The Influence of histamine H1-receptor on liver functions in immunized rabbits

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Abstract This study was designed to investigate the functional roles of histamine and histamine H1-receptor agonist and antagonist in the development of liver function impairment in immunized rabbits. The study comprised of six groups containing 18 rabbits each. Group III–VI received histamine (100 μg/kg, s.c.), H1R-agonist (HTMT, 10 μg/kg, s.c.), H1R-antagonist (pheniramine, 10 mg/kg, i.m.), and H1R-antagonist (pheniramine, 10 mg/kg, i.m.) plus histamine (100 μg/kg, s.c.), respectively, b.i.d. for 10 days. Group I (negative control) and group II (positive control) received sterile distilled water intramuscularly b.i.d. for 10 days. Group I (negative control) and group II (positive control) received sterile distilled water intramuscularly b.i.d. for 10 days. Groups II–VI were immunized on day 3 with intravenous injection of SRBC (1 × 10⁹ cells/ml). Blood samples were collected on
1. Introduction

Histamine H1 receptor (H1R) expression has been demonstrated to change in the disorders such as allergic rhinitis, autoimmune myocarditis, rheumatoid arthritis and atherosclerosis (Tripathi et al., 2010a). Histamine H1R antagonists have a long history of clinical efficacy in a variety of allergic disorders. Several studies have reported changes in the expression of H1R in pathological situations. Increased expression of H1R was observed in the nasal mucosa of patients with allergic rhinitis and in cultured aortic intimal smooth muscle cells of the patients suffering from atherosclerosis (De Backer et al., 1998). Also, it has been documented that up-regulation of the H1R expression might have a role in the initiation and progression of cardiovascular disease. Increased H1R expression has been reported in the inflamed joints of rheumatoid arthritis patients and in the heart of mice with autoimmune myocarditis (De Backer et al., 1998). Moreover, it has been demonstrated that the exacerbation of asthmatic symptoms experienced by some women during pregnancy is due to the enhanced expression of H1R in nasal epithelial cells on induction by female sex hormones (De Backer et al., 1998).

H1R antihistamines, known as histamine H1R blockers or antagonists, are specific for the H1-receptor (De Backer et al., 1998). Some H1R antagonists inhibit transmission through the muscarinic, a-adrenergic, and serotonin receptors and also through ion channels. The H1R-antihistamines have been reclassified as inverse agonists, rather than as H1R antagonists, which is consonant with an increased understanding of their molecular pharmacologic features (Simons, 2004). More than 40 H1R antagonists are available worldwide, indeed these H1R ligands are among the most widely used of all medications. However, the H1R antagonists (astemizole and terfenadine), which are associated with cardiac toxic effects, are no longer approved for use (Simons, 2004). Both health care professionals and consumers assume that all the approved H1R antagonists have been shown to be efficacious and safe, but several medications in this class have not been optimally studied in randomized, double-blind, controlled trials (Simons, 2004).

First and second-generation H1R antagonists are reported to cause adverse effects, the mechanisms for which are incompletely understood. These effects include fixed-drug eruption, photosensitivity, urticaria, fever, elevation of liver enzymes and hepatitis, and agranulocytosis (Simons, 2004).

Recently, it has been demonstrated that histamine receptors (HR) on induction via their specific agonist can stimulate hypertrophy and hyperplasia of respiratory tract epithelium, and suggested HR agonists’ role akin to growth stimulating factor (Dilkash et al., 2010).

Liver is a vital organ for the biotransformation of xenobiotics and therefore is also exposed to drug toxicity including histamine and HR ligands (Tripathi et al., 2010b). Histamine acting via histamine H2R pathway is believed to protect the liver against early alcohol-induced liver injury in rats (Hornyak et al., 2003) and also plays a role in the treatment of endotoxin-induced hepatic injury and related inflammatory disorders (Masaki et al., 2005). Interestingly, different drugs acting through the same receptors show different roles on the same biological process e.g., H2R antagonists like cimetidine and ranitidine have inhibitory effect on liver regeneration but famotidine has no such effect (Kanashima and Kobayashi, 1989). It has also been demonstrated that modest inflammation predisposes liver to ranitidine toxicity in rats and suggested a role for the inflammation in idiosyncratic reactions to ranitidine (Luyendyk et al., 2003).

