Original Article

Reversal of difluoromethylornithine effects by the administration of putrescine in rats

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Abstract

Objective: To assess the reversal of Di-fluoromethyl ornithine (DFMO) effects by administration of putrescine on thyroid glands in rats.

Methods: The study was conducted on female rats weighing 248 to 320 grams at Quaid-e-Azam University, Islamabad in November end 2006. They were divided into three groups namely control group I treated with normal saline, DFMO treated group II at a dose of 50 mg/rat and DFMO and Putrescine group III received a combination of 50mg/rat of DFMO and 150µg of Putrescine, subcutaneously for five consecutive days. On sixth day, blood was collected by cardiac puncture and radioimmunoassay was performed to measure Serum T3, T4 and TSH levels in all groups.

Results: In group II there was a fall in T3, T4 concentration with significant rise in TSH concentration as compared to the control group. The combined administration of Putrescine and DFMO resulted in a rise in serum T3 and T4 with negligible fall in TSH.

Conclusion: DFMO induced hypothyroidism was reversed by the administration of Putrescine. It is thus concluded that hormone mediated response in thyroid tissue can be altered by altering ODC responsiveness of target tissue of female rats.

Keywords: Di-fluoromethylornithine, Putrescine, Rats, Thyroid (JPMA 61:752; 2011).

Introduction

Polyamines such as putrescine, spermidine and spermine are aliphatic amines that act as nucleotide precursors.[1] Present in all mammalian cells; they are essential for maintenance of cell growth and function.[2-6] The initial step of polyamine synthesis is the decarboxylation of L-ornithine. Their synthesis require an essential enzyme Ornithine-decarboxylase (ODC) that is induced by different stimuli such as high amino acid intake, viral infection, chemical carcinogens; tumour promoters various stressful and regenerative stimuli.[7,8] Based on these observations, inhibition of ODC has been considered to be an effective approach in cancer chemotherapy.[5] DFMO is a specific potent mechanism based irreversible inhibitor of ODC.[5,9] It was synthesized by Metcalf;[10] is water soluble and has a molecular weight of 236.65 amu. Since DFMO inhibits cell replication strikingly.[11] it has been used as chemoprotective agent in clinical trials sponsored by the National Cancer Institute against cancers of colon, breast, cervix, oesophagus and bladder.[2] The compound has a potential value as anti-tumour, anti-viral and anti-protozoal agent. This aim is acquired by significant changes in chromatin and DNA structure.[6] The current research was done with the background that DFMO acts as an antizyme for ODC activity on pituitary tissues.[12,13] Treatment with alpha-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), depletes the putrescine and spermidine content, and reduces the growth rate of Ehrlich ascites tumour cells.[14] The purpose of the study was to see whether these effects on Thyroid gland can be reversed by administration of putrescine or not.

Materials and Methods

Randomized controlled trial was carried out at Quaid-e-Azam University Islamabad after fulfilling the requirements of Ethical Review Board of the University in November 2006. Thirty female rats with an average weight of 248 to 320 gms were procured from National Institute of Health Islamabad. They were kept in the animal house in Department of Biological Sciences, at Quaid-e-Azam University Islamabad. They were provided with food at libitum and constant supply of drinking water at a temperature of 21± 3°C and lighting 10hr/14hr light/dark cycle. On the basis of treatment given they were divided into three groups namely; control (I), DFMO (II), Putrescine and combined treatment (III). Group I was given 0.5 ml normal saline. Group II was given a dose of 50 mg/rat of DFMO; which was achieved by dissolving 250mg/ml of drug in 0.9% saline. Group III received putrescine 150µg/rat achieved by dissolving 300µgm/ml of the compound in 0.9% saline; together with DFMO (50mg/rat). All doses were given at 10.00 hours subcutaneously for five days. Cardiac puncture of all the groups was done on the sixth day at 1400 hours by a longitudinal incision along the left side of sternum. Blood samples were collected from the heart. Serum was separated and stored at -20°C until analysis.

Radioimmunoassay of T3, T4 and TSH was performed independently using competitive radio-immunoassay technique. Thyroxine concentration was determined by dispensing 50 µ liter of standard or sample was with 500 µ litre of Amerilex-M antibody suspension in Amerilex-M T4 kit. The tubes were incubated for 45 minutes at 18° to 28°C and then were decanted and allowed to drain for 5 minutes. Bound radioactivity was counted in a LKB (DP 5500) gamma counter.

T3 immunoassay was done with Amerilex-M. T3 RIA.100 µliter of standard sample was dispensed with 500 µliter of trace and 500 µliter of Amerilex-M antibody suspension. They were incubated for 60 minutes at 37°C. Following incubation all the tubes were decanted and allowed to drain. Bound radioactivity was counted in a LKB (DP 5500) gamma counter.

Thyrotropin concentration in serum sample was determined by solid phase (Iodine 125) radioimmunoassay.100 µ litre of sample or control was incubated with 100 µ litre of tracer reagent for 90 minutes at 37 ± 1°C. After 1 to 2 minutes tubes were decanted again. A total of three washes were done. Bound radio-activity was counted in a LKB (DP5500) gamma counter.

