Differential effects of 2-difluoromethylornithine and methylglyoxal bis(guanyldihydrazone) on the testosterone-induced growth of ventral prostate and seminal vesicles of castrated rats

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1. 2-Difluoromethylornithine totally prevented any increases in putrescine and spermidine concentrations in the ventral prostate of castrated rats during a 6-day testosterone treatment. Prostatic ornithine decarboxylase activity was inhibited by 80%, whereas *S*-adenosylmethionine decarboxylase was stimulated by more than 9-fold. 2. In seminal vesicle, the inhibition of putrescine and spermidine accumulation, as well as of ornithine decarboxylase activity, was only minimal, and no stimulation of *S*-adenosylmethionine decarboxylase was observed. 3. Administration of methylglyoxal bis(guanyldihydrazone) to castrated androgen-treated rats resulted in a marked increase in concentrations of all prostatic polyamines. Prostatic ornithine decarboxylase activity was nearly 2 times and adenosylmethionine decarboxylase activity 9 times higher than that of the testosterone-treated animals. 4. In contrast with ventral prostate, methylglyoxal bis(guanyldihydrazone) treatment inhibited moderately the accumulation of spermidine and spermine in seminal vesicle, although both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities were stimulated. 5. Difluoromethylornithine inhibited significantly the weight gain of ventral prostate, but methylglyoxal bis(guanyldihydrazone) produced a substantial increase in prostatic weight. These changes were largely due to the fact that the volume of prostatic secretion was greatly decreased by difluoromethylornithine, whereas methylglyoxal bis(guanyldihydrazone) increased the amount of secretion. 6. Treatment with difluoromethylornithine strikingly increased the methylglyoxal bis(guanyldihydrazone) content of both ventral prostate and seminal vesicle, but even under these conditions the drug concentration remained low in comparison with other tissues. 7. The results indicate that a combined use of these two polyamine anti-metabolites does not necessarily result in a synergistic growth inhibition of the androgen-induced growth of male accessory sexual glands.

Difluoromethylornithine is a specific inhibitor of putrescine and spermidine biosynthesis in both cell cultures and animal tissues (for references see Heby & Jänne, 1981), and it has the apparent advantage of being well tolerated by experimental animals. Another inhibitor of polyamine synthesis, methylglyoxal bis(guanyldihydrazone), has been widely used in combination with difluoromethylornithine. The rationale of this combination is based on the fact that a prior treatment with difluoromethylornithine greatly enhances the uptake of methylglyoxal bis(guanyldihydrazone) (Alhonen-Hongisto *et al.*, 1980). This drug combination exerts a distinct cytotoxic effect on cultured cells,

transplanted tumours, hyperproliferative mouse skin and in human leukaemia (Seppänen *et al.*, 1981*a,b*; Siimes *et al.*, 1981; Käpyaho *et al.*, 1983).

Ventral prostate of the rat contains exceptionally high concentrations of spermidine and spermine (Pegg *et al.*, 1970; Danzin *et al.*, 1982), which are also components of the prostatic secretion (Mann & Lutwak-Mann, 1981). Castration results in a swift involution of male reproductive glands, and this effect can be reversed by testosterone administration. Among the first responses to the androgen is the stimulation of enzymes catalysing the synthesis of the polyamines (Pegg *et al.*, 1970; Piik *et al.*, 1977; Käpyaho *et al.*, 1980). Prostatic

ornithine decarboxylase activity appears to be extremely sensitive to inhibition by difluoromethylornithine (Danzin *et al.*, 1979a,b), and this compound also inhibits moderately the growth of ventral prostate of developing young rats (Danzin *et al.*, 1982) or of castrated rats treated with testosterone (Danzin *et al.*, 1979b). The last authors have also reported experimental findings which indicate that difluoromethylornithine may specifically inhibit the growth of prostatic acini and the secretory epithelial cells lining these acini. They suggested that in rat ventral prostate polyamines would be essential not so much for the growth of the gland itself as for the development of its secretory function. In contrast with prostate, testosterone-induced spermidine and spermine accumulation in seminal vesicle is only marginally inhibited by difluoromethylornithine, and the androgen-induced growth of this tissue is not affected by the drug (Danzin *et al.*, 1979b).

