

Effect of Histidine on Autotaxin Activity in Experimentally Induced Liver Fibrosis

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ABSTRACT

*The aim of the present study was to explain whether serum autotaxin activity might be a target for regulation of liver fibrosis and to evaluate the hepato-protective and anti-fibrotic effects of histidine in thioacetamide induced liver fibrosis in rats. The study was carried out on 100 albino rats, classified into 5 groups each of 20 rats: group I (control group), group II: rats were given histidine intra-peritoneally, group III: rats were injected intra-peritoneally with thioacetamide, group IV: rats were injected with L-histidine together with thioacetamide, group V: rats were injected with TAA for one month then treated with intra-peritoneal injection of L-histidine for another month. At the end of experiment, blood and liver were collected for determination of some liver enzymes, plasma total antioxidant capacity, serum autotaxin activity and liver tissue hydroxyproline. Thioacetamide treatment caused significant increases in liver enzymes, autotaxin activities and liver hydroxyproline, but with a significant decrease in plasma total antioxidant capacity. Upon treatment with histidine significant decreases in liver enzymes, autotaxin activities and liver hydroxyproline were observed with a significant increase in plasma total antioxidant capacity in group IV and significant decrease in group V. **Conclusion:** histidine as an antioxidant has a protective effect on thioacetamide-induced liver fibrosis, its beneficial effects in rats might be not only by inhibition of collagen synthesis and increasing total antioxidant capacity, but also by inhibition of autotaxin activities, thus reducing its capacity to produce lysophosphatidic acid which has a role in liver fibrosis.*

Keywords: autotaxin, histidine, liver fibrosis, thioacetamide, total anti-oxidant capacity.

INTRODUCTION

Liver fibrosis is characterized by altered hepatic function and excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases^[1].

Previous experiments showed that treatment of rats with thioacetamide (TAA) resulted in liver cell damage, fibrosis and/or cirrhosis, associated

with increased oxidative stress and activation of hepatic stellate cells (HSCs)^[2].

The development of fibrosis involves a multitude of events and factors, the majority of these molecules were found to be proteins or peptides^[3]. More recent data show significant involvement of phospholipids in the development of fibrosis. These phospholipids include for example lysophosphatidic acid (LPA)^[4], which can be generated from

phospholipids by the action of lysophospholipase D [LPLD or autotaxin (ATX)], which cleaves the choline group from lysophosphatidylcholine (LPC) to generate LPA, so that ATX plays a pivotal role in the production of LPA¹⁵. LPA and its receptor can play an important role in the development of fibrosis as it involves stimulation of fibroblast migration, increased vascular permeability and connective tissue growth factor secretion by a number of cells; all events known to be involved in the fibrotic process.¹³

The progression of liver fibrosis often develops into irreversible cirrhosis and is associated with liver cancer. Therefore, it is urgent to reduce the damage to the liver and to slow down the progress of liver injury to fibrosis and cancer¹⁶.

ATX is implicated in a variety of biological processes during normal development and under pathological conditions. For instance, it has been described as being a key in the metastatic process.^{17,81} So, the presence of ATX might turn cancer cells into very aggressive, metastasis-induced tumors, with poor prognoses¹⁹.

Several studies have demonstrated the beneficial effect of antioxidants in protecting the liver against TAA-induced injury¹⁰¹. Histidine in addition to routine metabolic role as a protein building block, it is an effective scavenger of toxic oxygen species by direct interactions of the histidine imidazole ring with singlet oxygen or by interfering with the redox reactions involving metal ions that produce the hydroxyl radical¹¹¹. Therefore, it is

useful in the protection of cells and tissues from a variety of inflammatory disease processes¹²¹.

So, the aim of this study was to explain whether serum ATX activity might be a target for regulation of liver fibrosis and to evaluate the hepatoprotective and antifibrotic effects of histidine in TAA induced liver fibrosis in rats.

MATERIAL & METHOD

The current study was carried out on 100 albino rats, weighing 150-250g. During the study, animals were housed in wire mesh cages and were fed standard rat chow and allowed free access to water. They were kept under constant environmental conditions (25°C and 12 h dark light cycle). Rats were acclimatized prior to experimental study by one week. All animals were weighed at the beginning and at the end of the study. Died rats were excluded from the study. Rats were classified into 5 groups each of 20 rats : group I (control group); group II (histidine group): rats were given intra-peritoneal injection of histidine (170 mg/kg twice a week for one month), group III (TAA group): rats were injected intra-peritoneally with TAA (200 mg/kg twice a week for one month), group IV (TAA plus histidine group): rats were injected L-histidine (170 mg/kg) together with TAA (200 mg/kg) twice a week for one month), group V (TAA then histidine group): rats were injected with TAA (200 mg/kg twice a week for one month then treated with intra-peritoneal injection of L-histidine (170 mg/kg twice a week for another one month).

