

Status of Thyroid Hormone During 3-Methylcholanthrene Induced Carcinogenesis with Thyroid Stress

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Abstract: Hormone act as a promoter in the carcinogenic process by inciting cell to produce substances that are carcinogenic. The thyroid provides the simplest example of association of cancer and hormone through its profound mutagenic effects on the growth and development of the body. However, the relationship between thyroid hormones and carcinogenesis is still not very conclusive. This investigation was therefore conducted to evaluate further the effect of thyroxine on T3, T4 and TSH status during 3-methylcholanthrene (3MC) induced carcinogenesis. In this investigation a significantly higher mean values of T3, T4 and TSH ($P < 0.01$) were observed in the group of animals treated with 0.5 mg 3-methylcholanthrene from the 10th day onwards to the 120th day, the end of the experiment suggesting a hypersecretion of thyroxine. Daily administration of $10\mu\text{g}$ thyroxine alone exhibited biphasic trend and inhibitory effect on T3 and T4 content where T3 showed steep decline of -1.75% on 120th day and T4 with -26.72% below the normal base line. On the contrary there is highly significant initial increase ($P < 0.01$) in the mean TSH values with percent deviation of 320.17%. In the animal group treated with thyroxine-3MC combination, a decrease in the thyroid function i.e. both T3 and T4 content throughout the experimental period up to 120th day was observed which may be due to some amount of suppression in the thyroid function caused by supplementation of thyroxine. However, comparative decrease of TSH content after combined treatment of thyroxine-3MC than that of 3MC alone may suggest some amount of recovery with both 3MC which act as a thyroid function enhancer and thyroxine supplementation though it did not touch the normal base line.

Key words: Thyroxine • 3-Methylcholanthrene (3MC) • Carcinogenesis

INTRODUCTION

The status of hormones as inducer of malignant transformation and the role of hormones in controlling growth and character of tumors are well established [1, 2]. Data from animal experimental works and clinical observation suggested that thyroid secretion plays an important role in the evolution of cancer, particularly of the genitalia and the breast [3, 4]. Hyperthyroidism has been described in various carcinomas as chorion carcinoma, embryonal cell carcinoma, urogenital carcinoma and laryngeal carcinoma [5, 8]. The thyroid provides simplest example of tumor formation through hypofunction and feed back failure. Thyroid hormone regulates proliferation and differentiation of cells, via its nuclear thyroid receptors (TR). These processes have been shown to be abnormally regulated during carcinogenesis [9]. Mutate or truncated forms of certain

members of the thyroid receptor superfamily have oncogenic potential. TRs are over expressed in several tumor types and act as a cancer promoting factors [10, 11]. Increase of thyroid hormone level induces thyroid receptors in the cell nucleus to increase DNA transcription which increases the synthesis of specific mitochondrial proteins and ultimately act as a major factor for the origin of neoplastic cell [12-14]. Importantly, research workers have identified high frequencies of mutations of thyroid hormone nuclear receptors alpha (TR- α) and beta (TR- β) genes in human hepatocellular carcinoma, renal clear cell carcinoma and papillary thyroid carcinoma [15-17]. However, the relationship between thyroid hormone and carcinogenesis is still not very conclusive. Therefore; the objective of this study was to evaluate further the effect of thyroxine on T3, T4 and TSH during 3-methylcholanthrene induced carcinogenesis.

MATERIALS AND METHODS

20 healthy male albino mice weighing between 55gm-60gm were taken as the experimental animals. Animals were randomly divided into five groups - Group I - Normal control group, Group II- Castor oil control, Group III- Thyroxine treated, Group IV- 3-methylcholanthrene treated(3MC), GroupV- 3-methylcholanthrene + Thyroxine combination treated. Each animal of the castor oil group was exposed to single dose of 0.25ml (250µl) of castor oil by intraperitoneal injection. Thyroxine treated group consist of male albino mice each of which was fed daily with 10µg of thyroxine solution till the end of the experiment. In the 3- methylcholanthrene treated group each animal was administered intraperitoneal a single dose of 0.5 mg of 3-methylcholanthrene in 0.25ml of castor oil. On the onset of the experiment in the thyroxine supplemented. 3-methylcholanthrene treated group all animals were intraperitoneal administered with a single dose of 0.5mg 3-methylcholanthrene with simultaneous daily oral administration of 10 µg of thyroxine. All animals were kept under the same environmental conditions and were fed with the same standard diet.

All chemicals were used of highest grades available. For estimation of T3, T4 and TSH, ELISA (solid phase sandwiched enzyme immunoassay) method was adopted by using the set of reagent supplied in the form of a kit by Syntron Bioresearch, Inc.

The data obtained during the period of investigation were statistically analyzed The mean, the standard error of mean, coefficient of variation (%) and the percentage

deviation for each set of data were calculated and compared with different set of data by applying standard statistical procedure to evaluate the changes among different groups in the study.

The level of significance between two sets of data was calculated according to student “t” test. Probability i.e. p value <0.01 for two sets of data were taken as significant.

