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Effect of administration of tamoxifen citrate on xanthine oxidase activity of liver during 3-methylcholanthrene induced carcinogenesis in female albino mice

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Abstract
Tamoxifen citrate, the non-steroidal anti-estrogen and the first selective estrogen receptor modulator is the treatment of choice for patients with all stages of estrogen receptor positive breast cancer. Although Cytochrome P-450s (CYP) are well known to be responsible for much of its metabolism, fragmentary studies have been carried out to analyze the activity of xanthine oxidase, a non-CYP drug metabolizing enzyme, in tamoxifen metabolism. So, the present investigation is aimed to ascertain the effect of tablet and nano-formulation of tamoxifen on xanthine oxidase expression during 3-methylcholanthrene-induced carcinogenesis. It is found that oral administration of tamoxifen in both the formulations significantly ($p < 0.01$) decreases the xanthine oxidase activity in liver tissue of female mice. We speculate our results to be beneficial for the better understanding of drug metabolism and intensity, which in turn will pave the way for new and efficient nano-formulations of drug coming into therapeutic use.

Keywords: Tamoxifen citrate; xanthine oxidase; drug metabolism; carcinogenesis.

Introduction

Tamoxifen citrate has long been the only option for the treatment of hormone-dependent breast cancer. Tamoxifen citrate (TMX) belongs to a class of non-steroidal triphenylethylene derivatives and is considered the first selective estrogen receptor modulator (MacGregor and Jordan 1998; Marcsek *et al.* 2004). The compound administered to patients is the citrate form of tamoxifen, i.e., tamoxifen citrate. The chemical structure shows that the core is composed of a C-20 skeleton with ethyl group at C-2 and *N*, *N*-dimethyl ethane – amino oxy group at C-4 as subsequent. The structure of TMX has been elucidated by IR spectroscopy, Raman spectroscopy, X-ray powder diffraction techniques (Kojima *et al.* 2007; Gamberini *et al.* 2007) .The antitumor effect of tamoxifen is related to growth arrest and induction of apoptosis (Mandlekar and Kong 2001) mediated by linkage to the intracellular estrogen receptor on breast cancer cells, blocking of steroid hormone action (Jordan 1993) and inhibition of protein kinase C (O'Brian *et al.* 1985). Tamoxifen has some major side-effects following long-term therapy in postmenopausal breast cancer patients, including higher incidence of endometrial cancer, liver cancer, thromboembolic disorders, and development of drug resistance (Jordan 1995). Tamoxifen citrate is characterized by spectrophotometric (UV, MS, GC-MS) and chromatographic (HPLC, GC, TLC) method of analysis (Adam *et al.* 1981; Sastry 1992). Mixed-function oxygenase (MFO) enzymes are mostly represented by cytochrome P-450s, Aryl hydrocarbon hydroxylase and xanthine oxidase which play a crucial role in the biotransformation, metabolism, and detoxification of xenobiotics and foreign compounds that are introduced in our body system. MFO includes a group of enzymes which plays an essential role in the metabolism of a broad range of xenobiotics including carcinogens and endogenous and exogenous substrates (Guengerich and Liebler 1985). Both xanthine oxidase and xanthine dehydrogenase, collectively called xanthine oxidoreductase, is known as the rate-limiting enzyme in purine and pyrimidine catabolism. Their role in metabolism of a large number of other physiological compounds has been reported (Hille and Nishino 1995). Tamoxifen is metabolized in the liver and several metabolites have been detected in serum (Wolf and Jordan 1992). The metabolism of tamoxifen is complex (Lien and Lonning 2000) and a wide inter-individual variation in tamoxifen metabolizing enzyme activity has been reported (Hu *et al.* 2003). Recent studies have demonstrated the ability of xanthine oxidases to metabolize xenobiotics including drugs (Strolin *et al.* 2006) and a number of

anti-cancer compounds to their active metabolites (Pritsos 2000).