Histidine and TAA were obtained from Sigma-Aldrich Chemical Co., USA and were dissolved in distilled water. At the end of treatment schedules, animals were scarified by decapitation, samples (blood/liver) were collected; part of blood was collected in plain tubes (no anticoagulant) to obtain serum, and the other part was collected on K₂EDTA coated tubes to obtain plasma, then all samples centrifuged at 3000 rpm for 20 min at 4°C and samples were transferred to polypropylene micro-tubes and stored at - 70°C until analysis.

□ **Serum samples** were used for assessment of:

1. Liver enzymes: ALT and GGT by Randox kit according to Reitman and Frankel^[13] and by spectrum kit according to Saw et al.^[14] respectively.
2. ATX activity according to (Sajdok et al., 1995)^[15]. It is based on colorimetric determination of the product of ATX reaction, choline, using choline oxidase/peroxidase system, by microtitration plate technique at 490 nm using ELISA reader

□ **Plasma samples** were used for assessment of total antioxidant capacity (TAC) according to Benzie and Strain^[16]. It measures the ferric reducing ability of plasma (FRAP). At low pH, when a ferric tripyridyltriazine (FeIII-TPTZ) complex is reduced to the ferrous form (FeII), an intense blue color with an absorption maximum at 593 nm develops, and the rate limiting factor of FeII-TPTZ, and hence color

formation is the reducing ability of the sample.

- **Liver** was removed, washed with ice cold saline to remove extraneous materials, and stored at -70°C for assessment of hydroxyproline content according to Bergman and Loxley,^[17] which was modified by Medugorac^[18], in which hydroxyproline was oxidized into pyrrole followed by coupling with p-dimethyl-amino benzaldehyde forming a red color, which was measured spectrophotometrically at 456 nm.

N.B. Liver fibrosis was confirmed by histological examination of hematoxylin and eosin stained liver sections.

Statistical analysis

All data were expressed as mean \pm standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA), and post-hoc comparisons were carried out using Tukey's t-test. Pearson correlation test was used to find out the correlation between the studied parameters. P values <0.05 were considered as significant.

RESULTS

Results are shown in tables 1, 2 and 3 according to this, TAA treatment caused significant increases in ALT, GGT serum levels, ATX activities and liver hydroxyproline tissue levels with, significant decrease in plasma TAC. Upon treatment with histidine either simultaneously with TAA or after induction of hepatic fibrosis, significant decreases in ALT,

GGT serum levels, ATX activities and liver hydroxyproline tissue levels were observed, with a significant increase in plasma TAC in group IV and significant decrease in group V. In TAA treated group (group III) and in histidine treated groups (groups IV, V): ALT, GGT and liver

hydroxyproline tissue levels were significantly positively correlated with ATX, but significantly negatively correlated with TAC, with no significant correlation between ATX activity and TAC plasma level (tables 2, 3).

Table (1): Comparison between the studied groups as regards all the studied parameters

	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group IV (n=20)	Group V (n=20)	F	P
ALT(U/l)	5.7±1.6	22.2±2.3	35.7±2.7	6.2±1.8	7.2±2	789.5	<0.001 all groups are significant except I vs. IV, I vs. V and IV vs. V
GGT (U/l)	6.8±0.6	19±1.4	39.9±2.3	6.4±0.5	7.5±0.7	2557	<0.001 all groups are significant except I vs. IV, I vs. V and IV vs. V
ATX (nmol/ml/min)	0.29±0.01	0.65±0.1	2.6±0.7	0.7±0.2	0.66±0.4	124.8	<0.001 all groups are significant except II vs. IV, II vs. V and IV vs. V
TAC(µmol/l)	762.5±48.9	722.9±61.7	267.4±121.9	702.5±70.1	354.4±110.3	141.2	<0.001 all groups are significant except I vs. II, I vs. IV and II vs. IV
Hydroxproline (µg/g liver tissue)	103.8±5.9	129±8.2	183.4±45.6	125.8± 5.7	141.7 ± 8.6	37.8	<0.001 all groups are significant except II vs. IV, II vs. V and IV vs. V

Group I (control group), group II (Histidine group), group III (TAA group), group IV (TAA plus histidine), group V (TAA then histidine). Alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), autotoxin (ATX), total antioxidant capacity (TAC)

