Possible Involvement of Androgen Receptor and Insulin Growth Factor-1 in the Pathogenesis of Induced Hepatocellular Carcinoma in Male Rats

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Abstract

Objective: Hepatocellular carcinoma (HCC) is considered one of the most common hepatic tumors. It has been documented that males carries a higher incidence than females to be attacked by this fatal disease. In view of this concept, this work was conducted in an attempt to study the possible impact of androgen receptor and IGF-1 in the pathogenesis of induced hepatocellular carcinoma in male rat.

Material and Methods: A total of 24 male albino rats were used in this study weighting 150-200 grams. These rats were divided into the following groups: Group I: (Control group) represented the vehicle-treated rats, Group II: (HCC group) represented the induced untreated hepatocellular carcinoma (HCC) group, Group III: (HCC + flutamide) represented the induced hepatocellular carcinoma group supplemented with flutamide (androgen receptor blocker). Blood samples were obtained for assessment of α-fetoprotein, testosterone and IGF-1. Also liver tissues were evaluated for estimation of gene expression of androgen receptor.

Results: The impact of flutamide treatment in the present study was observed in decreasing the serum levels of AFP (tumor marker), IGF-1 and androgen receptor gene expression that was reflected in the attenuation of histopathological state of HCC.

Conclusion: Modulation of the efficacy of androgens through their receptors by administration of flutamide in male rats had been associated with braking the progression of hepatocellular carcinoma suggesting its protective role especially at the early stage of the disease.

Key Words: Hepatocellular carcinoma – Testosterone – IGF-1.

Introduction

HEPATOCELLULAR carcinoma (HCC) is considered one of the most common fatal hepatic tumors. Almost, allover the world, males have a higher incidence than females. This is in turn, has been explained by gender differences in immune response and gene expression [1].

Sexual dimorphism affects the metabolic function of the liver and also affect the hepatic ability to resist toxins in both sexes [2].

Testosterone the predominant androgenic hormone is secreted mainly by the testicles in males. It has also been secreted in females by lesser amount from ovaries and adrenal gland [3]. This hormone has a wide variety of actions that affect many organs other than those involved in reproduction [4].

The physiological actions of the androgens result from its association with its nuclear receptor that consequently regulates gene transcription [5].

The final target of gene transcription is the output of certain proteins including insulin-like growth factor I [6].

The radical importance of insulin-like growth factor I have been detected from intrauterine life and even throughout life following birth. Therefore, its association with the pathogenesis of several diseases has been studied, including growth disorders, metabolic disturbance and many malignant tumors [7-9]. Thus, focusing on insulin-like growth factor I pathway may be beneficial on discovering new therapeutic design that could be at least help in the attenuation of pathological states of several diseases [10].
Material and Methods

The study was carried out in the Animal House of Physiology and Biochemistry Departments, Faculty of Medicine, Cairo University during 2013. Twenty four male adult albino rats aged approximately 10-12 weeks and of body weights ranging from 150 to 200 grams were included in this study. The animals were left for a few days to acclimatize to ordinary environmental living conditions in the Animal House, as regards humidity, temperature and dark/light cycles. They were kept in wire-mesh cages and had free access to food and water. The rats were then randomized into three groups:

Group I (n=8): (Control group): Represented the placebo group of the vehicle-treated rats. The animals were supplied by a single intra-peritoneal injection of isotonic saline at a dose of 0.1ml, then followed by single weekly subcutaneous injection of isotonic saline in a dose of 3ml/Kg body weight for 6 weeks.

Group II (n=8): (HCC group): Represented the induced untreated HCC group.

Group III (n=8): (HCC + flutamide): Represented the induced HCC rats which were supplied with flutamide “an anti-androgen” in a dose of 25mg/kg/day orally prepared from androxin tablets, Sigma Pharmaceutical Industries, Egypt. Each tablet which is equivalent to 250mg flutamide, was dissolved in a few drops of alcohol. The dissolved drug powder was then diluted with 100ml of their drinking water [11]. The concentration of flutamide was 2.5mg/1ml. The rats received 1ml/100g body weight per day throughout the duration of the study [12]. Every week, the rats were weighed and the dose for each was adjusted accordingly.