The concept of the combined use of difluoromethylornithine and methylglyoxal bis(guanyldrazone) for growth inhibition has also been applied in prostatic neoplasia. Growth of prostatic cancer cells transplanted subcutaneously in rats was inhibited by combined treatment with these two polyamine anti-metabolites (Dunzendorfer *et al.*, 1983; Herr & Kleinert, 1983).

Our results indicate that polyamine biosynthesis in rat ventral prostate is extremely sensitive to difluoromethylornithine. The latter drug produced profound depletion of putrescine and spermidine in the ventral prostate of testosterone-treated castrated rats and prevented the development of the prostatic secretory function. Methylglyoxal bis(guanyldrazone), however, paradoxically enhanced the accumulation of polyamines in ventral prostate and apparently stimulated the secretory function of the organ during androgen-induced growth. In comparison with ventral prostate, seminal vesicle appears to be remarkably resistant to the action of difluoromethylornithine, but responded to the treatment with methylglyoxal bis(guanyldrazone) in a more 'normal' fashion as regards the accumulation of polyamines.

Experimental

Chemicals

2-Difluoromethylornithine was generously given by the Centre de Recherche Merrell International (Strasbourg, France). Methylglyoxal bis(guanyldrazone) was synthesized and provided by Orion Pharmaceutical Co. (Espoo, Finland). DL-[1-¹⁴C]Ornithine (sp. radioactivity 56 Ci/mol) was purchased from Amersham International (Amersham, Bucks., U.K.), and S-adenosyl-L-[1-¹⁴C]methionine was prepared enzymically from L-

[1-¹⁴C]methionine (sp. radioactivity 5 Ci/mol; Amersham) by the method of Pegg & Williams-Ashman (1969). Testosterone propionate (Calbiochem, San Diego, CA, U.S.A.) was dissolved in olive oil.

Animals and treatments

Male rats of Sprague-Dawley and Wistar strains (280–350 g) were used. The rats were castrated through the scrotal route under light diethyl ether anaesthesia. At 8 days after the operation, testosterone treatment was commenced with daily subcutaneous injections of 2.5 mg of testosterone propionate in olive oil. The testosterone treatment was continued for 6 days, whereafter the animals were killed by decapitation. At the same time, some of the rats received 3% (w/v) difluoromethylornithine in drinking water, starting 6 h before the first testosterone injection, and/or methylglyoxal bis(guanyldrazone) (25 mg/kg intraperitoneally, in 0.9% NaCl) administered concomitantly with the testosterone injection.

Preparation of the tissue extracts

After decapitation, ventral prostate, seminal vesicle, liver, kidney and part of the small intestine were removed. Seminal fluid was squeezed out from the seminal vesicles and weighed (for the collection of prostatic secretion, see the detailed description below). Half of each prostate and seminal vesicle was homogenized in 25 mM-Tris/HCl buffer, pH 7.2, containing 0.1 mM-EDTA and 5 mM-dithiothreitol, and centrifuged at 100 000 g for 20 min in the cold. The enzyme activities and the content of difluoromethylornithine were assayed in the supernatant fraction. The other half of the tissues was homogenized in 0.2 M-HClO₄, and centrifuged at 2000 g for 5 min. Polyamines and methylglyoxal bis(guanyldrazone) were determined in the supernatant fractions, and the pellet was used for the determination of DNA content.

Isolation of the prostatic secretion

The thin capsule surrounding the ventral prostatic lobes was removed, and the tissue was cut into pieces. The pieces were placed on a grid, which was mounted inside a centrifuge tube. The tubes were centrifuged at 3000 g for 10 min in the cold. During the centrifugation the secretion was squeezed to the bottom of the tube, which contained 0.1 ml of 25 mM-Tris/HCl buffer, pH 7.2, with 0.1 mM-EDTA and 5 mM-dithiothreitol. The volume of the secretion was measured and it was divided into two parts, one for enzyme and difluoromethylornithine assays and the other for the determination of polyamine, methylglyoxal bis(guanyldrazone), DNA and protein contents.