Table (2): Correlation between the studied parameters in TAA treated group (III)

TAA (n=20)	ALT	ATX	TAC	Hydroxyproline	
ALT (U/l)	r	-----	0.74*	-0.86*	0.63*
	P-value	-----	<0.001	<0.001	0.003
GGT (U/l)	r	0.77*	0.81*	-0.71*	0.72*
	P-value	<0.001	<0.001	0.001	<0.001
ATX (nmol/ml/min)	r	0.74*	----	-0.30	0.74*
	P-value	<0.001	----	>0.09	<0.001
Hydroxyproline (µg/g liver tissue)	R	0.63*	0.74*	-0.71*	-----
	P-value	0.003	<0.001	<0.001	-----

Alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), autotoxin (ATX), total antioxidant capacity (TAC)

Table (3): Correlation between the studied parameters in histidine treated groups (group IV, V)

Histidine treated (n=40)	ALT	ATX	TAC	Hydroxyproline	
ALT (U/l)	r	----	0.51*	-0.56*	0.74*
	P-value	-----	0.001	<0.001	<0.001
GGT (U/l)	r	0.66*	0.40*	-0.85*	0.85*
	P-value	<0.001	0.010	<0.001	<0.001
ATX (nmol/ml/min)	r	0.51*	-----	-0.23	0.43*
	P-value	0.001	-----	0.156	0.006
Hydroxyproline (µg/g liver tissue)	r	0.74*	0.43*	-0.89*	-----
	P-value	<0.001	0.006	<0.001	-----

Alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), autotoxin (ATX), total antioxidant capacity (TAC)

DISCUSSION

Oxidative stress might play an important role in TAA-induced liver injury^{19,20}. In the current study, hepatocyte necrosis and inflammation induced by TAA resulted in significant increase of ALT and GGT levels (which are commonly used as biochemical markers of liver injury), as compared to control group^{21,22}.

It is well known that liver fibrosis is the result of increased

collagen synthesis which can be reflected on the hydroxyproline content²³. In the current study in TAA induced liver fibrosis group, liver hydroxyproline levels were significantly increased in comparison to control. This finding came in accordance to those of Lai et al., Wang et al., Das et al.^{24,22,25}.

A common link between chronic liver damage and hepatic fibrosis may be related to oxidative stress^{26,27}. Living organisms have developed a

complex antioxidant network to counteract reactive species that are detrimental to life. So, it is essential to measure these antioxidants,

However, the number of different antioxidants makes it difficult to measure each antioxidant separately. The possible interaction among different antioxidants *in vivo* could also make the measurement of any individual antioxidant less representative of the overall antioxidant status. Therefore, several methods including FRAP assay, have been developed and used to determine the TAC of various biologic samples^[28].

In the current study, the TAA induced liver fibrosis group showed significant decrease in TAC in comparison to control group, that finding came in accordance with that of Horoz et al.^[29], who stated that TAC was significantly lower in fibrotic group than in normal one.

From another point of view, ATX has been gaining attention because it could be involved in cancer invasion and metastasis as an autocrine motility factor^[30,31]. ATX is a key enzyme in the synthesis of LPA, a lipid mediator with a wide range of biological actions including the stimulation of proliferation and contraction in HSCs, a pivotal player in hepatic fibrosis^[32].

In the present study, ATX activity was significantly increased in TAA- induced liver fibrosis group as compared to control one. This result came in accordance with that of Watanabe et al.^[33], who reported that LPA level and serum ATX activity were increased in rats with various liver injuries; suggesting that plasma LPA level and serum ATX activity

were increased in liver injury in relation to its severity and that ATX activity is one of the determinants of LPA. Moreover, Yanase et al., Ikeda et al.^[34,35,36] added that LPA stimulates proliferation, contraction, migration and inhibits apoptosis in rat HSCs, also LPA inhibits DNA synthesis induced by growth factors in rat hepatocytes in primary culture. These *in vitro* findings raise the possibility of involvement of LPA in the mechanism of liver fibrosis or regeneration. However, it was speculated that ATX mRNA in the liver was not altered in rats treated with carbon tetrachloride for 8 weeks, indicating that ATX production was not increased at the transcriptional level. In the present study, serum ATX activity was increased correlatively with the extent of liver fibrosis, suggesting that serum ATX activity may be increased in relation to the reduced liver function. This suggestion is supported by the finding of Watanabe et al.^[37] who found quick elevation of serum ATX activity at 3 h after hepatectomy so that the increase of serum ATX activity in liver injury might be due to the decrease of clearance of ATX, not to the increase of its production, possibly in the liver but, the exact mechanism of the enhanced serum ATX activity needs to be further elucidated.

