Research Article

NandroloneDecanoate Administration Elevates Aryl Hydrocarbon Hydroxylase Activities in Liver and Kidney Tissues of Male Albino Mice

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ABSTRACT
Aryl hydrocarbon Hydroxylase (AHH), a cytochrome P-450 dependent monoxygenase, appears to play an important role in the metabolism of xenobiotics and carcinogen. Although much studies has been conducted on drug metabolism by cytochrome P-450s and other enzymes but fragmentary works are reported on the role of AHH on anabolic steroid metabolism. In the present study, it is found that intramuscular administration of 2.5 mg NandroloneDecanoate on male albino mice elevates the activities of liver and kidney AAH. Enhancement of AHH activity is found highly significant ($p<0.01$) from the normal control group of animals in both liver and kidney tissues. Although previous studies established the role of AHH in liver cancer but its role in drug metabolism in liver and kidney tissues is highly significant which indicates the potential role of the enzyme in drug metabolism and detoxification that can be of great pharmacological importance.

Keywords: Aryl hydrocarbon hydroxylase (AHH), drug metabolism, kidney, liver, mice.

INTRODUCTION

Metabolism of anabolic steroidsand xenobiotics in the body results in the activation of Phase I drug metabolizing enzymes. Cytochrome P-450 (CYP), aryl hydrocarbon hydroxylase (CYP) and xanthine oxidase (XOD) are some important phase I metabolizing enzymes which are involved in drug metabolism, xenobiotic detoxification and drug–induced toxicity[1,2]. These biomarkers, also called mixed function oxygenase (MFO),are often used to evaluate xenobiotic toxicity[3] which plays a crucial role in xenobiotic detoxification by carrying out a series of oxidation reactions whereby relatively insoluble organic compounds are converted into water soluble metabolites that are further conjugated and excreted in urine or bile[3-5].MFO play central role in the biotransformation, metabolism and detoxification of xenobiotics or foreign compounds that are introduced to the human body and thereby protect or defend the body against the potential harmful insults from the environment[6].

Aryl hydrocarbon hydroxylase (AHH) representsa large group of cytochrome P-450 monooxygenases that complex withNAD(P)H-flavinoxidoreductase in numerous mixed-function oxidations of aromatic compounds. AHH catalyze hydroxylation of a broad spectrum of substrates and are important in the metabolism of steroids, drugs, and toxins, carcinogens, and insecticides.AHH appears to play an important role in the activation of polycyclic hydrocarbons into reactive moieties that can bind to DNA and that may directly induce cancer[7].

Studies have demonstrated that certain chemicals can either stimulate or inhibit the activities of AHH or they may have little or no effect, depending on the molecule involved. The aryl hydrocarbon receptor (AhR) reported to mediate a variety of biological responses to ubiquitous environmental pollutants[8].The level of AhRinduction depends on the potency of xenobiotic metabolizing enzymes inducer[8]. Studies show that AHH is responsible for the initial oxidative steps in the metabolism of a variety of polycylic hydrocarbons, endobiotics and exobiotics. Results of various studies suggest that Ah receptor-specific and promoter-specific elements regulate the expression of the human CYP1A2 and CYP1A1 gene in hepatoma cells and human liver[9,10]. However, the precise relationship between AHH activity, inducibility of the enzyme in target tissues and susceptibility to chemical carcinogenesis by polycyclic hydrocarbon remains controversial.

Anabolic-androgenic steroids (AAS) are some of the most frequently misused drugs in human sports[11]. Liver and kidney tissues are most affected by the anabolic steroids[12,13]. Long term uses of AAS are reported to cause fibroids, hepatoid tumours[14] and hepatocellular carcinoma[15].These are also responsible for peliosis hepatic, subcellular changes of hepatocytes and hepatocellular adenomas[12].

AHH is a promising marker of lung cancer, especially for lung squamous carcinoma, and it is used in clinical diagnosis, monitoring and prognosis estimation in patients with lung cancer[16].

Heavy exposure to polychlorinated biphenyls (PCBs) and steroidsinduces the activities of aryl hydrocarbon hydroxylase (AHH) [17]. AAS are one of the xenobiotics that either increases or decreases the activities of Phase I drug metabolism enzymes. Many steroids are found to alter the activities of AHH.However the mode of action of Nandralonedecanoate, the most commonly abused anabolic steroid, on AHH activities is still fragmentary. Therefore the present study is aimed to access the effect of intramuscular administration of Nandralonedecanoate on the activity of AHH in male albino mice and to find out potential role of AHH in drug metabolism which is less understood till now.