At the present time, much attention is being paid towards the design and synthesis of composite nanoparticles (Pappas and Jordan 2002) whose application may find great significance in the pharmaceutical industry where they can be used in drug delivery. Using nanoparticles (NPs) for drug delivery of anti-cancer agents has significant advantages such as the ability to target specific locations in the body, the reduction of the overall quantity of drug used, and the potential to reduce the concentration of the drug at non-target sites resulting in fewer unpleasant side-effects. The cross-linked guar gum nanoparticles loaded with tamoxifen citrate are reported to exhibit sustained release of the drug and delayed apoptosis over a long period of time making it suitable for cancer treatment (Sarmah *et al.* 2012).

Although it is well demonstrated that xenobiotics induces the activities of drug metabolizing enzymes, the effect of tamoxifen citrate on xanthine oxidase activity during carcinogenesis is still sporadic. The present investigation is therefore aimed to examine the xanthine oxidase activity during 3-methylcholanthrene-induced carcinogenesis with simultaneous treatment of tamoxifen citrate in its two formulations, i.e., the tablet formulation and the nano-formulation.

Materials and Methods

All the chemicals used in this study were of analytical grade and were obtained from Sigma-Aldrich, Merck and Spectrum Chemicals Pvt. Ltd.

(i) Drugs: Tamoxifen citrate, which is sold under the trade name 'Nolvadex', was purchased locally. Pure tamoxifen citrate which is required for the preparation of cross-linked guar gum nanoparticles was gifted by CDL, Kolkata, India and was prepared by a co-worker.

(ii) Animals: The present study is conducted on healthy female albino mice weighing 25–30 g which are acclimatized in the animal room for 4 weeks and fed on standard animal diet. Adequate measures were taken to minimize the discomfort to the mice. The handling and the experiments were carried out in accordance with the International standards on animal welfare as well as being compliant with local (Ethical Committee of Animal Welfare of Guwahati University, Guwahati, Assam, India) and national regulations. As per the plan of the study, the targeted numbers of animals are randomly divided as follows:

(a) Group I *(Normal control group):* 10 healthy female albino mice without any sign of deficiencies are randomly selected for normal control group and maintained throughout the whole period of experiment in the same condition.

(b) Group II *(Castor oil control group):* 10 healthy animals are randomly selected for this group and each animal of the group is exposed to single dose of 0.5 mL of castor oil by intra-peritoneal injection.

(c) Group III *(3-methylcholanthrene [3-MC] treated group)*: This group consists of randomly selected animals from the normal healthy already acclimatized animal pool. A single dose of 0.5 mg of 3-MC in 0.5 mL of castor oil is administered intra-peritoneally to each animal of this group. During the whole period of experiment this group received normal standard diet.

(d) Group IV (*3-methylcholanthrene [3-MC] & tamoxifen [Tablet formulation] treated group):* The animals for this group are selected from the acclimatized general pool. They are intra-peritoneally administered with a single dose of 0.5 mg of 3-MC dissolved in 0.5 mL of castor oil and orally fed with 0.01 mg of tablet form of TMX daily.

(e) Group V (*3-methylcholanthrene [3-MC] & tamoxifen [Nanoformulation] treated group):* The animals for this group are selected from the acclimatized general pool. They are intra-peritoneally administered with a single dose of 0.5 mg of 3-MC dissolved in 0.5 mL of castor oil and orally fed with 0.01 mg of nano-formulation of TMX daily.

(iii) Collection of tissues: The mice are anesthetized by diethyl ether and dissected to collect liver tissue. The tissue is dried over a filter paper and immediately weighed and recorded. The tissue homogenate is prepared in deionized water with the help of homogenizer. The tissue is collected from the normal control as well as experimental mice on the desired days, i.e., 15th, 30th, 45th, 60th, 75th, 90th, and 120th days of treatment.

