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Decreased aryl hydrocarbon hydroxylase activity in liver tissue during Tamoxifen citrate treatment in 3-methylcholanthrene induced carcinogenesis

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ABSTRACT

The anti- estrogenic drug Tamoxifen is widely used in the treatment of breast cancer. Aryl hydrocarbon hydroxylases are important drug metabolizing enzymes and activity of these enzymes is important for the understanding of drug metabolism and intensity. So, a study has been carried out to ascertain the effect of Tamoxifen citrate (tablet and nanoformulation) on Aryl hydrocarbon hydroxylase activity during 3-methylcholanthrene induced carcinogenesis. It is found that oral administration of Tamoxifen significantly ($p<0.01$) decreases the Aryl hydrocarbon hydroxylase activity in liver tissue of female mice. Altered enzyme activity is noticed during oral administration of Tamoxifen citrate in its nanoformulation too. Because alterations in the enzyme activity correspond to the pharmacological action of drugs, our assessment may help in the field of cancer treatment.

KEYWORDS: *Aryl hydrocarbon hydroxylase; Carcinogenesis; Drug metabolism; 3-methylcholanthrene; Tamoxifen citrate; Xenobiotic metabolizing enzymes.*

INTRODUCTION

Tamoxifen citrate (TMX), a triphenylethylene derivative, is a major therapeutic agent for breast cancer. Tamoxifen citrate is the citrate salt of an antineoplastic nonsteroidal selective estrogen receptor modulator (SERM). It acts like estrogen in some tissues (bone, liver, and uterus) and as an antiestrogen in other tissues (the breasts). It was first synthesized by Dora Richardson.

TMX is a synthetic compound that competitively blocks estrogen receptors with a mixed antagonist- agonist effect. The manifestation of these different actions depends on each species, organ, tissue and cell types considered [1].

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 [2,3]. Tamoxifen citrate is characterized by spectrophotometric (UV, MS, GC-MS) and chromatographic (HPLC, GC, TLC) method of analysis [4, 5].

Mixed function oxygenase (MFO) system includes a group of enzymes that play a critical role in Xenobiotic detoxification by carrying out a series of oxidation reactions whereby relatively insoluble organic compounds are converted into water soluble metabolites which may be further conjugated and excreted in urine or bile [6,7].

Aryl hydrocarbon hydroxylase (AHH) is a component of the mixed function mono-oxygenase system which was first identified in liver tissues [8]. AHH has been identified in liver and in many extra hepatic tissues including the lungs [9]. The microsomal cytochrome P-450 dependent Aryl hydrocarbon hydroxylase is important in the detoxification of polycyclic hydrocarbons as well as their activation to cytotoxic or carcinogenic derivatives [10]. The level of activity of the enzymes

of this group is extremely sensitive to fluctuations in the environment of the animals and varies with age, sex and species.

Tamoxifen is metabolized in the liver and several metabolites have been detected in serum [11].The metabolism of Tamoxifen is complex [12], and a wide interindividual variation in Tamoxifen metabolizing enzyme activity has been reported [13].TMX is metabolized similarly in rats and humans to a variety of products, principally the 4-hydroxy-N-desmethyl and N-oxide derivatives[14].Hepatic cytochrome P-450- dependent monoxygenases and related enzymes like Aryl hydrocarbon hydroxylases are known to be responsible for much of this metabolism.

At the present time, much attention is being paid towards the design and synthesis of composite nanoparticles [15, 16] 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 anticancer 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.

Although various studies have been made in this field i.e. the relationship between TMX and the various metabolizing enzyme activity, still there is a blurred picture regarding the changes in the AHH activity during Tamoxifen citrate treatment in 3-methylcholanthrene (3-MC) induced carcinogenesis. Therefore, the present study is aimed to examine the AHH activity during the above mentioned conditions along with the relationship between Tablet formulation of Tamoxifen and nano formulation of Tamoxifen induced changes in the AHH enzyme activity.

MATERIALS AND METHODS

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

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 a gift from CDL, Kolkata, India and was prepared by a co-worker.

Animals: The present study is conducted on healthy female albino mice weighing 25 to 30 grams which are acclimatized in the animal room for four 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 International standards on animal welfare as well as being compliant with local(Ethical committee of animal welfare of Gauhati University, Guwahati, Assam, India) and national regulations. As per the plan of the study the targeted numbers of animals are randomly divided as follows:

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.

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 intraperitoneal injection.

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 intraperitoneally to each animal of this group. During the whole period of experiment this group received normal standard diet.

Group IV (*3-methylcholanthrene [3-MC] &Tamoxifen citrate [Tablet formulation] treated group):* The animals for this group are selected from the acclimatized general pool. They are intraperitoneally 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.

Group V (*3-methylcholanthrene [3-MC] &Tamoxifen citrate [Nano formulation] treated group):* The animals for this group are selected from the acclimatized general pool. They are intraperitoneally 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.