Blood Collection: Before initiation of the experimental part blood samples were collected from the whole general pool of acclimatized animals to get a normal baseline on the day “zero” of the experimental period. Subsequently, blood samples were collected from each of the individual group along with the normal group on 10th, 15th, 20th, 25th, 30th, 45th, 60th, 75th, 90th and 120th days of treatment.

RESULTS

The results obtained in the present investigation were summarized in fig. 1,2 and 3.

In the animal group receiving daily dose of thyroxine the trend line of serum T3 exhibited a biphasic character with -20% to -50% initial decrease up to 15th day of thyroxine treatment and a decrease of -21.9% from the normal base line on 45th day and with 20% to 80% increase between 20th to 30th and 60th to 90th days of thyroxine treatment (Fig. 1). But there was a steep decline of -1.75% on 120th day below the normal baseline. The serum T3 values exhibited similar biphasic trend line with maximum depletion on 45th day in case of solitary administration of 3-methylcholanthrene.

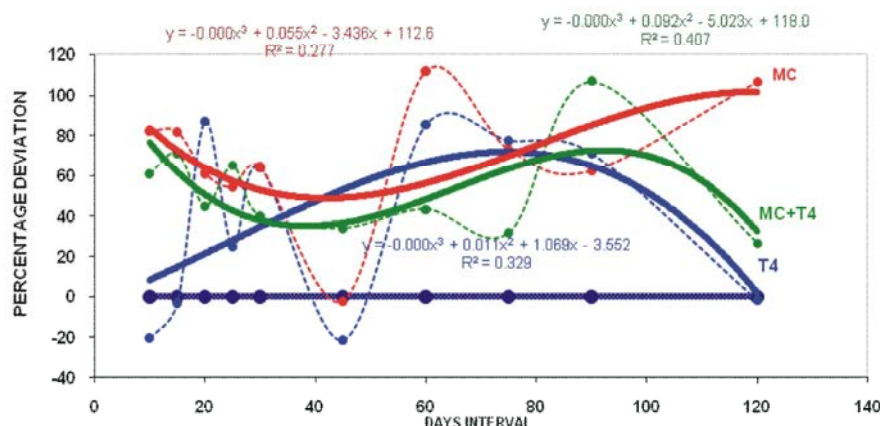


Fig. 1: Presenting the percentage deviation of triiodothyronine (T₃) in serum of different experimental groups from the mean values of the normal control. (T₄: Thyroxine; MC+T₄: 3- Methylcholanthrene+Thyroxine; MC: 3- Methylcholanthrene)

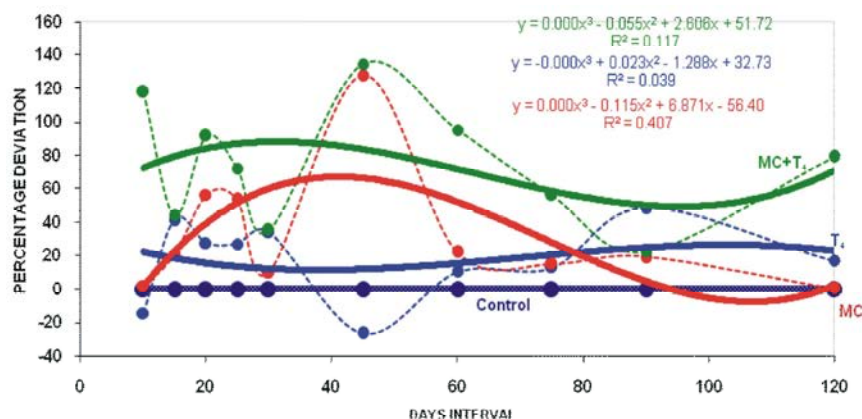


Fig. 2: Presenting the percentage deviation of thyroxine (T_4) in serum of different experimental groups from the mean values of the normal control.

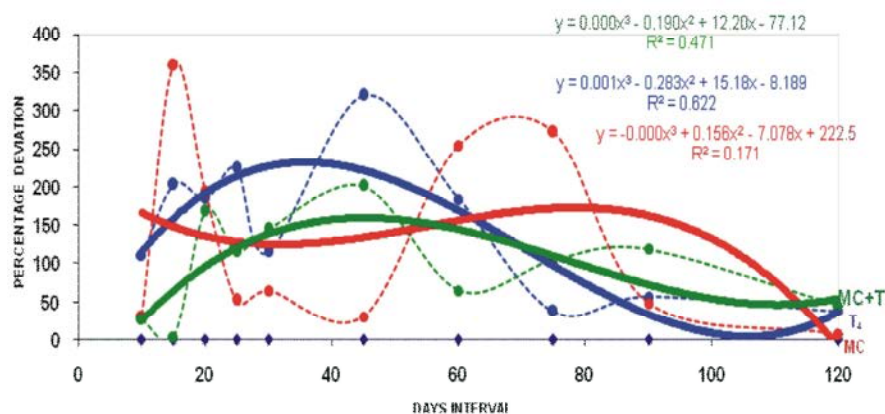


Fig. 3: Presenting the percentage deviation of thyroid stimulating hormone (TSH) in serum of different experimental groups from the mean values of the normal control.