Induction of hepatocellular carcinoma: An experimental model of hepatocellular carcinoma (HCC) was induced chemically by a single intra-peritoneal injection of diethylnitrosamine at a dose of 200mg/kg body weight. This was followed by a single weekly subcutaneous injection of carbon-tetrachloride (CCI4) at a dose of 3ml/kg body weight for 6 weeks [13].

Preparation of diethylnitrosamine (DEN): Diethylnitrosamine (the initiator of carcinogenesis) was purchased from Sigma-Aldrich Egypt, number C 1900 in a solution form and was given diluted with castor oil [13].

Preparation of carbon-tetrachloride (CCI4): Carbon-tetrachloride which is the propagator of DNA damage, was purchased from Sigma-Aldrich Egypt, number C 1900.

Mortality rate: Throughout the period of the study 3 rats died, possibly from the induction of HCC and were replaced.

Biochemical measurements: The plasma was separated by centrifugation and stored at \( \leq -20^\circ C \). Serum levels of alpha-fetoprotein, Tetestosterone, IGF-1 were assayed by Enzyme Linked Immuno-Sorvent Assay (ELISA) (Quantiquine R&D system) according to the instructions of manufacturers. The animals were then sacrificed by cervical dislocation; their livers were dissected and divided into two samples for histopathological assessment using hematoxylin and Eosin (H&E) stain, Masson’s trichrome stain, Periodic acid Schiff stain. The other sample for biochemical assessment of liver tissues for estimation of gene expression of androgen receptor through Real-time quantitative polymerase chain reaction (qPCR) [14].

Statistical methods: Data was summarized using mean and standard deviation and subjected to statistical analysis using statistical package SPSS version 16. Comparisons between groups were done using analysis of variance (ANOVA). \( p \)-values less than 0.05 were considered as statistically significant [15].

Results

Upon induction of HCC, the present study revealed that serum levels of AFP and testosterone were significantly elevated in group II (induced HCC group) compared to group I (control group) as observed in Table (1) and Fig. (1). Moreover, serum levels of IGF-1 showed significant increase in group II (induced HCC group) compared to group I (control group) as observed in Table (1) and Fig. (2). Also androgen receptor gene expression was significantly elevated in group II (induced HCC group) compared to group I (control group) as seen in Table (2) and Fig. (3).

When observing serum levels of AFP and testosterone in group III (induced HCC group supplied with flutamide), as seen in Table (3) and Fig. (4), our results recorded a marked significant decrease in their serum levels when compared to group II. Moreover, serum levels of IGF-1 showed significant decrease in group III (induced HCC group supplied with flutamide) compared to group II (induced HCC group) as observed in Table (3) and Fig. (5). Interestingly, AR gene expression significantly decreased in group III (induced HCC group supplied with flutamide) compared to group II (induced HCC group) as shown in Table (4) and Fig. (6).
Table (1): Comparison of serum levels of AFP, testosterone, IGF-1 among group I (control group) and group II (induced HCC group).

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>HCC group</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>AFP (pg/ml)</td>
<td>0.41±0.10</td>
<td>1.96±0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>1.66±0.37</td>
<td>2.76±0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGF-1 (pg/ml)</td>
<td>31.51±4</td>
<td>108.78±6.72</td>
<td>&lt;0.001</td>
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Table (2): Comparison of androgen receptor gene expression among group I (control group) and group II (induced HCC group).

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>HCC group</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Androgen receptor</td>
<td>0.12±0.03</td>
<td>1.49±0.41</td>
<td>&lt;0.001</td>
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</tbody>
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Table (3): Comparison of serum levels of AFP, testosterone, and IGF-1 among group II (induced HCC group) and group III (induced HCC group supplied with flutamide).

<table>
<thead>
<tr>
<th></th>
<th>HCC group</th>
<th>HCC + flutamide group</th>
<th>p-value</th>
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<tbody>
<tr>
<td>AFP (pg/ml)</td>
<td>1.96±0.50</td>
<td>0.82±0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>2.76±0.47</td>
<td>1.14±0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGF-1 (pg/ml)</td>
<td>108.78±6.72</td>
<td>69.81±14.90</td>
<td>&lt;0.001</td>
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Table (4): Comparison of AR gene expression among group II (induced HCC group) and group III (induced HCC group supplied with flutamide).