Collection of tissues -The mice are anaesthetized by diethyl ether and dissected to collect liver tissue. The tissue is dried over a filter paper and immediately weighted and recorded. The tissue homogenate is prepared in deionised water with the help of homogenizer. The tissue is collected from 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

Aryl hydrocarbon hydroxylase enzyme activity in hepatic tissue is estimated by the method of Nebert and Gelboin [17] and the final results are expressed as Unit/ mg of protein. The total protein in the hepatic tissue is determined by Lowry's method [18].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 ± S.E. 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 AND DISCUSSION

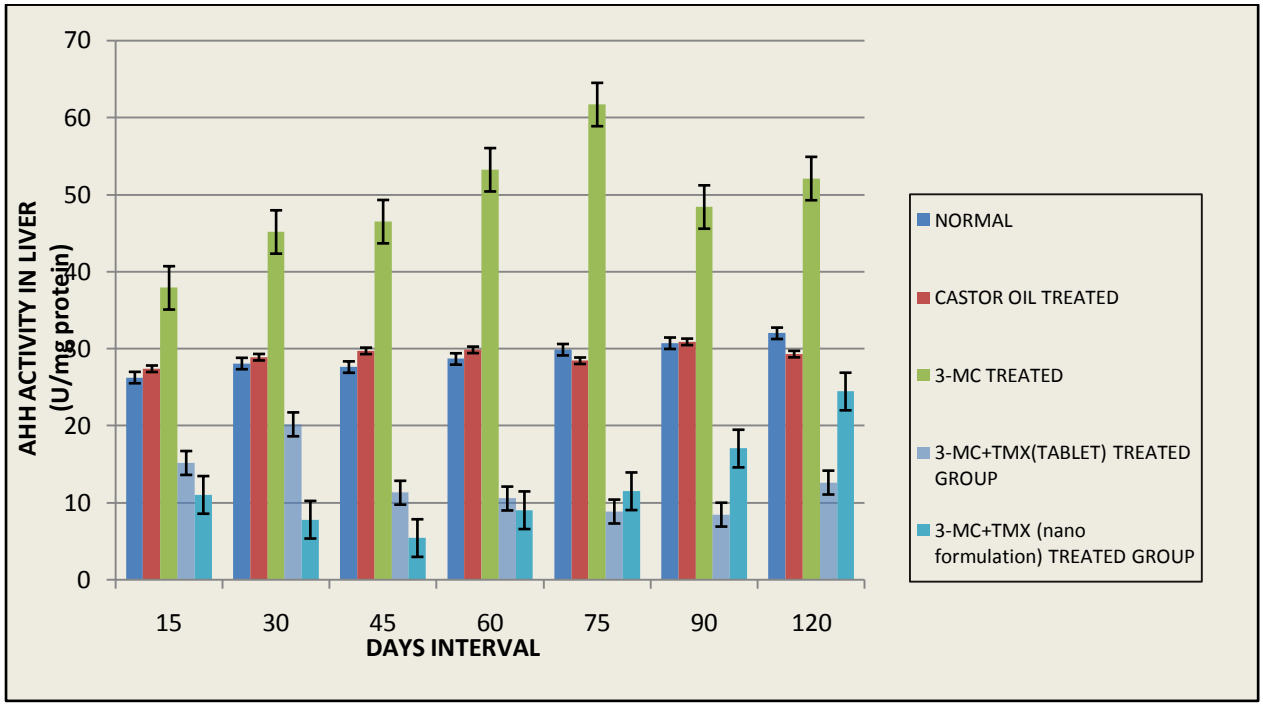
Figure 1 shows the mean values of AHH activity in liver tissue in normal control group along with the other experimental groups. The mean AHH activities in the normal control group of animals ranges from 26.28±0.64 to 32.02±0.38 Unit/ mg protein. In castor oil control group the values lie between 27.42±0.50 to 30.91±0.38 Unit/ mg protein. In the group of animals treated with 3-MC alone, an increasing trend of mean AHH activity is found up to 75th day of treatment, a decline in enzyme activity is observed on 90th day which is again found to be increased on 120th day of treatment. However, the enhancement of enzyme activity is found highly significant ($p<0.01$) **(Table 2)** with the normal control and castor oil control group. Earlier investigations reported 50 fold induction of AHH activity on administration of single intraperitoneal injection of 3-methylcholanthrene in mice [19]. Studies report that 3-methylcholanthrene elicits co-ordinated and sustained induction of phase II genes presumably via persistent activation of aryl hydrocarbon receptor, a phenomenon that have implication for chemical induced carcinogenesis and chemopreventive strategies in human [20].