Methods of evaluation: Xanthine oxidase activity in hepatic tissue is estimated by the method of Fried and Fried (1974) and the final results are expressed as unit/mg of protein. The total protein in the hepatic tissue is determined by Lowry's method (Lowry *et al.* 1951). The estimations are done by using UV-visual spectrophotometric method utilizing assisted analytical system. The results

obtained are statistically analyzed and compared between different groups by applying standard statistical procedures. The data are expressed as mean value ± SE. Statistical significance of difference between various treatments are analyzed by Student's "t" test. P-values >0.05 and <0.01 were considered as statistically significant.

Results

The mean xanthine oxidase activities in the normal control group of animals are found to be 18.81 ± 0.41 on 15th day, 18.76 ± 0.38 on 30th day, 10.60 ± 0.33 on 45th day, 22.21 ± 0.35 on 60th day, 20.34 ± 0.38 on 75th day, 21.42 ± 0.32 on 90th day, and 18.78 ± 0.28 on 120th day of and 18.78 ± 0.28 on 120th day of treatment. In castor oil control group the xanthine oxidase activities ranges from 18.62 ± 0.30 to 22.26 ± 0.45 unit/mg protein (Figure 1).

In the 3-MC-treated group, the mean xanthine oxidase activities are 21.80 ± 1.05 on 15th day, 24.60 ± 1.18 on 30th day, 23.70 ± 0.62 on 45th day which increases to 25.80 ± 0.74 on 60th day, and 30.40 ± 0.89 on 75th day of treatment. The enzyme activity declines to 27.30 ± 0.98 on 90th day and 23.70 ± 1.19 unit/mg protein on 120th day of treatment (Figure 1).

In the group of animals treated with 3-MC and tablet form of tamoxifen together, the mean xanthine oxidase activity is observed as 5.71 ± 0.43 unit/mg protein on 15th day. The mean value is found to be highest as 9.22 ± 0.24 on 30th day of treatment which is found to be declining as 7.44 ± 0.43 on 45th day, 5.70 ± 0.23 on 60th day and 4.40 ± 0.39 unit/mg protein on 75th day of treatment. The enzyme activity is found to be increased to 7.63 ± 0.67 on 90th day of treatment which again decreases to 6.70 ± 0.77 unit/mg protein on 120th day of treatment (Figure 1).

In the group of animals treated with 3-MC and nano-formulation of tamoxifen citrate together, the mean xanthine oxidase activity is found to be lowest as 12.03 ± 0.81 on 15th day of treatment. The mean values of the enzyme activity are observed as 18.22 ± 0.68, 15.10 ± 0.75 and 17.17 ± 0.75 unit/mg protein on 30th, 45th, and 60th day of treatment respectively. The xanthine oxidase activity is found to be highest on 75th day of treatment as 21.91 ± 0.81 unit/mg protein which declines to 16.21 ± 0.68 on 90th day and 15.27 ± 0.70 on 120th day of treatment (Figure 1).