MATERIALS AND METHODS

The study is conducted on randomly selected healthy male albino mice weighing between 25 to 30 grams after getting clearance from Ethical committee of animal welfare of Gauhati University, Guwahati, Assam (India). Before the experimental procedure is started, all the animals are acclimatized in the animal room for four weeks and fed on standard animal diet. As per plan of the study the targeted number of animals are randomly divided into two groups as follows-

Group I (Normal control group)

10 healthy male albino mice having approximately same weight 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 (Nandralonedecanoate treated group)

Healthy mice are randomly selected for the group and each animal of the group is injected with 0.1 ml of 2.5 mg Nandrolonedecanoate (anabolic steroid) intramuscularly at 15 days intervalupto 90 days.Dose is selected on the basis of our previous study on Nandralone administrations on mice[18]. As one of the objectives of the present study is to assess the liver cirrhosis, tumor development and change in hepatocytes morphology, so a relatively 15 day's interval was chosen for dose administration upto 90 days.

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

Tissues are collected from normal control as well as experimental group on the desired days i.e. 15^{th},30^{th},45^{th}, 60^{th}, 75^{th} and 90^{th} days of treatment.

Aryl hydrocarbon hydroxylase (AHH) activity is determined by the method of Nebert and Gelboin[19].

The results obtained are statistically analyzed and compared between different groups of the study by applying standard statistical procedures to evaluate the changes among different groups.

RESULTS

The mean,SD,SEM and CV% values of Aryl hydrocarbon hydroxylase (Unit/mg) in liver and kidney tissues of normal and experimental animals are presented in **table 1** and the percentage deviation of experimental group from the normal control mean values are presented in **table 3** and the comparison of mean values with significance of variance are presented in **table 2**.

The meanAHHactivities in the liver tissues of normal control group of animals are 0.477 ± 0.010 on 15^{th} day, 0.491 ± 0.023 on 30^{th} day, 0.494 ± 0.032 on 45^{th} day, 0.483 ± 0.014 on 60^{th} day, 0.490 ± 0.019 on 75^{th} day and 0.478 ± 0.013 unit/mg on 90^{th} day of treatment.

In Nandralonetreated group, increasing trend of mean AHH a**c**tivitie**s** is found upto 75^{th} day of treatment in liver tissues. The mean value recorded are 0.510 ± 0.024 on 15^{th} day, 0.596 ± 0.018 on 30^{th} day , 0.677 ± 0.126 on 45^{th} day, 0.904 ± 0.153 on 60^{th} day,1.081 ± 0.061 on 75^{th} day of treatment highlighting the role of AHH in drug metabolism .A terminal decline in enzyme activity is observed as 0.854 ± 0.153 unit/mg on 90^{th} day of treatment.

The meanAHH activities in kidney tissues of the normal control group of animals are 0.485 ± 0.032 on 15^{th} day, 0.489 ± 0.045 on 30^{th} day, 0.486 ± 0.136 on 45^{th} day, 0.491 ± 0.117 on 60^{th} day, 0.487 ± 0.096 on 75^{th} day and 0.490 ± 0.107 unit/mg on 90^{th} day of treatment.

In Nandralone treated group, increasing trend of mean AHH a**c**tivitie**s** is found upto 75^{th} day

of treatment after which terminal decline in enzyme activity is observed in case of renal tissues. The mean value recorded are 0.497 ± 0.187 on 15th day, 0.648 ± 0.213 on 30th day and 0.696 ± 0.171 on 45th day., 0.877± 0.223 on 60th day and 0.948 ± 0.075 on 75th day a and 0.617 ± 0.057 unit/mg on 90th day of treatment .

DISCUSSION

Aryl hydrocarbon hydroxylase(AHH) is one of the important drug metabolizing enzymes that actively take part in drug and xenobiotic metabolism although it is well known fact that this enzyme is prominent serum cancer biomarker of lung and hepatic tissues. AHH is important marker for tumours and lung squamous carcinoma so it is widelyused in clinical diagnosis, monitoring and prognosis estimation in patients with lung cancer[16].

Some investigation shows that AHH is one of the member of Cytochrome family and represent it as CYP1A1[20-21] while some reports it to be CPY 1B1[22]. Although debate on this persists but from the present investigation it can be suggested that AHH plays an important role in drug metabolism which was previously not clearly underlined.

It is well known that drugs can modulate the expression of drug metabolizing enzymes and are useful in chemoprevention as well as therapy in cancer[23].In the present investigation; administration of Nandralonedecanoateshows a uniform enhancement of hepatic AHH activity. Doses of Nandralonedecanoateincreases the AHHactivity from the initial period of study and shows the maximum peak value on 75th day of experiment which later declines during the end part of investigation. Enhancement of AHHactivity is found highly significant ($p<0.01$) from the normal control group of animals.

The fluctuating trend of hepatic AHHactivity in Nandralonetreated group confirms that the enzyme involved in carcinogen metabolism is also involved in the metabolism of variety of substrates, and thus it may be said that the introduction of specific xenobiotics may change the operating level and the existence of other chemicals. The mechanism of modification of thisdrug metabolizing enzyme activity and its role in the activation and detoxification of xenobioticsthus need more study.