Analytical methods

Ornithine decarboxylase activity was assayed by the method of Jänne & Williams-Ashman (1971a) and S-adenosylmethionine decarboxylase as described by Jänne & Williams-Ashman (1971b). Polyamines were determined as their dansylated derivatives by the method of Seiler (1970) as modified by Dreyfuss *et al.* (1973). Methylglyoxal bis(guanyldrazone) was assayed in neutralized HClO₄ supernatants by the method of Seppänen *et al.* (1980). Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard, and DNA was determined after hot-trichloroacetic acid hydrolysis by the method of Giles & Myers (1965), with deoxyguanosine as the standard.

Enzymic determination of difluoromethylornithine

The determination of difluoromethylornithine was based on its ability to inhibit irreversibly ornithine decarboxylase activity. To obtain the enzyme preparation, Wistar rats were injected intraperitoneally with thioacetamide (150 mg/kg body wt.) to induce the hepatic ornithine decarboxylase activity. The animals were decapitated 20 h later, and their livers were removed and homogenized in 25 mM-Tris/HCl buffer, pH 7.2, containing 0.1 mM-EDTA and 5 mM-dithiothreitol. The homogenate was centrifuged at 100 000g for 20 min, and the supernatant fraction was used as source of the enzyme. A portion of the tissue extract containing difluoromethylornithine was evaporated to dryness in a hot-water bath to inactivate endogenous ornithine decarboxylase, and the residue was dissolved in 25 mM-Tris/HCl, pH 7.2, containing 0.2 mM-EDTA. Then 125 µl of the enzyme preparation, 50 µl of the difluoromethylornithine-containing sample and 45 µl of a mixture containing 22 mM-EDTA, 22 mM-dithiothreitol and 2.2 mM-pyridoxal phosphate in 0.55 M-

Tris/HCl buffer, pH 7.2, were incubated for 20 min at 37°C to allow the reaction between difluoromethylornithine and ornithine decarboxylase. Thereafter 10 µl of 25 mM-L-ornithine and 20 µl of DL-[1-¹⁴C]ornithine (0.8 µCi) were added, which effectively stops any further enzyme inhibition, and the residual ornithine decarboxylase activity was determined as described by Jänne & Williams-Ashman (1971a). The concentration of difluoromethylornithine in the tissue samples was calculated with the aid of a standard curve run in parallel by plotting log (1/enzyme activity) against the concentration of difluoromethylornithine.

Results

Accumulation of polyamines in ventral prostates and seminal vesicles during androgen-induced growth

Treatment with difluoromethylornithine totally prevented the accumulation of putrescine and spermidine in the ventral prostate of castrated testosterone-treated rats, and even the accumulation of spermine was inhibited by 64% (Table 1). In contrast, treatment with methylglyoxal bis(guanyldrazone) produced a paradoxical increase in the concentrations of all three polyamines (Table 1). When both treatments were combined, the concentration of spermidine remained low, although spermine was substantially higher than in response to difluoromethylornithine alone (Table 1).

The effects were much less pronounced in seminal vesicle. Difluoromethylornithine decreased moderately the contents of putrescine and spermidine, whereas methylglyoxal bis(guanyldrazone) treatment resulted in a distinct decrease in spermidine (and spermine) concentrations (Table 2). The combined treatment did not result in any appreciable potentiation of the drug effects, with the possible exception of a further decrease in spermine content (Table 2).

Table 1. *Effects of difluoromethylornithine, methylglyoxal bis(guanyldrazone) and their combination on polyamine accumulation in the ventral prostate of castrated rats treated with testosterone*

Castrated rats received daily injections of testosterone propionate (2.5 mg/animal subcutaneously in olive oil). Difluoromethylornithine was given in drinking water (30 g/l), starting 6 h before the first testosterone injection, and methylglyoxal bis(guanyldrazone) was given as daily intraperitoneal injections (25 mg/kg) concomitantly with testosterone. After 6 days' treatment, the animals were decapitated and polyamines were determined as described in the Experimental section. The results represent means ± S.E.M. for five animals in each group. Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanyldrazone); n.d., not detectable. **P* < 0.01 and ***P* < 0.001, in comparison with testosterone-treated group.