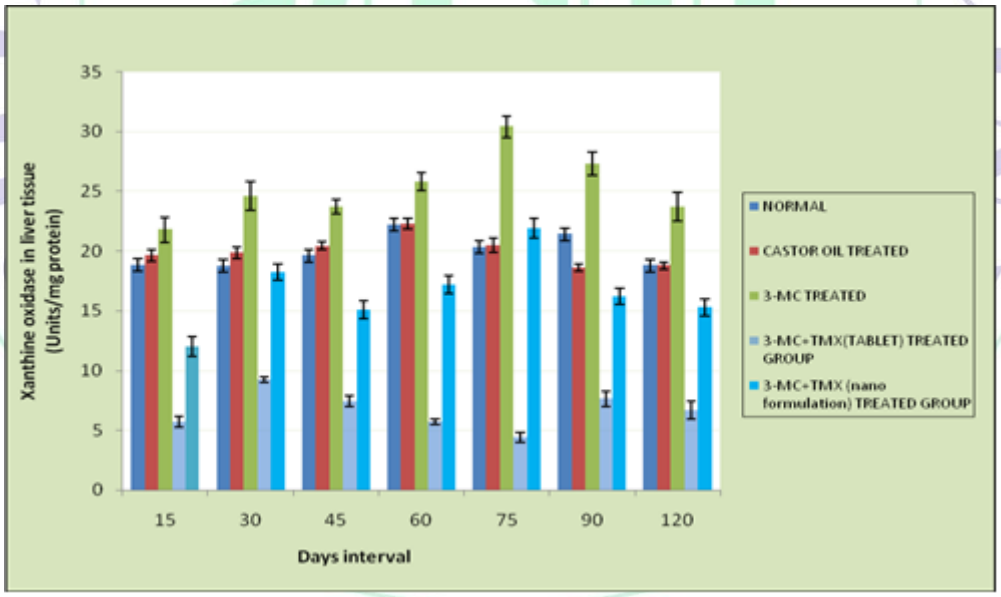

Figure 1: Presenting the mean values with SEM of xanthine oxidase (unit/mg protein) in liver tissue of different experimental groups at different days interval.

Discussion

Xanthine oxidases are some major oxidative enzymes which are involved in the metabolism of drugs and xenobiotics. Xanthine oxidase is a critical source of reactive oxygen species (ROS) in inflammatory disease (Kelly *et al.* 2010). Earlier findings revealed that xanthine Oxidase-derived ROS is a novel and critical component of hypoxia-inducible factor 1-α regulation in glycolytic-dependent (U251-MG) phenotype of glioma cells, pointing toward a more general role of this transcription factor in tumor progression (Griguer *et al.* 2006). In the present study, a single dose of 3-

methylcholanthrene injected intra-peritoneally increases the xanthine oxidase activity level 15% from normal base line to a maximum deviation of 49% on the 75th day of treatment (Table 2). However, this increasing trend is not regular and gradual. The percent increase of xanthine oxidase activity is found significant ($p < 0.01$) from normal control and castor oil control groups.

Although xanthine oxidase activity has been determined in a number of animal tumors and in a few human tumors, little is known about the expression of xanthine oxidase activity in cancer cells (Shannon *et al.* 2003; Stirpe *et al.* 2002). Simultaneous administration of tamoxifen citrate in tablet formulation in animals treated with single intra-peritoneal dose of 3-methylcholanthrene shows a significant decrease ($p < 0.01$) (Table 1) of xanthine oxidase activity level compared with 3-methylcholanthrene-treated group and even normal control and castor oil control group. However, the decrease in the enzyme activity is observed to be steady throughout the experimental period with deviations within the range of 50– 80% (Table 2). Acharjee *et al.* (2010) has also demonstrated declined xanthine oxidase activity in liver and kidney tissue in albino mice when intramuscularly injected with nandrolone decanoate, an anabolic steroid. Nandrolone decanoate which is considered as a xenobiotic like “tamoxifen” (non-steroidal) also tends to decrease the enzyme activity though it is steroidal in nature. Decreased xanthine oxidase activity is also linked with various types of cancer. Patients with breast cancer and different other types of cancer are reported with decreased oxidoreductase expression (Linder *et al.* 2006).

The animals of the combination group treated with tamoxifen-loaded nanoparticles with a single dose of 3-methylcholanthrene shows a slightly declined enzymatic activity than the normal control and castor oil control groups. Xanthine oxidase activity declines by 36% from the normal control baseline during the initial phase of study which is again observed to be reaching the normal base line on 30th and 75th day of treatment. When compared with the combination group of 3-methylcholanthrene and tablet formulation of tamoxifen citrate, the xanthine oxidase activity is much elevated ($p > 0.05$). However, it remains significantly lower ($p < 0.01$) than the 3-methylcholanthrene alone treated group (Table 1).