Similar effect was also seen in 3-methylcholanthrene and thyroxine combined group. But there were some irregularities in the serum T3 trend line in the terminal part of the experiment showing gradual decline with maximum of 26% above baseline from 45th day to the terminal part of the experiment and a steep and sudden increase of 106.85% above the normal base line on 90th day (Fig. 1).

The serum T_4 level in the thyroxine treated group also revealed similar biphasic trend line with step decline of -26.72% below the base line on 45th day of the experiment (Fig. 2). But an opposite result with a increased peak of 127.70% was observed in 3-methylcholanthrene treated group. In contrast to the T3 content, the T_4 values gradually decreases from 45th day onwards and touches the baseline on 120th day. However, simultaneous administration of 3-methylcholanthrene and thyroxine exhibited an irregular and fluctuating effect on T_4 level throughout the experimental period from 10th to 120th day

though the trend line was almost similar to that of the trend line with solitary 3-methylcholanthrene treatment (Fig. 2).

The TSH trend line exhibited a highly significant increase ($P < 0.01$) in the values between normal control and thyroxine treated group (Fig. 3). On daily administration of solitary thyroxine, the TSH trend line attains its peak on 45th day with percent deviation 320.17% and afterwards gradually decline in the later part of the experimental period. On single dose administration of 3-methylcholanthrene the TSH content reached its highest peak much earlier that is on 15th day of the experimental period with 360.48% deviation and maintained this increasing trend significantly ($P < 0.01$) on 60th and 70th day with percent deviation 272.58% from normal baseline. However, during the terminal part of the experiment these increase in TSH content were not sustained (Fig. 3). On simultaneous administration of

3-methylcholanthrene and daily dose of thyroxine a contrasting behavior was observed in the TSH trend line up to 90th day when compared with that of the solitary 3-methylcholanthrene treated animal group except the terminal part of the experiment (Fig. 3).

DISCUSSION

Experimental works have established the close interconnection between disturbances of hormone equilibrium and the appearance of tumors [19]. Disorders of the hormone equilibrium may act as a powerful endogenous factor contributing to genesis of tumors [20, 21].

The phase of the present experiment concerned with the monitoring of the thyroid related hormone profile under the different combinations of the experimental set up showed that daily administration of 10 μ g dose of thyroxine throughout the experimental period maintains a circulating thyroxine level which is not more than 20% above the basal thyroxine level of the control group. The T4 elevation trend in the thyroxine treated group showed a symmetrical harmony around 60th day which is the mid point of experimental duration (Fig. 2). The symmetrical distribution of the gradual and moderate fluctuation in circulating T4 level on daily thyroxine administration tallies perfectly with the established and expected behaviour of feed back mediated control between the T4 –TSH duplex of hormonal regulation (Figs. 2 and 3).

In total contrast to the trend of T4, on daily administration of thyroxine, the circulating thyroxine under single dose administration of 3-methylcholanthrene was elevated to much higher extent reaching the peak value between 42 to 45th days of initial 3-methylcholanthrene exposure (Fig. 2) which subsequently declines to the basal level of the control group near the terminal phase of the experiment. The observed trend of circulating T4 on 3-methylcholanthrene exposure suggests 3-methylcholanthrene as a potent stimulator for T4 synthesis (Fig. 2) which also produces proportionate simultaneous depression of TSH production (Fig. 3). The effect of 3-methylcholanthrene administration on T3 the other member of thyroid hormone with higher biological activities but lower circulating concentration appeared to be proportionately more augmentive than on T4 as observed from the trends of the experiment (Fig. 1).

The simultaneous administration of single dose of 3-methylcholanthrene and a daily dose of 10 μ g of thyroxine depicts an irregular and fluctuating effect on T3 and T4 content. However, these irregularities were more prominent in the terminal part of the experiment in T3 content. On the other hand the TSH trend exhibited some suppressing effect under 3-methylcholanthrene-thyroxine combination in comparison to solitary 3-methylcholanthrene treatment which is either due to 3MC or thyroxine treatment or combined effect of both. The effective correlation between the level of TSH content and thyroxine (T4) was clearly evident in the present study throughout the experimental period from 10th to 120th day except slight fluctuation in the intermediate phases of the experimental period.

CONCLUSION

The combination of cholanthrene and thyroxine appears to set the circulating thyroid hormone levels at a higher level than solitary administration of thyroxine along with proportionate alteration in TSH. The markedly elevated trend of hormone was the major metabolic adjustment under the influence of single dose of (0.5mg) 3-methylcholanthrene exposure which may suggest marked augmentation of thyroid activity associated with cholanthrene metabolism either for xenobiosis or carcinogenesis. The resulting effect of cholanthrene – thyroxine combination on the relative decrease of circulating thyroid hormone and its regulatory hormone may be termed as stabilization but at a slightly hyperthyroid state.

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