In contrast to studies on histamine and HR agonists and antagonists in various pathological conditions the data on H1R agonists and antagonists on hepatic functions are elementary in the existing literature. This prompted us to look for their roles in hepatotoxicity (if any) in albino rabbits by using liver function test.

2. Materials and methods

2.1. Experimental design

To evaluate the serum level of liver enzymes and bilirubin, 108 (54 male and 54 female) New Zealand adult healthy albino rabbits of either sex weighing 1.0–1.5 kg were randomized equally into six treatment groups. Each group contained 18 (9 male and 9 female) rabbits. All groups were immunized with sheep red blood cells (SRBC). Group I (negative control) and group II (positive control) received vehicle (sterile distilled water, 1 ml/kg b.i.d.) while groups III, IV, V, and VI were treated with histamine, H1R agonist, H1R antagonist and H1R antagonist + histamine, respectively.

They were housed in a well maintained animal facility at Central Animal House, J. N. Medical College, Aligarh Muslim University, Aligarh, in the Bioresources unit under 12 h light/
dark cycle, temperature (22 ± 2°C) and were allowed free access to standard laboratory diet, including green vegetables, and tap water until experimentation. All the studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee.

2.2. Drugs

Histamine dihydrochloride was purchased from Himedia Laboratories Pvt. Limited, India. H1R agonist (histamine trifluoro-methyl toluidide (HTMT)-dimaleate) was kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom; H1R antagonist [Avil® (pheniramine maleate)] in injection I.P. from Unimark Remedies, India.

2.3. Dosage regimen

Histamine dihydrochloride 100 µg/kg and H1R agonist (HTMT-dimaleate) 10 µg/kg were administered through subcutaneous (s.c.) route, and H1R antagonist (pheniramine maleate) 10 mg/kg was administered through intramuscular (i.m.) route; twice in a day [12 hourly (8 am and 8 pm)] for 10 days (starting from 3 days prior to immunization until 7 days after immunization). All doses referred to the weight of the salts used.

2.4. Antigen

Sheep blood diluted 1:1 in sterile Alsevier’s solution was obtained from the Department of Microbiology, J. N. Medical College, A.M.U., Aligarh, and washed with PBS (pH 7.4) thrice by centrifugation. The cell suspensions were adjusted to the desired concentration in terms of hemoglobin, lysis of a 1% SRBC suspension (2 × 10⁸ cells/ml) with 14 volumes of 0.1% Na₂CO₃ develops an optical density of 0.135 at 541 nm in a spectrophotometer (Systronics, UV–visible double beam spectrophotometer-2101, India) (Tripathi et al., 2010c–g). Finally the concentration was adjusted to 5% (1 × 10⁹ cells/ml) in PBS for immunization before use.

2.5. Immunization of rabbits

All the rabbits in the study groups (II–VI) were immunized on day 3 intravenously (i.v.) with 1 ml of 5% (1 × 10⁹ cells/ml) sheep red blood cells (SRBC) in PBS.

2.6. Biochemical analyses

To determine the biochemical levels for liver function, blood samples were collected from rabbits through the marginal ear veins prior to immunization (day 0), as well as on days 7-, 14-, 21-, 28-, and 58-post-I. Blood samples were kept at room temperature for 2 h and then at 4°C overnight. Blood samples were centrifuged for 10 min at 580xg, and serum was separated and heated at 56°C for 30 min to inactivate the complement proteins and stored in aliquots containing sodium azide as a preservative at −20°C. Serum levels of liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin [total bilirubin (TB), direct bilirubin (DB), and indirect bilirubin (ID)] were determined using an automatic analyzer (Transasia XL 300, Germany).

2.7. Statistical analyses

Data were summarized as Mean ± SD. Groups were compared by using repeated measures (subjects within groups) two-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test. A two-tailed (α = 2) probability p < 0.05 was considered to be statistically significant. Analyses were performed on SPSS for windows (version 12.0, Inc., Chicago, IL).

3. Results

The changes in the mean values of serum level of serum enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) in group I–VI are shown in Figs. 1–6, respectively.

On each experimental day, the mean values of serum level of serum enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) in group I (negative control) and group II (positive control) showed no significant changes, when compared with the experimental values of serum enzymes and bilirubin within the group, and also compared with the corresponding values of each other. In groups III–VI, these enzymes and bilirubin levels showed changes on each experimental day, when compared with the experimental values of serum enzymes and bilirubin within the group.