Oxidative stress induces collagen synthesis and plays a major role in hepatic fibrosis. Consequently, antioxidants have emerged as potent anti-fibrotic agents. Previous and recent findings on the anti-fibrotic potential of antioxidants could attenuate hepatic fibrosis in rodents and may exert beneficial effects in

patients with chronic liver diseases by retarding the progression of experimental hepatic fibrosis through inhibition of collagen synthesis and decreasing oxidative stress^[22].

According to the mentioned suggestions, L-histidine was chosen as antioxidant to detect its effect on the present TAA-induced liver fibrosis.

In the current study, in histidine treated groups; histidine had the capabilities of not only returning ALT and GGT serum levels, but also, decreased hydroxyproline level, to near control values. Also, in histidine treated group, it prevented the decrease of TAC demonstrating the antioxidant effect of histidine. Such effect cannot be detected when histidine is given after induction of liver fibrosis, which could be explained on the bases that oxidative stress is out of its anti-oxidant capacities. These results came in accordance with those of Liu et al.^[38], who concluded that histidine could elevate the activity of catalase and glutathione peroxidase (GPX), as well as enhance the mRNA expression of catalase and suppress CYP2E1 activity which causes ROS overproduction. These findings indicated that histidine possessed enzymatic antioxidant activities, which definitely contributed to hepatic antioxidant protection. Also, it could exhibit antioxidant activities via non-enzymatic actions such as scavenging free radicals and binding divalent metal ions.^[11]

Consistently, the data presented herein showed that histidine had the capabilities of decreasing ATX serum activities in a significant manner as compared to TAA group but with still

significant differences with the control. Clair et al., (2005)^[39] stated that L-histidine-induced inhibition of the ATX enzymatic activities is predominantly noncompetitive, i.e. Vmax is decreased significantly, while Km is not significantly affected. Free L-histidine, appears to be unique in its ability to inhibit lysophospholipase D (LPLD), and hence regulate a major source of LPA, in living systems. Since LPA has significant pathological effects, the regulation of its formation is of considerable interest. Furthermore, they reported that L-histidine inhibition of LPLD is not a simple stoichiometric chelation of metal ions but is more likely a complex interaction with a variety of moieties, including the metal cation, at or near the active site.

Moreover, in the present study, ALT, GGT and liver hydroxyproline tissue levels were significantly positively correlated with ATX, but significantly negatively correlated with TAC, with no significant correlation between ATX activity and TAC plasma level which came in accordance with the findings of Watanabe et al.^[33], who reported that the serum ATX activity was significantly correlated positively with serum hyaluronic acid and with the histologic stage of fibrosis.

Finally, in the present study following intra-peritoneal injection of histidine alone a significant increase in the serum levels of ALT and GGT and increased liver hydroxyproline level were observed which indicate that histidine alone may induce cell injury. Rauen et al.^[40] stated that histidine toxicity is mediated by an

iron dependent pathway. Histidine can chelate Fe^{2+} ions and also can form adduct with H_2O_2 . Therefore, it is possible that simultaneous binding of Fe^{2+} ions and H_2O_2 to histidine can facilitate hydroxyl radical generation in the close neighborhood of DNA molecules and results in their enhanced degradation. Such finding is supported by studies showing enhancing effect of histidine on single-strand breaks in DNA, cytotoxicity of H_2O_2 ^[41] and superoxide- Fe^{2+} -ascorbate- dependent lipid peroxidation^[42,43].

Mozdzan et al.^[44] found that histidine strongly augmented the DNA degradation. Addition of histidine and alanine to carnosine that mimics partial carnosine hydrolysis caused the decrease in DNA protection. Also, equimolar mixture of histidine and alanine that resembles total carnosine hydrolysis enhanced the degradation of DNA molecules. These indicate that carnosine may have biphasic activity; first intact dipeptide serves as antioxidant protecting DNA molecules and second degraded carnosine due to pro-oxidant activity of released histidine can enhance the DNA destruction. Therefore, the exact mechanism of histidine alone on liver injury needs further investigations. Until further investigations designed discern the role of L-histidine in liver fibrosis are conducted, explanations for the preferential histidine effect are only speculative.

Recommendations

1. Further studies will be needed to evaluate the role of histidine administration in human as adjuvant therapy in cases of liver fibrosis.