Treatment	Putrescine (nmol/mg of DNA)	Spermidine (nmol/mg of DNA)	Spermine (nmol/mg of DNA)
None	12 ± 1	137 ± 9	228 ± 34
Testosterone	57 ± 5	2340 ± 218	1460 ± 168
Testosterone + DFMO	2 ± 1***	139 ± 20**	666 ± 83**
Testosterone + MGBG	101 ± 13**	3880 ± 361**	2590 ± 250**
Testosterone + DFMO + MGBG	n.d.	248 ± 30***	1320 ± 131

Table 2. *Effects of difluoromethylornithine, methylglyoxal bis(guanylhyazone) and their combination on polyamine accumulation in seminal vesicles of castrated rats treated with testosterone*

For experimental details see the legend for Table 1. Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhyazone). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, in comparison with testosterone-treated group.

Treatment	Putrescine (nmol/mg of DNA)	Spermidine (nmol/mg of DNA)	Spermine (nmol/mg of DNA)
None	9 $\pm$ 1	183 $\pm$ 17	158 $\pm$ 8
Testosterone	44 $\pm$ 6	1220 $\pm$ 119	475 $\pm$ 42
Testosterone + DFMO	22 $\pm$ 1***	895 $\pm$ 71*	466 $\pm$ 42
Testosterone + MGBG	28 $\pm$ 10	753 $\pm$ 32**	375 $\pm$ 36
Testosterone + DFMO + MGBG	39 $\pm$ 10	859 $\pm$ 47*	233 $\pm$ 32*

Table 3. *Effects of difluoromethylornithine, methylglyoxal bis(guanylhyazone) and their combination on the activities of ornithine decarboxylase and adenosylmethionine decarboxylase in ventral prostate and seminal vesicles of castrated rats treated with testosterone*

For experimental details see the legend for Table 1. Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhyazone). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, in comparison with testosterone-treated group.

Treatment	Ornithine decarboxylase activity (pmol/h per mg of protein)		Adenosylmethionine decarboxylase activity (pmol/h per mg of protein)	
	Prostate	Seminal vesicle	Prostate	Seminal vesicle
None	18 $\pm$ 10	24 $\pm$ 6	391 $\pm$ 95	195 $\pm$ 32
Testosterone	8780 $\pm$ 1343	1050 $\pm$ 150	2590 $\pm$ 320	1030 $\pm$ 237
Testosterone + DFMO	1640 $\pm$ 237***	769 $\pm$ 132	23700 $\pm$ 738***	747 $\pm$ 49
Testosterone + MGBG	15900 $\pm$ 3680	2320 $\pm$ 164**	23500 $\pm$ 8660	12700 $\pm$ 3080***
Testosterone + DFMO + MGBG	1290 $\pm$ 228**	1340 $\pm$ 148	47500 $\pm$ 1960***	44000 $\pm$ 2880***

As illustrated in Table 3, ornithine decarboxylase activity was only partially inhibited by difluoromethylornithine in both tissues. Difluoromethylornithine strikingly (10-fold) increased the activity of adenosylmethionine decarboxylase in ventral prostate, but the drug had no effect on the enzyme activity in seminal vesicle (Table 3). The stabilization of adenosylmethionine decarboxylase by methylglyoxal bis(guanylhyazone) was reflected by a 10-fold stimulation of the enzyme activity in both tissues (Table 3). Methylglyoxal bis(guanylhyazone) also doubled the activity of ornithine decarboxylase in both tissues (Table 3). The combination of the two drugs produced additive stimulation of adenosylmethionine decarboxylase activity in ventral prostate. Although difluoromethylornithine did not stimulate adenosylmethionine decarboxylase activity in seminal vesicle, the combined treatment further enhanced the enzyme activity in comparison with methylglyoxal bis(guanylhyazone) alone (Table 3).

The activities of both ornithine decarboxylase and adenosylmethionine decarboxylase in ventral prostatic secretion reflected the activities of the tissue. Only traces of ornithine decarboxylase activity were found in the secretion, whereas the activity of adenosylmethionine decarboxylase rep-

resented 10% of that found in prostate (results not shown). This may indicate better stability of adenosylmethionine decarboxylase towards proteolytic enzymes present in the secretory fluid.

