EFFECT OF DIFLUOROMETHYLORNITHINE ON THYROID FUNCTION IN RATS

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Background: A variety of stimuli cause a rapid increase in polyamine synthesis by increasing an enzyme ornithine decarboxylase required for the biosynthetic pathway of protein synthesis. Di-fluoromethyl ornithine is a selective inhibitor of this enzyme and hence arrests cell replication strikingly. Its effects on thyroid gland are studied with respect to change in animal's weight and levels of Triiodothyronine, Thyroxine and Thyroid stimulating hormone. The study was conducted to evaluate the inhibitory effects of Di-fluoromethyl ornithine (DFMO) administration on polyamine metabolism of thyroid gland in rats. **Methods:** The study was conducted on rats weighing 248 to 320 grams, divided into control and DFMO treated group. A dose of 50 mg/rat was administered subcutaneously to the treated group for 5 consecutive days and placebo (normal saline) injections to control group. On sixth day, blood was collected by cardiac puncture and serum was separated. Serum T3, T4 and TSH were analyzed with the help of radioimmunoassay in both groups. **Results:** In treated group there was a fall in T3, T4 concentration with significant rise in TSH concentration as compared to control group. **Conclusion:** DFMO (Difluoro methyl ornithine) decreases cellular proliferation of thyroid gland as is assessed by decrease in thyroid hormone levels. The hypothalamo pituitary thyroid axis however remains intact as is shown by a feedback rise in TSH concentration. DFMO can thus be employed for anti-neoplastic clinical trials on account of interference with activity of ODC (Ornithine Decarboxylase) fundamental for polyamine biosynthesis.

Keywords: DFMO, ODC, thyroid, rats, radioimmunoassay

INTRODUCTION

Difluoro Methyl Ornithine (DFMO) was synthesized by Metcalf.[1] It is water soluble & has a molecular weight of 236.65.It is a potent mechanism based inhibitor of ODC.[2] ODC is the first and a key (rate-limiting) enzyme in the biosynthes of polyamines, specifically putrescine, spermidine and spermine.[3] These polyamines are nucleotide precursors present in all mammalian cells: essential for maintenance of cell growth and function.[4] Polyamine synthesis occurs during the G1 phase of cell cycle.[5] The initial step in polyamine synthesis is the decarboxylation of L-Ornithine by ODC which results in conversion of ornithine to putrescine.[6] DFMO (2-Difluoromethylornithine) acts specifically on ODC & has no actions on rest of the enzymes. It was developed for treatment of *Trypanosoma brucei gambiense* the parasite which causes African sleeping sickness and is effective via suppression of Trypanosoma DNA synthesis.[6–8] It has been used to inhibit levels of polyamines in variety of cells both in vivo & vitro.[9] Since DFMO inhibits cell replication strikingly[10] it has been used in Phase-II clinical trials sponsored by the National Cancer Institute as a chemoprotective agent against cancers of colon, breast, cervix and oesophagus & phase-III clinical trials for prevention of Bladder carcinoma.[4] Published clinical trials of this compound raised considerable interest in therapeutic use of DFMO. Its therapeutic dose was adjusted with the help of previous clinical trials.[11] Main aim of the study was to evaluate its role in biosynthetic inhibition of polyamine synthesis on Thyroid functions.

MATERIALS AND METHODS

For this study twenty female rats were procured from National Institute of Health Islamabad. They were kept in animal house in Department of Biological Sciences, Quaid-e-Azam University Islamabad. They were provided with food libitum and constant supply of drinking water at a temperature of 21±3 ºC and lighting 10 hr/14 hr light/dark cycle. They were divided in control & DFMO treated groups. Group I had an average weight of 248–320 gm. They were given 0.5 ml normal saline at 1000 hours subcutaneously for 5 days. Group-II (DFMO) treated group had a dose of 50 mg/rat which was achieved by dissolving 250 mg/ml of drug in 0.9% saline.

Cardiac puncture of both the groups was done on the sixth day at 1400 hours by a longitudinal incision along left side of the sternum. The blood samples were collected from the heart. Serum was separated and stored at -20 ºC until analysis. Radioimmunoassay of T4 was done by Amerlex-M T4 kit which involves a competitive radioimmunoassay technique. For this 50 µL of standard or sample was dispensed with 500 µL of Amerlex-M antibody suspension. They were incubated for 45 minutes at 18–28 ºC. Following incubation all the tubes were decanted and allowed to

drain for 5 minutes. Bound radioactivity was counted in a LKB (DP5500) gamma counter. T3 immunoassay was done with Amerlex-M T3 RIA100 µl of standard sample was dispensed with 500 of tracer and 500 µL of Amerlex-M antibody suspension. They were incubated for 60 minutes at 37 ºC. Following incubation all the tubes were decanted & allowed to drain. Bound radioactivity was counted in a LKB (DP5500) gamma counter.

