THE INFLUENCE OF α-DIFLUOROMETHYLORNITHINE ON THE ACTIVITY OF WOOL FOLLICLES

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Introduction

The polyamines, spermidine and spermine, are involved in nucleic acid and protein synthesis, especially in actively dividing cells (Pegg and McCann, 1982), and are likely to be important in wool follicle bulb cells. Their synthesis requires the formation of putrescine from ornithine catalyzed by ornithine decarboxylase, and the addition of an aminopropyl group derived from methionine. α-Difluoromethylornithine (DFMO) is a specific and potent inhibitor of ornithine decarboxylase (Pegg and McCann, 1982). In order to ascertain whether DFMO influenced wool growth and follicle activity, sheep were given intravenous infusions of DFMO.

Materials and Methods

Four Merino sheep, body weight 38-44 kg, were given a daily ration of 800 g sheep pellets (Milling Industries, SA) which provided approx. 9 g nitrogen and 5.5 MJ metabolizable energy. DFMO (supplied by Merrell Dow Research Institute) was dissolved in sterile, isotonic saline and infused continuously via a jugular vein by means of a peristaltic pump for 4 days. Two sheep received DFMO at a rate of 75 mg/kg body weight/day and two at a rate of 100 mg/kg/day.

Tracer doses of L-[^{35}S] cysteine were injected into a jugular vein at intervals of 4 days before, during and after the infusion of DFMO to enable measurement of length growth rate and diameter of wool fibres by the technique of autoradiography. Fibres were sampled from four sites along one side of each sheep; measurements were made on 120 fibres per sheep with approximately equal numbers from each site. Mitotic activity in wool follicle bulbs was estimated in midside skin biopsy samples following intradermal injections of colchicine to achieve metaphase arrest (Hynd et al., 1986; Hynd, 1989). At least 300 random bulb counts were made in skin taken 90 and 240 minutes after colchicine administration to give estimates of mitoses/hour, before DFMO infusion commenced and at the end of the 4-day infusion.

Results and Discussion

DFMO caused a reduction in fibre length growth rate (L) of 14-22% (mean 19.2%) in the 4 days after the infusion stopped; recovery was rapid thereafter (figure 1). The effect appeared to be slightly greater with the higher dose rate. Fibre diameter (D) increased by 1-2 μm during DFMO infusion and then declined to pre-treatment values or below (figure 1). These changes in L and D post-infusion caused a reduction in the volume of wool grown of 10-18% (mean 13.8%).

The differential effects of DFMO on L and D caused a considerable decrease in the ratio of mean length of fibre grown per day to mean fibre diameter (L/D ratio) during the 4-day period after the infusion (8-12 d, table 1). These effects are in contrast to the relative constancy of the L/D ratio for individual sheep over a wide range of wool growth rates induced by variations in nutrition (Downes and Sharry, 1971). However, similar changes in the L/D ratio have been observed with thyroid hormones and cold exposure (Downes and Sharry, 1971; Hynd, 1989).

In view of the effects of DFMO on wool growth, measurements were made of mitotic activity in follicle bulbs. Overall, there was little

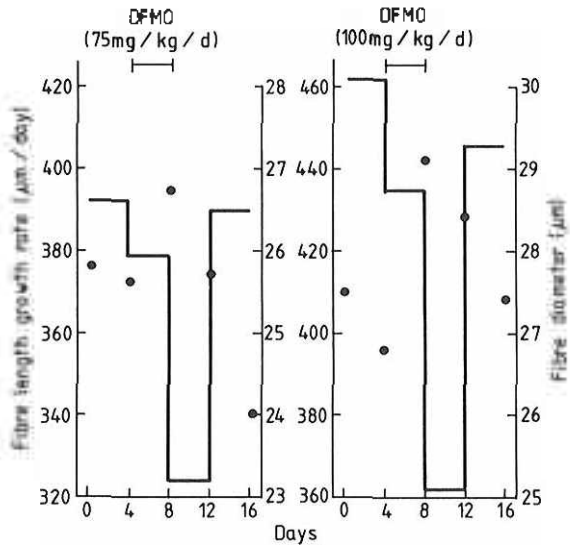

Figure 1. Effect of intravenous infusion of DFMO on length growth (—) and diameter (●) of wool fibres. Values are means for two sheep.

change in mitotic activity due to DFMO treatment, although mitotic rate decreased in both sheep given the higher dose whereas it increased with the lower dose (table 1). A tentative conclusion would be that DFMO alters the L/D ratio of fibres by means other than bulb cell division. This conclusion is in accord with the observation that DFMO did not influence DNA synthesis in skin slices *in vitro* (P.I. Hynd, unpublished). There is some evidence that colchicine may underestimate mitotic rate by 20-40% (P.I. Hynd and B.K. Applebee, unpublished). Nevertheless, colchicine has proved satisfactory for measuring changes in mitotic rate in response to nutrition and mitotic inhibitors (Hynd et al., 1986; Hynd, 1989).

Reis (1989) reported that DFMO perturbed the process of fibre formation; the present results confirm this observation and thus provide presumptive evidence for a role of spermidine and spermine in the wool follicle. DFMO may influence protein synthesis or cell migration rather than cell division, but further work will be required to understand its effects on wool growth.

TABLE 1. LENGTH/DIAMETER RATIO OF WOOL FIBRES AND MITOTIC RATE IN FOLLICLE BULB CELLS

Sheep	Dose rate of DFMO[a] (mg/kg/d)	Length/diameter ratio				Mitoses/hour	
		0-4 d	4-8 d	8-12 d	12-16 d	Before DFMO	After DFMO
1	75	13.8	13.9	11.7	14.3	0.87	1.07
2	75	16.6	15.2	13.2	17.2	1.19	1.49
3	100	15.4	14.1	11.4	14.7	1.96	1.31
4	100	18.6	17.2	14.0	17.4	1.55	1.15
Mean		16.1	15.1	12.6	15.9	1.39	1.26

[a]DFMO was infused during days 4-8

(Key Words: α-Difluoromethylornithine, Mitotic Activity, Wool Growth)

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