Effects of difluoromethylornithine and methylglyoxal bis(guanylhyazone) on the testosterone-induced growth of ventral prostate and seminal vesicle

Treatment of castrated rats with testosterone propionate for 6 days increased the weight of the ventral prostate and seminal vesicles 5-fold and 4-fold respectively (Table 4). Difluoromethylornithine alone significantly inhibited the androgen-induced weight gain of ventral prostate, whereas methylglyoxal bis(guanylhyazone) produced an opposite effect: the prostatic weight was further increased by 34% (Table 4). During combined treatment, the weight of ventral prostate increased as in the animals receiving testosterone alone. Neither drug, alone or in combination, had any significant effect on the growth of seminal vesicles (Table 4).

Effect of polyamine anti-metabolites on the secretory activity of ventral prostate

Table 5 shows that a treatment with difluoromethylornithine effectively prevented the develop-

ment of the prostatic secretory function. Both the volume of the secretion and the concentration of protein secreted were only about 20% of that of the androgen-treated controls. In striking contrast, methylglyoxal bis(guanylhydrazone) treatment resulted in an enhanced production of prostatic fluid, whereas the concentrations of protein and polyamines secreted were not significantly increased (Table 5). The increased secretion volume of prostates from animals treated with both drugs reflected the effect of methylglyoxal bis(guanylhydrazone) rather than that of difluoromethylornithine (Table 5). However, the concentrations of secreted spermidine remained low even during the combined treatment.

Concentrations of difluoromethylornithine and methylglyoxal bis(guanylhydrazone) in male accessory sexual glands and other tissues in testosterone-treated castrated rats

Table 6 shows that intraperitoneal injections of

methylglyoxal bis(guanylhydrazone) resulted in the highest tissue concentration of the drug in liver. The drug concentrations in kidney and small intestine were about half of that found in liver, whereas the content of methylglyoxal bis(guanylhydrazone) in ventral prostate and seminal vesicle remained very low (about one-tenth of the liver concentration) (Table 6). Concomitant treatment with difluoromethylornithine only slightly enhanced the accumulation of methylglyoxal bis(guanylhydrazone) in liver, kidney and small intestine, but it produced a strikingly enhanced drug accumulation in ventral prostate (7-fold) and seminal vesicle (3-fold) (Table 6). However, even then the methylglyoxal bis(guanylhydrazone) concentrations remained lower than in the other tissues. Methylglyoxal bis(guanylhydrazone) treatment appeared to have no effect on the concentration of difluoromethylornithine, as shown in Table 7. Further, when the rather large statistical

Table 4. *Effects of difluoromethylornithine, methylglyoxal bis(guanylhydrazone) and their combination on the weight of ventral prostate and seminal vesicles of castrated rats treated with testosterone*

The animals were treated as described in the legend for Table 1. The weights are means $\pm$ S.E.M. for four animals. Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone). * $P < 0.05$, and ** $P < 0.01$, in comparison with testosterone-treated group.

Treatment	Prostate (mg)	Seminal vesicle (mg)
None	87 $\pm$ 8	166 $\pm$ 4
Testosterone	473 $\pm$ 43	698 $\pm$ 119
Testosterone + DFMO	253 $\pm$ 16**	733 $\pm$ 70
Testosterone + MGBG	635 $\pm$ 43*	701 $\pm$ 50
Testosterone + DFMO + MGBG	413 $\pm$ 40	767 $\pm$ 71

Table 6. *Effect of difluoromethylornithine treatment on the accumulation of methylglyoxal bis(guanylhydrazone) in some tissues of castrated rats receiving testosterone*

The experimental details are as in Table 1. Abbreviations used: MGBG, methylglyoxal bis(guanylhydrazone); DFMO, difluoromethylornithine. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, in comparison with the group not receiving difluoromethylornithine.