<table>
<thead>
<tr>
<th></th>
<th>HCC group</th>
<th>HCC + flutamide group</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>AR</td>
<td>1.49±0.41</td>
<td>0.66±0.22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. (1): Comparison of serum levels of AFP, testosterone, among group I (control group) and group II (induced HCC group).

Fig. (2): Comparison of serum levels of IGF-1 among group I (control group) and group II (induced HCC group).

Fig. (3): Comparison of androgen receptor gene expression among group I (control group) and group II (induced HCC group).

Fig. (4): Comparison of serum levels of AFP, testosterone, among group II (induced HCC group) and group III (induced HCC group supplied with flutamide).
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Fig. (7): Section in the liver tissues in group I (control group), showing normal architecture, normal hepatocytic appearance and normal portal tract appearance with no signs of inflammation (H & E, x 100).

Fig. (8): Section in the liver tissues in group I (control group), showing normal liver architecture, adequate glycogen content and normal portal tract appearance (Periodic acid schiff, x 200).

Fig. (11): Section in the liver tissues in group II (induced HCC group), showing decreased hepatocytic glycogen content with hydropic changes (Periodic acid schiff, x 200).

Histolopathological Results

Fig. (5): Comparison of serum levels of IGF-1 among group II (induced HCC group) and group III (induced HCC group supplied with flutamide).

Fig. (6): Comparison of AR gene expression among group II (induced HCC group) and group III (induced HCC group supplied with flutamide).

Histolpathological Results

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Fig. (8): Section in the liver tissues in group I (control group), showing normal liver architecture, adequate glycogen content and normal portal tract appearance (Periodic acid schiff, x 200).

Fig. (9): Section in the liver tissues in group I (control group), showing normal liver architecture and normal portal tract appearance (Masson's trichrome, x 100).

Fig. (10): Section in the liver tissues in group II (induced HCC group), showing distorted hepatic architecture associated with high grade of dysplastic changes in the form of dark nuclei, minimal dark cytoplasm and increased nuclear/cytoplasmic ratio (H & E, x 100).

Fig. (11): Section in the liver tissues in group II (induced HCC group), showing decreased hepatocytic glycogen content with hydropic changes (Periodic acid schiff, x 200).
Fig. (12): Section in the liver tissues in group II (induced HCC group), showing cirrhotic nodule surrounded by fibrous bands (Mason's trichrome, x 200).

Fig. (13): Section in the liver tissues in group III (induced HCC group supplied with flutamide), showing preserved architecture and widening of portal tract with infiltration of inflammatory cells (H & E, x 100).

Fig. (14): Section in the liver tissues in group III (induced HCC group supplied with flutamide), showing preserved architecture and healthy glycogen appearance filling hepatocytes (Periodic acid schiff, x 200).

Fig. (15): Section in the liver tissues in group III (induced HCC group supplied with flutamide), showing normal liver architecture and normal portal tract appearance (Mason's trichrome, x 200).
Discussion

Diethylnitrosamine and CCl4 have yielded in the present study histo-pathological alterations and biochemical changes related to HCC, which were observed at the serum as well as the hepatic tissue levels. Thus, the present findings recorded a significant elevation in the estimated levels of AFP (a tumor marker of HCC) in the main experimental group of HCC. These results are matching with the previous reports which concluded that serum AFP is a beneficial marker for monitoring of hepatic tumor progression and treatment response [16-18].

Interestingly, the current observation in the present study about the increased serum levels of testosterone during early hepatocarcinogenesis, supports a previous study done by De Maria et al., [19] which emphasized that elevated serum levels of testosterone have been documented in the development of many malignant tumors as HCC.

It has been suggested by Yu et al., [20] that the disorders of genes responsible for regulation of androgens, is also involved in the HCC etiology. This explain the positive correlation between the serum testosterone levels and HCC incidence.

In the present study, it has been found that the serum IGF-1 level was significantly elevated with induction of HCC suggesting its significant sharing in the sequence of the disease.