Thyrotropin concentration in serum sample was determined by solid phase (I125) radioimmunoassay. 100 µL of sample or control was incubated with 100 µL of tracer reagent for 90 minutes at 37±1 ºC. After 1–2 minutes tubes were decanted again. A total of three washes were done. Bound radioactivity was counted in a LKB (DP5500) gamma counter.

RESULTS

For statistical comparison of body weights, serum T3, T4 and TSH levels the Mean±SD was calculated. Treated group results were compared with control group using Student's *t*-test. The values were considered significant when $p<0.01$ and highly significant when $p<0.001$. Results labelled to be insignificant when $p>0.05$.

In control group mean body weight of rats increased to 290±53 gm from 280±53 gm as shown in Table-1. After 5 days of treatment with DFMO their body weights increased to 295±50 gm (Table-1). However change was found to be non-significant statistically.

A significant decrease in concentration of T4 was observed in DFMO treated group i.e. 23.52±6.42 as compared to 30.06±5.51 in control group as shown in Table-2.

As far as TSH is concerned DFMO caused an increase from 0.14±0.02 µIU/ml (control) to 0.17±0.01 µIU/ml as shown in Table-3.

Table-1: Effect of DFMO 50 mg/rat on Mean Body Weight of Rats

Group	Initial Weight	Weight after treatment
Control	280±53	290±53
DFMO	280±50	295±50

Table-2: Effect of DFMO on T3 T4 Concentration

	Treatment	T_3 (ηmol/litre)	T_4 (ηmol/litre)
Control	0.9%Saline	0.60±0,13	30.06±5.51
DFMO	50mg/rat	0.44±0.08	23.52±6.42

Table-3: Effect of DFMO on TSH Concentration

	Treatment	TSH (µIU/ml)
Control	0.9% saline	0.14±0.02
DFMO	50 mg/rat	0.17±0.01

DISCUSSION

Our results show that administration of DFMO at a rate of 50 mg/animal caused an increase in mean body weight, fall in T3 (free) and T4 (total) with a rise in TSH concentration. The observation tally well with a study[12] who found that a decrease in Thyroid hormone stimulates Pituitary gland to secrete more TSH that in turn stimulates the gland. These endocrine changes are because of positive stimulation of Hypothalamo-pituitary thyroid axis.[13]

It was proved that DFMO blocks enhanced ODC (Ornithine-decaboxylase) activity.[14] This inhibition leads to cellular deprivation of Putrescine a polyamine that acts like an intracellular messenger and mediates responses to hormonal stimuli in target organs.[15] DFMO has been used by several workers to retard, arrest or prevent an increase in polyamine.[16] DFMO was found to be associated with decrease in LH output induced by repeated exposure to LHRH of pituitary tissue[17] removed on pro-oestrous. It has been found that administration of DFMO results in a cytostatic effect in a human breast adenocarcinoma model[18] and inhibits glioma cell growth and proliferation.[19]

CONCLUSION

It may be concluded that DFMO (Difluoro methyl ornithine) decreases cellular proliferation of Thyroid gland. This drug however does not disturb Hypothalamo-pituitary feed back system as shown by a rise in TSH concentration. This drug can be further investigated for its affectivity on other endocrine glands. Its hypothyroid effects can be reversed when given in combination with Putrescine which enhances thyroid cell growth and proliferation.[20] From a clinical perspective, polyamine metabolism inhibition could be a target for anti-neoplastic therapy.[21]

It is thus suggested that DFMO (Difluoromethyl ornithine) which is a specific inhibitor of ODC and in turn putrescine biosynthesis may be tried as an experimental model. Indeed clinical trials with DFMO, a selective inhibitor of Ornithine decarbo, oxylase show promise as inhibitors of carcinogenesis.[21–24] In order to support further development of DFMO as an antiproliferative, chemopreventive agent, preclinical safety studies on animals should be undertaken to evaluate potential for developmental toxicity.

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