Tissue	MGBG concentration (μ M)	
	-DFMO	+DFMO
Liver	391 $\pm$ 106	505 $\pm$ 97
Kidney	175 $\pm$ 51	322 $\pm$ 64
Small intestine	159 $\pm$ 20	308 $\pm$ 26**
Ventral prostate	30 $\pm$ 7	221 $\pm$ 27***
Seminal vesicle	49 $\pm$ 10	151 $\pm$ 41*

Table 5. *Effects of difluoromethylornithine, methylglyoxal bis(guanylhydrazone) and their combination on the secretory activity of ventral prostate of castrated rats treated with testosterone*

The separation of the prostatic secretion and the determination of proteins and polyamines are described in the Experimental section. Percentages in parentheses are percentages of those for the respective testosterone-treated group. The prostates of castrated control rats contained no secretion. Further, polyamine content of the testosterone + DFMO-treated group could not be estimated because of the small amount of the prostatic secretion. For other experimental details see the legend for Table 1. Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, in comparison with testosterone-treated group.

Treatment	Secretion volume (μ l/g of tissue)	Secretory protein (mg/g of tissue)	Secretory polyamines (nmol/g of tissue)		
			Putrescine	Spermidine	Spermine
Testosterone (3)	293 $\pm$ 9 (100%)	7.8 $\pm$ 0.6 (100%)	31 $\pm$ 6	457 $\pm$ 36	154 $\pm$ 15
Testosterone (3) + DFMO	66 $\pm$ 30** (23%)	1.4 $\pm$ 0.6** (18%)	—	—	—
Testosterone (4) + MGBG	726 $\pm$ 65** (248%)	10.3 $\pm$ 1.7 (132%)	95 $\pm$ 41	675 $\pm$ 186	255 $\pm$ 72
Testosterone (4) + DFMO + MGBG	599 $\pm$ 57* (204%)	7.5 $\pm$ 0.6 (96%)	6 $\pm$ 1**	62 $\pm$ 9***	124 $\pm$ 38

Table 7. Concentrations of difluoromethylornithine and methylglyoxal bis(guanylhydrazone) in ventral prostate, prostatic secretion and seminal vesicles of castrated rats treated with testosterone

All animals were treated as in Table 1. After 6 days' treatment the animals were killed by decapitation. The isolation of the prostatic secretion and the determination of the drug concentrations are described in the Experimental section. Results are means $\pm$ S.E.M. for four animals (except the secretion of the prostates treated with difluoromethylornithine alone, where difluoromethylornithine could be determined from two specimens only). Abbreviations used: DFMO, difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone). ** $P < 0.01$, in comparison with the group not receiving difluoromethylornithine.

Sample	Treatment with	Concn. of			
		DFMO (μ M)		MGBG (μ M)	
		DFMO	DFMO + MGBG	MGBG	DFMO + MGBG
Ventral prostate		382 $\pm$ 56	731 $\pm$ 292	21 $\pm$ 8	248 $\pm$ 59**
Prostatic secretion		815	811 $\pm$ 223	5 $\pm$ 2	30 $\pm$ 6**
Seminal vesicle		167 $\pm$ 26	163 $\pm$ 41	33 $\pm$ 11	219 $\pm$ 102

variation within each experimental group is considered, the molar concentration of difluoromethylornithine in prostatic tissue and secretion showed little difference. This was in distinct contrast with methylglyoxal bis(guanylhydrazone), which was preferably accumulated in the prostatic tissue as compared with the secretion (Table 7).

Discussion

Methylglyoxal bis(guanylhydrazone) has been used clinically as an anti-cancer drug in the early sixties (Regelson & Holland, 1961; Mihich, 1963). Although experimental evidence suggested that the mechanism of action of the drug was possibly linked to the metabolism of spermidine (Mihich, 1963), it was not until 1972 that Williams-Ashman & Schenone discovered that methylglyoxal bis(guanylhydrazone) is an extremely potent inhibitor of eukaryotic adenosylmethionine decarboxylase and hence inhibits the synthesis of spermidine and spermine.