Under the experimental set up with doses of tablet formulation of TMX with an initial load of 3-MC, the hepatic AHH activity is found to be significantly ($p<0.01$) **(Table 2)** decreased than the 3-MC alone treated group. The enzyme activity is observed as 15.20±0.44 on 15th day which increases to 20.21±0.6 on 30th day. A declining trend in enzyme expression is noticed from 30th to 90th day which rises to initial level as 12.66±0.52 on 120th day of treatment. This fall in the AHH activity of this combination group is significantly lower ($p<0.01$) **(Table 2)** than the normal control and castor oil control group. The effect of single intraperitoneal injection of 3-MC and daily oral administration of TMX nanoparticles on hepatic AHH activity is found similar to the group treated with 3-MC and tablet formulation of TMX because the mean values are significantly lower ($p<0.01$) **(Table 2)** than the 3-MC group, normal control and castor oil control group.AHH activity is found 11.06±0.69 on 15th day of treatment which follows a declining trend up to 60th day and shows a gradual increase in the terminal part of the study.

Table 1 shows the percentage deviation of mean values of different experimental groups from the normal control group. Initial dose of 3-MC shows an elevation from the initial phase of study, where hepatic AHH activity shows 106 percent increase from normal base line. In the experimental group of animals treated with 3-MC and tablet form of TMX, the enzyme activity declined by 42 percent initially followed by 72 percent on 90th day of treatment. The AHH activity level drops by 80 percent from the normal control base line in liver tissues of animals treated with 3-MC and nanoformulation of TMX. The fluctuating trend of hepatic aryl hydrocarbon hydroxylase activity in different experimental groups confirms that the enzymes involved in carcinogen metabolism are also involved in the variety of substrates, and thus the introduction of specific xenobiotics may change the operating level and the existence of other chemicals. The mechanism of modification of drug metabolizing enzyme activities and their role in the activation and detoxification of

xenobiotics and carcinogens thus need more study. In general, throughout the entire period of study a decrease in the enzyme activity is noticed in the Tamoxifen citrate treated groups from the 3-MC treated group which are in conformity with the previous findings [21].

Figure 1: Presenting the mean values with SEM of Aryl hydrocarbon hydroxylase (Unit/ mg protein) in liver tissue of different experimental groups at different days interval.



ABBREVIATIONS: AHH=Aryl hydrocarbon hydroxylase **MC=**Methylcholanthrene **TMX=**Tamoxifen citrate

Table 1: Presenting percentage deviation of Aryl hydrocarbon hydroxylase (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	26.28	28.10	27.64	28.69	29.89	30.73	32.02
Castor oil control group	% deviation	4.34	2.92	7.60	4.11	-4.78	0.58	-8.43
3-MC treated group	% deviation	44.25	60.71	68.23	85.53	106.39	57.50	62.68
3-MC +TMX(Tablet) treated group	% deviation	-42.16	-28.08	-58.97	-63.09	-70.22	-72.34	-60.46
3-MC +TMX(Nanoformulation) treated group	% deviation	-57.91	-72.09	-80.25	-68.39	-61.42	-44.45	-23.58

ABBREVIATIONS: MC=Methylcholanthrene **TMX=**Tamoxifen citrate

Table 2: Presenting significance of difference in the mean values of Aryl hydrocarbon hydroxylase (Unit/ mg protein) in liver tissue between different experimental groups at different day's interval.

GROUPS		DAYS OF TREATMENT						
		15th day	30th day	45th day	60th day	75th day	90th day	120th day
Between normal control and castor oil	t	-1.40	-1.18	-2.72	-1.27	2.07	-0.21	4.82
	P	>0.05	>0.05	<0.01	>0.05	>0.01	>0.05	<0.01
	df	18	18	18	18	18	18	18
Between normal control and 3-MC	t	-7.55	-21.59	-21.19	-15.63	-25.44	-11.4	-17.76
	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(Tablet)	t	14.38	14.61	30.18	29.67	51.19	39.69	30.25
	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)	t	16.36	45.02	48.21	38.47	27	20.69	15.41
	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	t	-7.04	-18.88	-15.96	-13.82	-24.79	-10.66	-19.8
	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 (Tablet)	t	18.51	10.75	23.58	22.16	30.56	29.48	24.86
	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)	t	19.47	37.64	34.19	25.68	20.15	16.48	9.33
	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(Tablet)	t	15.45	27.72	39.06	27.69	43.27	26.08	33.13
	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)	t	17.21	54.88	48.28	29.24	37.71	20.08	24.88
	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)	t	5.11	19.95	12.51	3.62	-4.24	-5.49	-10.64
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18	18

ABBREVIATIONS: MC=**Methylcholanthrene** TMX=**Tamoxifen citrate** df =**Degrees of freedom**

CONCLUSION

The present study confirms that Tamoxifen citrate in its tablet formulation inhibits the Aryl hydrocarbon hydroxylase activity in liver tissue during 3- methylcholanthrene induced carcinogenesis. Here, for the first time, we have demonstrated the nature of AHH activity during treatment with nano formulation of Tamoxifen citrate in similar conditions. As the nature of Xenobiotic metabolizing enzymes is an important determinant of the duration and intensity of the pharmacological action of drugs, it can be concluded from our study that nanoparticle formulations of TMX could provide increased therapeutic benefit. However, a much more investigations have to be carried out in the field of drug delivery and bioavailability to ascertain the activity.

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