This present finding is in accordance with a previous report that concluded that the insulin-like growth factor-1 (IGF-1) pathway is involved in the development of HCC. Moreover, focusing on this pathway may help in finding new beneficial therapeutic design as IGF-1 has been believed to control cell proliferation and also has been involved in its malignant transformation in tissue culture [21,22].

Previous studies suggested that the birth weight are related to IGF-1 level, and the infants with a higher birth weight for their percentile are more prone to develop more malignancies such as prostate and breast cancers later in their life [23,24].

Evidence suggesting that IGF-1R is vital in the pathogenesis of malignant tumors derived from its ability to induce anti-apoptosis and increase tumor cell survival following its activation. Also, onco-genes as Akt kinase and Src kinase is evident to increase the gene expression of IGF-1R [25].

Interestingly, there are many therapeutic methods involving IGF pathway in the HCC treatment. The first strategy is to reduce the ligand activity, the second is to impair or block its receptors, and the third targets the downstream signals of its pathways [26].

Aleem et al. [27] concluded that an upregulation of IGF-IR has been early detected following administration of hepatocarcinogens, therefore the sensitivity of hepatocytes is increased to the circulatory IGF-1. Later on, with the advance of the disease, the IGF-1 levels are reduced, and other growth factors such as tumor growth factor-a may be responsible for the future sequence [28].

Previous report suggested that the developing of liver diseases that could consequently progress in HCC could be detected from monitoring of IGF-I concentrations [29].

It has been clearly observed in the present study that androgen receptor gene expression was increased upon induction of HCC. This is agreeable with previous studies on HCC tissues and animal models of induced HCC that highlighted the importance of sex hormones and their receptors in HCC pathogenesis [19,30].

The present finding in induced HCC male rats supports the findings of a previous report that revealed that in malignant cells, androgen receptors expression is present in greater concentrations than that for estrogen receptors [31]. Difference in the expression of sex hormones receptors has also been observed in HCC and normal liver, suggesting a strong association between sex hormones and development of HCC [32].

The expression of both AR and ER in animal studies increases during early preneoplastic lesions and thereafter, with cancer development, ER expression is present in greater concentrations than that for estrogen receptors [31]. Difference in the expression of sex hormones receptors has also been observed in HCC and normal liver, suggesting a strong association between sex hormones and development of HCC [32].

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of several growth factors genes that affect hepatocytes proliferation and growth as transforming growth factor beta-1 and vascular endothelial growth factor [40,41].

These facts together suggest that a new therapeutic design against HBV-related HCC might be developed from targeting of AR [42].

In the present study, it has been clearly observed that flutamide supplementation in the induced HCC male rats had been associated with significant decrease in gene expression of androgen receptor. Interestingly, the impact of flutamide treatment in the present study, was also observed in decreasing the serum levels of IGF-1 that was reflected in the attenuation of histopathological state as well as biochemical analysis as regards the decline in the serum level of AFP (tumor marker). This improvement was depicted consequently in the serum levels of sex hormones as regards decreasing the serum levels of testosterone.

The current result was supported by previous experimental studies that have suggested a stimulus effect of androgens on malignant tissue growth [43], which may be restrained through castration or an anti-androgen therapy [44]. Furthermore, anti-androgen flutamide was shown to suppress the activity of AR as revealed by Kai and Levenson, [45].

The observed results are also in accordance with a previous report which, estimated that the flutamide was shown to reduce the IGF-1 expression in cultured cells which was exacerbated by trenbolone acetate [46].

Thus, upon flutamide supplementation in the induced HCC male rats in the present study, an inhibition of the cross talk between IGF-1 and AR signaling was implicated as implied by previous reports [47,48].

The strength of the present study comes from evaluation of AR status at pre-malignant stages for crucial understanding of their involvement in HCC pathogenesis. Nevertheless, the precise mechanisms of sex hormones and/or their receptors in HCC is needed more to be focused on and clarified in future studies. Further investigations are also required to study the fluctuation in the serum levels of sex hormones and aromatase expression especially at the pre-malignant stage.

These results emphasize the suggestion that HCC depends for its progression on sex hormones receptors thus, hormonal therapy could be a potential prophylactic agent in preventing this disease. However, further investigations and trials on both animal and human studies are required to support the current findings.

References
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