Methylglyoxal bis(guanylhydrazone) apparently utilizes the cellular carrier system designed for natural polyamines for its transport into cells (Dave & Caballes, 1973; Seppnen *et al.*, 1980). This fact, combined with the finding that polyamine depletion produced by inhibitors of ornithine decarboxylase, most notably by difluoromethylornithine, greatly enhances the uptake of polyamines offered the rationale for a sequential combination of these two drugs (Seppnen *et al.*, 1981a, b). The cytotoxicity exerted by the combination appears to show some tumour selectivity as regards the tissue distribution of methylglyoxal bis(guanylhydrazone) after a prior treatment with difluoromethylornithine (Seppnen *et al.*, 1983).

The view that difluoromethylornithine selectively stimulates the putative polyamine carrier in tumour tissues has been supported by findings of

Chaney *et al.* (1983) showing that EMT6 tumours transplanted in mice took up most effectively, in comparison with host tissues, labelled putrescine in response to a prior difluoromethylornithine treatment.

Androgen-induced growth of prostatic tissue of castrated rodents is a useful model for studies with polyamine anti-metabolites, since this organ not only contains high concentrations of polyamines but also secretes large amounts of these compounds. Furthermore, the involution as well as the regrowth of this organ is accompanied by striking changes in polyamine synthesis and accumulation (Pegg *et al.*, 1970; Piik *et al.*, 1977).

By preventing the accumulation of putrescine and spermidine with injections of difluoromethylornithine during testosterone treatment of castrated rats, Danzin *et al.* (1979b) concluded that polyamines are involved in the maintenance of functional secretory epithelium. Our present results also point in this direction, as the volume of prostatic secretion was radically decreased in response to difluoromethylornithine treatment (Table 5). In distinct contrast with this treatment, daily injections of methylglyoxal bis(guanylhydrazone) resulted in a more than 2-fold increase in the volume of prostatic secretion (Table 5). Whether this is related to the peculiar rise in the tissue polyamine concentrations after treatment with methylglyoxal bis(guanylhydrazone) (Table 1), or whether the latter drug can mimic the possible action of polyamines on prostatic secretory function, is not known. In fact, the finding (Table 1) that combined treatment with the two drugs decreased prostatic polyamines to the concentration found after difluoromethylornithine alone, yet the volume of prostatic secretion did not decrease (Table 5), may even support the view that methylglyoxal bis(guanylhydrazone) directly stimulates the secretory function of the gland.

Polyamine accumulation in rat ventral prostate appears to be extremely sensitive to difluoromethylornithine, whereas that in seminal vesicle is remarkably insensitive in this respect. The opposite is true for methylglyoxal bis(guanyldihydrazine): in ventral prostate this drug paradoxically enhanced the accumulation of all three polyamines (Table 1), whereas in seminal vesicle the concentrations of all three polyamines decreased in response to the treatment with the drug (Table 2). One may try to find some kind of explanation for these phenomena in the different enzyme patterns of the two sexual accessory organs. In ventral prostate the androgen-induced accumulation of polyamines apparently relies on the striking (almost 500-fold) stimulation of ornithine decarboxylase activity, which was about 3 times higher than the maximally stimulated activity of adenosylmethionine decarboxylase (Table 3). In seminal vesicle, the activities of the two decarboxylases were stimulated exactly to the same extent (Table 3). The possibility that the differences would result from different tissue drug concentrations remains open, since ventral prostate accumulated difluoromethylornithine more effectively than did seminal vesicles (Table 7).

Although the concentration of methylglyoxal bis(guanyldihydrazine) in the accessory sexual glands of testosterone-treated castrated rats was by far the lowest of all tissues analysed (Table 6), treatment with difluoromethylornithine produced the most striking stimulation of the drug's accumulation in prostate (7-fold) and seminal vesicle (3-fold) (Table 6). The latter observation is in good agreement with the report by Kadmon *et al.* (1982) demonstrating that prostatic putrescine uptake was enhanced by more than 10-fold when castrated rats were treated with a combination of testosterone and difluoromethylornithine.