Furthermore, the levels of serum enzymes and bilirubin showed significant difference (p < 0.05) in groups III–VI on each experimental day, when compared with the corresponding values of each other, and also compared with the corresponding values of groups I and II.

4. Discussion

It has been demonstrated that liver of rabbits was markedly damaged by histamine and HR (H1R, H2R, H3R, and H4R) agonist (Tripathi et al., 2010b). The damage caused by short-term exposure to histamine and HR agonist appeared quite specific for each of the agonist (Tripathi et al., 2010b). In general, increased incidence of hepatocyte polyplody (bi- and multinucleated), frequent occurrence of Kupffer cells (KC), variable grades of necrosis, congestion, and lympho-cytic infiltration constituted the hallmark of injuries were observed in histamine and HR agonist treated rabbits (Tripathi et al., 2010b). Moreover, it has been shown that the elevation of endogenous histamine by amodiaquine (a histamine N methyltransferase inhibitor) plays a protective role through the regulation of TNF-α production in endotoxin-induced hepatic injury in mice (Yokoyama et al., 2007).

Imoto et al. (1985) suggesting that histamine by H1R plays an important role in liver function. In addition to this, we have designed our study to explore histamine and H1R agonist and antagonist induced liver functions in immunized [with SRBC, a T cell-dependent test antigen (Tripathi et al., 2010c–g)] and non-immunized rabbits.

The abnormalities in liver function tests (LFTs) are elevated levels of static biochemical tests, including liver enzymes
(ALT, AST, and alkaline phosphatase) and bilirubin, which are the biochemical parameters of liver damage (Masaki et al., 2005; Thapa and Walia, 2007). The biotransformation of xenobiotics takes place in liver (Maciejewska-Paszek et al.,

**Figure 1** Effects of histamine and histamine H1-receptor agonist and antagonist on serum ALT levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman–Keuls post hoc test revealed that the effect of treatments ($F = 1163.937, \text{DF} = 5102; p < 0.01$) and days ($F = 237.363, \text{DF} = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments \(\times\) days) effect of ($F = 285.644, \text{DF} = 25,510; p < 0.01$) these on SRBC was also found to be significant.

**Figure 2** Effects of histamine and histamine H1-receptor agonist and antagonist on serum AST levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman–Keuls post hoc test revealed that the effect of treatments ($F = 482.721, \text{DF} = 5102; p < 0.01$) and days ($F = 122.566, \text{DF} = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments \(\times\) days) effect of ($F = 177.821, \text{DF} = 25,510; p < 0.01$) these on SRBC was also found to be significant.
The Influence of histamine H1-receptor on liver functions in immunized rabbits

2007), therefore, the LFTs are the most common blood tests required by clinical practitioners for examining liver damage. The most common abnormalities of LFTs in an asymptomatic person are increased ALT and AST. The upper normal limits

Figure 3  Effects of histamine and histamine H1-receptor agonist and antagonist on serum alkaline phosphatase (ALP) levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman–Keuls post hoc test revealed that the effect of treatments ($F = 696.678$, $DF = 5102; p < 0.01$) and days ($F = 742.821$, $DF = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments $\times$ days) effect of ($F = 270.182$, $DF = 25,510; p < 0.01$) these on SRBC was also found to be significant.

Figure 4  Effects of histamine and histamine H1-receptor agonist and antagonist on serum total bilirubin (TB) levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman–Keuls post hoc test revealed that the effect of treatments ($F = 775.278$, $DF = 5102; p < 0.01$) and days ($F = 199.418$, $DF = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments $\times$ days) effect of ($F = 134.927$, $DF = 25,510; p < 0.01$) these on SRBC was also found to be significant.
of ALT and AST differ from lab to lab (Giannini et al., 2005). AST and ALT reflect liver degeneration. Both AST and ALT may be raised in patients with muscle injury (Hasan and Owed, 2003). In most liver diseases, AST/ALT ratio is < 1.

Figure 5 Effects of histamine and histamine H1-receptor agonist and antagonist on serum direct bilirubin (DB) levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F = 469.121, DF = 5102; p < 0.01$) and days ($F = 142.24, DF = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments $\times$ days) effect of ($F = 111.402, DF = 25,510; p < 0.01$) these on SRBC was also found to be significant.