2. Assessment of the effect of another imidazole containing drug (carnosine) on hepatic fibrosis.

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تأثير الهستيدين على نشاط الاوتوتكسين في التليف الكبدى المفتعل

منال البطش ، وفاء إبراهيم ، سها زكريا

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إن التليف الكبدى هو عملية إنتنام إستجابة لإصابات مزمنة مختلفة مثل إلتهاب الكبد الفيروسي، المناعة الذاتية، الكحوليات، وما إلى ذلك حيث يتميز بالإنتاج الزائد وترسب الكولاجين خارج الخلية نتيجة تنشيط وتحول الخلايا النجمية نتيجة الإلتهايات بالإضافة الى الأكسدة مما يؤدي إلى التليف الكبدى. وقد لوحظ وجود صلة مشتركة بين التليف الكبدى و الإجهاد التأكسدى. ولتوضيح الوضع الكلى المضاد للأكسدة، يجب تقييم مكونات مضادات الأكسدة. و من جانب اخر يعتبر الهستيدين واحدا من الأحماض الأمينية الأساسية، بالإضافة لدوره فى بناء البروتين، وجد أنه يمكن أن يحفز الأنشطة المضادة للأكسدة و أن له تأثير مثبت على نشاط الأوتوتكسين بطريقة غير تنافسية. ولقد تم اكتشاف إنزيم الأوتوتكسين فى سرطان خلايا الجلد، و قد حظى بالإهتمام لأنه قد يكون سببا فى غزو السرطان.

ويهدف هذا البحث الى دراسة تأثير الهستيدين على نشاط الأوتوتكسين كهدف لتنظيم تليف الكبد. قد أجريت هذه الدراسة على خمس مجموعات من الجرذان: المجموعة الأولى (المجموعة الضابطة): و تشمل ٠ ٢ جرذ، المجموعة الثانية (مجموعة الهستيدين): و شملت على ٢٠ جرذ تم حقنهم بمادة الهستيدين مرتين فى الأسبوع لمدة شهر، المجموعة الثالثة (مجموعة الثيوأسيتيميد): و شملت ٢٠ جرذ تم حقنهم بمادة الثيوأسيتيميد مرتين فى الأسبوع لمدة شهر، المجموعة الرابعة (مجموعة الثيوأسيتيميد والهستيدين معا): و شملت على ٢٠ جرذ تم حقنهم بمادة الثيوأسيتيميد والهستيدين معا مرتين فى الأسبوع فى وقت واحد لمدة شهر، المجموعة الخامسة (مجموعة الثيوأسيتيميد ثم الهستيدين): و شملت ٢٠ جرذ تم حقنهم بالثيوأسيتيميد مرتين فى الأسبوع لمدة شهر ثم الهستيدين مرتين فى الأسبوع لمدة شهر اضافى. وقد تم قياس مايلى لمجموعات الدراسة الخمس: إنزيمات الكبد فى مصل الدم، مستوى الهيدروكسى برولين فى نسيج الكبد، الفحص الهستوباثولوجى لعينة من نسيج الكبد، نشاط الأوتوتكسين فى مصل الدم، قدرة البلازما المضادة للأكسدة.

وقد أسفرت الدراسة عن ظهور زيادة ذات دلالة إحصائية فى نشاط إنزيمات الكبد و فى نشاط الأوتوتكسين و محتوى الكبد للهيدروكسى برولين مؤشرا على زيادة الكولاجين فى مجموعة التليف الكبد (الثالثة) مقارنة بالمجموعة الضابطة. أيضا ظهور نقص ذا دلالة إحصائية فى قدرة البلازما المضادة للأكسدة فى مجموعة تليف الكبد مقارنة بالمجموعة الضابطة. بالإضافة الى ذلك توصلت النتائج الى وجود نقص ذا دلالة إحصائية فى نشاط إنزيمات الكبد ومحتوى الكبد للهيدروكسى برولين و نشاط الأوتوتكسين فى المجموعة التى تم علاجها بالهستيدين سواء أثناء أو بعد إحداث التليف الكبدى. و ظهور زيادة ذات دلالة إحصائية فى قدرة البلازما المضادة للأكسدة فى المجموعة التى تم علاجها بالهستيدين سواء أثناء إحداث التليف الكبدى ولكنها لم تتحسن فى المجموعة التى تم علاجها بالهستيدين بعد إحداث التليف الكبدى. هذه الدراسة تدعم دور الهستيدين فى تنظيم حالة التليف الكبدى من خلال قدرته المضادة للأكسدة و من خلال قدرته على نقص نشاط الأوتوتكسين الذى قد يكون سببا فى تحويل التليف الكبدى الى السرطان الكبدى