In contrast to these rhythmic fluctuations,elevated AHH activity is also observed in kidney tissues.In experimental group exposed to the initial dose ofNandralonedecanoate , the renal AHH activity shows a gradual increase from 15th day and this increasing trend continues upto 75th day which later declines in the end of the experimental period. Although, the initial increase of AHH activity is very marginal, but it peaks up on 75th day where about 94 percent increase is observed from the normal control base line.Most of the studies reports on the activity of AHH on liver tissues but present investigation highlight that kidney tissues also get stressed upon drug administration.

In general, throughout the entire period of study an elevated kidney AHHactivity is noticed from the normal control groups of animal. The results of the present study are in conformity with the previous findings of increase in renal aryl hydrocarbon hydroxylase activity by carcinogen (beta napthoflavone) administration[24]. Study also demonstrates that aryl hydrocarbon hydroxylase activity declines rapidly or shows no activity in the terminal part of study upon heroin administration[1].Present investigation also shows conformity with finding thatfew tested drugs, namely phenyl butazone, ketoprofen, piroxicam, and acetaminophen, caused an increase the activity of aryl hydrocarbon hydroxylase[25].

Among the two tissues (liver and kidney) the activity of AHH is more marked in liver tissues indicating liver tissues are much stressed underxenobiotic load. It is demonstrated that although other body organs are also involved in drug biotransformation and metabolism, the liver is the predominant organ of metabolism for a wide range of endogenous compounds and xenobiotics[26]. A rhythmic fluctuation in the enzyme activity is well marked as the level of activity of the AHHis extremely sensitive to fluctuations in the environment of the animals and varies with age, sex, and species. The exposure to a variety of foreign compounds (such as pharmacologically active drugs, carcinogens and insecticides) and changes in nutrition and hormonal balance alter the levels of this enzyme in the whole animal[27].

The present investigation shows significant involvement of AHH in drug metabolism which was previously less understood as AHH is basically known to be a cancer marker and is involved in carcinogen and polyhydrocarbon metabolism. The present study thus can suggest that AHH probably plays an important role in drug metabolism and hence a much more elaborate investigation is needed to explore its potential roles that can be of pharmacological and therapeutic significance.

Table 1: Presenting the values of mean aryl hydrocarbon hydroxylase (Unit/mg) in liver and kidney tissue of normal and experimental group in different days interval

Tissues	Groups		Days of treatment					
			15th	30th	45th	60th	75th	90th
LIVER	Normal control (n = 10)	Mean	0.477	0.491	0.434	0.483	0.490	0.478
		SD±	0.010	0.023	0.032	0.014	0.019	0.013
		SEM±	0.003	0.007	0.101	0.004	0.006	0.004
		CV%	2.09	4.68	6.47	2.89	3.88	2.72
	Nandralone treated (n = 10)	Mean	0.510	0.596	0.667	0.904	1.081	0.854
		SD±	0.024	0.018	0.126	0.153	0.061	0.078
		SEM±	0.007	0.005	0.039	0.048	0.019	0.024
		CV%	4.70	3.02	18.89	16.92	5.64	9.13
KIDNEY	Normal control (n = 10)	Mean	0.485	0.489	0.486	0.491	0.487	0.490
		SD±	0.032	0.045	0.136	0.117	0.096	0.107
		SEM±	0.01	0.014	0.043	0.037	0.03	0.033
		CV%	6.59	9.2	27.98	23.82	19.71	21.83
	Nandralone treated (n = 10)	Mean	0.497	0.648	0.696	0.877	0.948	0.617
		SD±	0.187	0.213	0.171	0.223	0.075	0.057
		SEM±	0.059	0.067	0.054	0.07	0.023	0.018
		CV%	37.62	32.87	24.56	25.42	7.91	9.23

SD = Standard deviation , SEM = Standard error of mean , CV = Coefficient of variance

Table 2: Presenting significance of difference in the mean values of aryl hydrocarbon hydroxylase (Unit/mg) in liver and kidney tissues between normal and experimental group at different days interval

Tissues	Groups		Days of treatment					
			15th	30th	45th	60th	75th	90th
LIVER	Between normal control and Nandralone	t	-4.33	-12.21	-1.59	-8.74	-29.66	-15.45
		p	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01
		df	18	18	18	18	18	18
KIDNEY	Between normal control and Nandralone	t	-0.2	-2.32	-3.04	-5.04	-12.19	-3.38
		p	>0.05	<0.05	<0.01	<0.01	<0.01	<0.01
		df	18	18	18	18	18	18

t = test of significance, df = degree of freedom , p = level of significance at 0.01 and 0.05

Table 3: Presenting percentage deviation of aryl hydrocarbon hydroxylase (Unit/mg) in liver and kidney tissues of experimental group from the mean values of normal control group

Tissues	Groups	% Deviation	Days of treatment					
			15th	30th	45th	60th	75th	90th
LIVER	Normal control group	Mean	0.477	0.491	0.494	0.483	0.490	0.478
	Nandralone treated group	% deviation	6.91	21.38	35.02	87.16	120.61	78.66
KIDNEY	Normal control group	Mean	0.485	0.489	0.486	0.491	0.487	0.490
	Nandralone treated group	% deviation	2.47	32.51	42.59	78.61	94.66	25.91

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