Figure 6 Effects of histamine and histamine H1-receptor agonist and antagonist on serum indirect bilirubin (IB) levels. The results demonstrate mean ± s.d. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F = 71.109, DF = 5102; p < 0.01$) and days ($F = 6.737, DF = 5510; p < 0.01$) on SRBC were statistically significant. The interaction (treatments $\times$ days) effect of ($F = 7.876, DF = 25,510; p < 0.01$) these on SRBC was also found to be significant.
However, in alcoholic liver disease (alcoholic steatosis, alcoholic hepatitis, and cirrhosis), AST/ALT is > 1, and is usually > 2 (Sorbi et al., 1999). Mild to moderate elevations in serum ALT and AST levels may be observed in virtually all liver diseases, and thus are generally nonspecific (Mathiesen et al., 1999). ALT and AST levels exceeding 15 times the upper limit of normal are usually found in a limited number of conditions (i.e. drugs, acute viral hepatitis, ischemic hepatitis, autoimmune hepatitis, common bile duct stones and muscle injury) (Fortson et al., 1985; Johnson et al., 1995; Mathiesen et al., 1999). ALT and AST are sensitive markers of hepatocellular injury but they lack specificity as they are also present in muscle (cardiac and skeletal), kidney and red blood cells (Yap and Aw, 2010). Serum alkaline phosphatase (ALP) activity may derive from liver, bone, intestine, and placenta. Various hepatobiliary (such as drugs, bile duct obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, liver metastasis, infiltrative liver disease, viral hepatitis, alcoholic hepatitis, and cirrhosis) and non-hepatic (bone disease, pregnancy, chronic renal failure, congestive heart failure, childhood growth, and malignancy) causes are related with raised ALP. However, low serum ALP in patients with biochemical evidence of liver dysfunction may be a clue to Wilson’s disease. Also, low ALP may be encountered in hypothyroidism, zinc deficiency, pernicious anemia, and congenital hypophosphatemia (Hasan and Owied, 2003). The results of this study revealed that groups III–VI show impaired serum levels of ALT, AST, and ALP. This study shows moderate alterations in serum levels of ALT, AST, and ALP in group IV (HTMT) while group III (histamine), group V (pheniramine), and group VI (pheniramine + histamine) show mild impairment. Thus, serum levels of ALT and AST impairment represent liver damage in the order: HTMT > pheniramine > pheniramine + histamine > histamine, when compared with positive control and negative control.

Furthermore, serum bilirubin is a mixture of α, β, γ, and δ fragments which are unconjugated, singly conjugated, doubly conjugated, and covalently bound to albumin, respectively (Yap and Aw, 2010). Although δ bilirubin measurement is available, it has not gained wider utility. In most cases a total bilirubin (TB) assay suffices for LFT, but fractionation may be required in isolated increases in bilirubin and neonatal jaundice. Direct bilirubin (DB) refers to the conjugated bilirubins that react directly with the diazo reagent, while indirect bilirubin (IB) is a derived value obtained from the difference of TB and DB. DB assays measure only 70–90% of the conjugated and δ bilirubins, and may underestimate the severity of jaundice (Yap and Aw, 2010). Fractionation of serum bilirubin is useful in separating the causes of jaundice. In prehepatic jaundice due to hemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin. In hepatic and post-hepatic jaundice, there is increased conjugated and δ bilirubin (Yap and Aw, 2010). When the liver function tests are abnormal and the serum bilirubin levels more than 17 μmol/L suggest underlying liver disease (Friedman et al. 2003; Thapa and Walia, 2007). The bilirubin results of this study revealed that the serum levels of TB, DB, and IB in groups III–VI show liver function impairment in the order: histamine > HTMT > pheniramine + histamine > pheniramine, when compared with positive control and negative control.

It is therefore concluded that histamine, HTMT (H1R agonist), pheniramine (H1R antagonist), and combination of pheniramine + histamine cause hepatic function impairment in terms of altered serum enzymes and bilirubin levels. Thus, the present findings suggest that HTMT causes moderate liver function impairment while others show mild impairment warranting further long-term studies. This study would definitely stimulate active discussions about the effects of histamine and its HR agonists and antagonists on hepatic functions. To the best of our knowledge, this is the first report describing a comparative study of histamine and H1R agonist and antagonist on liver function in immunized and non-immunized rabbits.

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References