These enzymes are reported to produce therapeutically active metabolites and modulate the efficacy of therapeutically active drugs or contribute to detoxification (Strolin *et al.* 2006). In general, throughout the entire period of study a decline in the enzymatic activity is observed in the groups of animals treated with tamoxifen citrate in its two formulations compared with the animals treated with 3-MC only. A correlation between enhanced growth of tumor, enzymatic protein degradation, and dropping of xanthine oxidase activity as demonstrated by previous findings (Shmarakov and Marchenko 2009) is in conformity with our present results.

Conclusion

In conclusion, results from the present investigation establish that tamoxifen citrate in both the formulations, is a potential inhibitor of xanthine oxidase enzyme activity in liver of female mice during chemical carcinogenesis. For the first time, we have reported the nature of xanthine oxidase activity during tamoxifen citrate treatment in its nano-formulation. The trend of enzyme expression of tamoxifen in its nano-formulation seems to be significantly elevated when compared with its tablet formulation in all the experimental groups. The relevance of our findings in relation to the therapeutic use of tamoxifen in its nano-formulation is still unclear and therefore we suggest much more detailed investigation in this area of research.

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Ethical Approval

This work was approved by the ethical committee of Department of Zoology, Gauhati University.

Conflict of Interests

Authors have no conflicting interests.

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Table 1: Significance of difference in the mean values of xanthine oxidase (unit/mg protein) in liver tissue between different experimental groups at different intervals.

Groups		Days of treatment						
		15th day	30th day	45th day	60th day	75th day	90th day	120th day
Between normal control and castor oil-treated group	t	-1.31	-1.69	-1.74	-0.09	-0.22	6.51	-0.02
	P	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01	>0.05
	df	18	18	18	18	18	18	18
Between normal control and 3-MC-treated group	t	-2.69	-4.67	-5.86	-4.38	-10.48	-5.71	-4.03
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18	18
Between normal control and 3-MC +TMX (Tablet)-treated group	t	23.39	19.87	22.52	40.27	30.07	18.63	14.73
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between normal control and 3-MC + TMX (Nanoformulation)-treated group	t	7.62	0.68	5.49	6.15	-1.76	6.95	4.68
	P	<0.01	>0.05	<0.01	<0.01	>0.05	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between castor oil and 3-MC-treated group	t	-1.88	-1.97	-4.68	-4.07	-9.44	-8.51	-4.02
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18	18
Between castor oil and 3-MC +TMX (Tablet)-treated group	t	21.40	19.00	24.30	33.12	23.66	15.05	14.74
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between castor oil and 3-MC +TMX (Nanoformulation)-treated group	t	7.99	1.93	6.49	5.85	-1.43	3.26	4.69
	P	<0.01	>0.05	<0.01	<0.01	>0.05	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between 3-MC and 3-MC + TMX (Tablet)-treated group	t	14.24	12.82	21.68	26.10	26.80	16.53	11.97
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between 3-MC and 3-MC + TMX(Nanoformulation)-treated group	t	7.34	4.69	8.86	8.22	7.07	9.32	6.11
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18	18
Between 3-MC + TMX (Tablet) and 3-MC + TMX (Nanoformulation)-treated group	t	-6.87	-12.50	-8.91	-14.70	-19.46	-9.03	-8.24
	P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18	18

Table 2: Percentage deviation of xanthine oxidase (unit/mg protein) in liver tissue of different experimental groups from the mean values of normal control group.

Groups	Mean % Deviation	Days of treatment						
		15th	30th	45th	60th	75th	90th	120th
Normal control group	Mean	18.81	18.76	19.60	22.21	20.34	21.42	18.78
Castor oil control group	% deviation	4.30	5.86	4.18	0.22	0.74	-13.07	0.05
3-MC-treated group	% deviation	15.89	31.13	20.92	16.16	49.46	27.45	26.19
3-MC +TMX (Tablet) treated group	% deviation	-69.64	-50.85	-62.04	-74.33	-78.37	-64.38	-64.32
3-MC +TMX (Nanoformulation) treated group	% deviation	-36.04	-2.88	-22.96	-22.69	-7.72	-24.32	-18.69

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