T3, T4 and TSH levels, and the mean ± standard error was calculated. The results of the groups treated were compared with the control group using Student significant t-test. The values were considered significant when the values of p were ± 0.01 and highly significant when P ± 0.001 values were obtained. Results were considered insignificant with $P < 0.05$. The T3, T4 and TSH levels of groups with DFMO and combined treatment with DMFO and Putrescine were compared with control group.

Results

The division of the groups with respective treatment are shown in Table-1 and the hormone analysis in Table-2.

1. Triiodothyronine (T3):

In Group II the concentration of T3 was reduced from (0.60±0.13 n mol/l) to (0.44±0.08nmol/l). However the level recovered to 0.66 ±0.10.082 n mol/l in combined treatment group (Figure-1).

Table-1: Division of groups.

No	Group	Treatment
I	Control	0.9%saline
II	DFMO	50mg/rat
III	Combined	150µg+50mg

2. Thyroxin (T4):

A significant decrease in the concentration of T4 was observed in DFMO treated group i.e. 23.52±6.42 nmol/l as compared to control group (30.06±5.51 nmol/l). A rise inT4 level was observed when putrescine was added with DFMO (33.16±7.37nmol/l) (Figure-2).

3. Thyrotropin (TSH):

Regarding TSH concentration, a significant fall was observed in Group II (0.17±0.01 n mol/l) which was raised to 0.15±0.03 n mol/l in combined action group but the p value was found to be statistically insignificant (Figure-3).

Table-2: Comparison of hormone analysis in all groups.

	T3	T4	TSH
I	0.60 ± 0.13	30.06 ±.51	0.14±0.02
II	0.44 ± 0.08	23.52 ± 6.42	0.17±0.01
III	0.66 ± 0.10.082	33.16 ± 7.37	0.15±0.03

Figure-1: Comparison of T3 Values.

Figure-2: Comparison of T4 Values.

Figure-3: Comparison of TSH Values.

Discussion

The study was conducted to integrate the effects of DFMO, ODC and polyamine metabolism on thyroid tissue. Administration of DFMO decreased thyroid hormone levels as it restricts cell replication strikingly.[11] From the clinical perspective polyamine metabolism inhibition could be a target for antineoplastic therapy.[15] Clinical trials with 2α-difluoromethyl-ornithine a selective inhibitor of ODC activity showed promise as inhibitors of carcinogenesis.[15,16] It is used in Phase II clinical trials sponsored by the National Cancer Institute as a chemopreventive agent against cancers of colon, breast, cervix and oesophagus.[17,18] In Phase III trials the compound has been tried for prevention of Bladder carcinoma[1] and has shown a potential value as anti-tumour, agent. Agreeing to the fact that over expression of ODC is characteristic of tumour development and progression,[19] inhibitors of ODC are thus required for restoration of normal phenotype.[20] The enzyme synthesis decreases in response to short-term exposure to ODC inhibitors which is not accompanied by any change in the mRNA content.[21] The synthesis of ODC protein can be regulated at the level of transcription and translation of ODC mRNA. DFMO has been used by several workers to retard arrest or prevent an increase in polyamine.[22] Polyamine depletion brought about by DFMO and other inhibitors leads to a large decrease in AdoMetDC activity (S-Adenosyl methionine decarboxylase). Keeping in mind DFMO, ODC and polyamine biology,it was analyzed that DFMO blocked the LHRH induced preovulatory surge of leutinizing hormone (LH)[12] in estrous

rats. This LH output occurs due to increased ODC activity of pituitary tissue, which is inhibited by the administration of DFMO. The inhibition was short-lived and was reversed by the simultaneous addition of putrescine.[12] Treatment with alpha-difluoromethylornithine (DFMO), depleted the putrescine and spermidine content in Ehrlich ascites tumour cells.[14] The addition of putrescine, rapidly restored the intracellular putrescine and spermidine pools and completely reversed the antiproliferative effect of DFMO on the growth rate of tumour cells.[14]

In another study TRH stimulated the secretion of prolactin from lactotrophs of anterior pituitary. This effect was brought about by stimulation of ODC activity which is associated with TRH-induced formation of IP^3 and DAG. In this way polyamines behave as intracellular messengers possibly by enhanced Ca^2 transport.[23] DFMO significantly reduced TRH-dependent prolactin release from pro-oestrous cells[24] as well as reduced putrescine content of the gland.[25]

In our study the administration of DFMO at a rate of 50mg/animal caused a significant, fall in T3 (free) and T4 (total) levels and an increase in TSH concentration. This observation matches findings of Schulze[9] who proposed DFMO as a potent irreversible and specific inhibitor of ODC: Ornithine- decarboxylase activity. In our research DFMO induced hypothyrodism was reversed by the administration of Putrescine.

Conclusion

It is thus concluded that hormone mediated response can be altered by altering ODC responsiveness of target tissue. The reversal of anti thyroid effects of DFMO by Putrescine brings a new insight for the combination of putrescine with DFMO in the management of neoplasms.

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