-fed cocks excreted 65% more bile acids than the controls; both differences were significant ($P < 0.05$).

Summary. Day-old cockerels were fed a control or a candicidin-containing diet (0.01%) with or without cholesterol in the form of whole egg powder. The birds were maintained for 18–27 months on their respective regimens and plasma cholesterol was measured at different intervals. At 6 and at 18 months cholesterol-fed birds were killed and the coronary arteries and the aorta evaluated histologically for atherosclerosis. The birds on the cholesterol-free diets were killed after 27 months and similarly evaluated. Initially, in the cholesterol-

fed birds, plasma cholesterol was drastically reduced below that of the controls by candicidin feeding. However, the plasma cholesterol level of the controls then decreased, and the differences in cholesterol concentration between candicidin-fed and control birds became smaller or nonexistent. No differences in plasma cholesterol occurred at any time in the cocks on the cholesterol-free diet. A lower incidence of coronary artery lesions and a lower severity and incidence of aortic atherosclerosis was observed in the candicidin-treated cocks, regardless of the presence or absence of dietary cholesterol.

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