Even though testosterone-induced growth of rat ventral prostate and seminal vesicle appears to respond poorly to the antiproliferative action of polyamine anti-metabolites (with the possible exception of difluoromethylornithine-induced diminution of prostatic secretion), a few reports indicate that experimental tumours of prostatic origin may be especially sensitive to the combined action of difluoromethylornithine and methylglyoxal bis(guanyldihydrazine) when transplanted subcutaneously (Dunzendorfer *et al.*, 1983; Herr & Kleinert, 1983). Of special interest is the report by Herr & Kleinert (1983) showing that the toxicity of methylglyoxal bis(guanyldihydrazine) can be dramatically decreased, without lowering the dose, by combining the drug with difluoromethylornithine.

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References

- Alhonen-Hongisto, L., Seppänen, P. & Jänne, J. (1980) *Biochem. J.* **192**, 941–945
- Chaney, J. E., Kobayashi, K., Goto, R. & Digenis, G. A. (1983) *Life Sci.* **32**, 1237–1241
- Danzin, C., Jung, M. J., Grove, J. & Bey, P. (1979a) *Life Sci.* **24**, 519–524
- Danzin, C., Jung, M. J., Claverie, N., Grove, J., Sjoerdsma, A. & Koch-Weser, J. (1979b) *Biochem. J.* **180**, 507–513
- Danzin, C., Claverie, N., Wagner, J., Grove, J. & Koch-Weser, J. (1982) *Biochem. J.* **202**, 175–181
- Dave, C. & Caballes, L. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 736
- Dreyfuss, G., Dvir, R., Harell, A. & Chayen, R. (1973) *Clin. Chim. Acta* **49**, 65–72
- Dunzendorfer, U., Relyea, N. M., Kleinert, E., Balis, M. E. & Whitmore, W. F. (1983) *Oncology* **40**, 57–62
- Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93
- Heby, O. & Jänne, J. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 243–310, Marcel Dekker, New York
- Herr, H. W. & Kleinert, E. L. (1983) *Proc. Annu. Meet. Am. Assoc. Cancer Res.* **24**, 1056
- Jänne, J. & Williams-Ashman, H. G. (1971a) *J. Biol. Chem.* **246**, 1725–1732
- Jänne, J. & Williams-Ashman, H. G. (1971b) *Biochem. Biophys. Res. Commun.* **42**, 222–229
- Kadmon, D., Heston, W. D. W., Lazan, D. W. & Fair, W. R. (1982) *J. Nucl. Med.* **23**, 998–1002
- Käpyaho, K., Pösö, H. & Jänne, J. (1980) *Biochem. J.* **192**, 59–63
- Käpyaho, K., Lauharanta, J. & Jänne, J. (1983) *J. Invest. Dermatol.* **81**, 102–106
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mann, T. & Lutwak-Mann, C. (1981) *Male Reproductive Function and Semen*, pp. 291–295, Springer-Verlag, Berlin, Heidelberg and New York
- Mihich, E. (1963) *Cancer Res.* **23**, 1375–1389
- Pegg, A. E. & Williams-Ashman, H. G. (1969) *J. Biol. Chem.* **244**, 682–693
- Pegg, A. E., Lockwood, D. H. & Williams-Ashman, H. G. (1970) *Biochem. J.* **117**, 17–31
- Piik, K., Rajamäki, P., Guha, S. K. & Jänne, J. (1977) *Biochem. J.* **168**, 379–385
- Regelson, W. & Holland, J. F. (1961) *Cancer Chemother. Rep.* **11**, 81–86
- Seiler, N. (1970) *Methods Biochem. Anal.* **18**, 259–337
- Seppänen, P., Alhonen-Hongisto, L., Pösö, H. & Jänne, J. (1980) *FEBS Lett.* **111**, 99–103
- Seppänen, P., Alhonen-Hongisto, L. & Jänne, J. (1981a) *Biochim. Biophys. Acta* **674**, 169–177
- Seppänen, P., Alhonen-Hongisto, L. & Jänne, J. (1981b) *Eur. J. Biochem.* **118**, 571–576
- Seppänen, P., Alhonen-Hongisto, L. & Jänne, J. (1983) *Cancer Lett.* **18**, 1–10
- Siimes, M., Seppänen, P., Alhonen-Hongisto, L. & Jänne, J. (1981) *Int. J. Cancer* **28**, 567–570
- Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295