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Investigation of the direct hepatic effects of intramuscular interleukin-8 injection in an experimental rabbit model

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Background: The aim of this study was to investigate the effects of intramuscular IL-8 injection on hepatic tissues using an *in vivo* histopathological animal model.

Material/Methods: Twelve New Zealand white rabbits were used for this randomized, controlled, single-blinded interventional study. For 6 days, 1 gluteus maximus muscle was injected daily with 1 mcg/kg of IL-8 in 6 rabbits (Group A). The remaining 6 rabbits (to determine to normal porto-hepatic morphology of the rabbit genus) were in the sham group (Group B). At the end of the 7th day, all rabbits were killed and livers were meticulously harvested. Microscopically, regional tissues were scored according to portal inflammation, focal necrosis, piecemeal necrosis, and total impact.

Results: Total impact score, portal inflammation, focal necrosis, and piecemeal necrosis were the histopathologic changes present in a higher incidence in the IL-8 group compared with the control group. The differences were significant when the groups were compared according to total impact score, portal inflammation, focal necrosis, and piecemeal necrosis according to Pearson's correlation ($p<0.05$). The most significant differences were detected at the total impact scores ($p=0.002$) and the portal inflammation scores ($p=0.008$).

Conclusions: Our results showed that IL-8 may damage hepatocytes. This can be the determined target for new therapeutic strategies. Further trials should be designed to obtain definitive results.

Key words: interleukin-8 • hepatotoxicity • liver

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Background

The liver may be considered as the most important organ in drug toxicity for 2 reasons: on the one hand, it is functionally interposed between the site of absorption and the systemic circulation and is a major site of metabolism and elimination of foreign substances; but on the other hand, these features also render it a preferred target for drug toxicity. Drug-induced liver injury therefore poses a major clinical problem. Certain drugs, when administered at therapeutic dose and some in overdose, may injure the liver [1,2]. A great majority of hepatic adverse reactions seen in clinical practice are unpredictable (unrelated to a drug's pharmacological characteristics) and basically result from an interaction of 3 circumstances: a drug with potential to generate hepatotoxic radicals in a genetically susceptible individual under certain environmental factors [3–6]. This type of reaction, which occurs only rarely, goes undetected during the drug development process, and hence typically manifests when tens of thousands of patients are exposed to it post-marketing; this still represents the leading cause for withdrawal of drugs from market.

Interleukin-8 (IL-8) is a neutrophil chemoattractant that stimulates proliferation and migration [7,8]. IL-8 can be produced by a various types of involved in inflammation, including monocytes and endothelial cells. Furthermore, the effects of IL-8 in acute phase reaction, inflammatory response, and angiogenesis stimulation have already been determined [7,9]. Additionally, several observations suggest that IL-8 pathways might also be involved in the pathogenesis of chronic liver disease [3,5,6,10]. For instance, IL-8 levels are increased intrahepatically and in the serum of patients with alcoholic liver disease, probably contributing to hepatic neutrophil accumulation and also exerting systemic actions [6,10]. In patients with chronic hepatitis C, IL-8 serum levels are associated with disease progression and relate to interferon unresponsiveness (10). Interestingly, experimental work raised the possibility that IL-8 may not only act as a mere chemoattractant protein in the liver, but also exerts direct profibrogenic functions. However, direct isolated IL-8 effects on hepatic tissues have not yet been sufficiently elucidated.

The aim of the present study was to investigate the effects of intramuscular IL-8 injection on hepatic tissues in an *in vivo* histopathological animal model.

Material and Methods

Study design

This randomized, controlled, single-blinded interventional study was approved by the Animal Ethics Committee (Reference Number: 2011/16) and was conducted in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 5377-3, 1996), Animal Ethics Committee." Twelve New Zealand white rabbits obtained from the Laboratory Animal Production Unit were used. Before surgery, rats were kept in the laboratory for 1 week in a temperature – (22±2°C) and humidity – (50±5%) controlled room for acclimatization with exposure to 12 hours of light and 12 hours of darkness. Rats were fed with tap water and standard rodent feed ad libitum. The rats were given only water during the 12 hours before start of the procedures.

Gluteal regions of the rabbits were shaved. For 6 days, 1 gluteus maximus muscle was injected daily with 1 mcg/kg (The dosages were standardized according to previous studies [9]) of IL-8 (BioVision recombinant human endothelial IL-8, cat. number 4149-25) in 6 rabbits (Group A). The remaining 6 rabbits (to determine to normal porto-hepatic morphology of the rabbit genus) were in the sham group (Group B). At the end of the 7th day, all rabbits were killed and livers were meticulously harvested.

Table 1. Histology activity index.

Periportal or periseptal interface hepatitis (piecemeal necrosis)	
None	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
None	0
One focus or less per 10× objective	1
Two to four foci per 10× objective	2
Five to ten foci per 10× objective	3
More than 10 foci per 10× objective	4
Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/ marked, all portal areas	3
Marked, all portal areas	4

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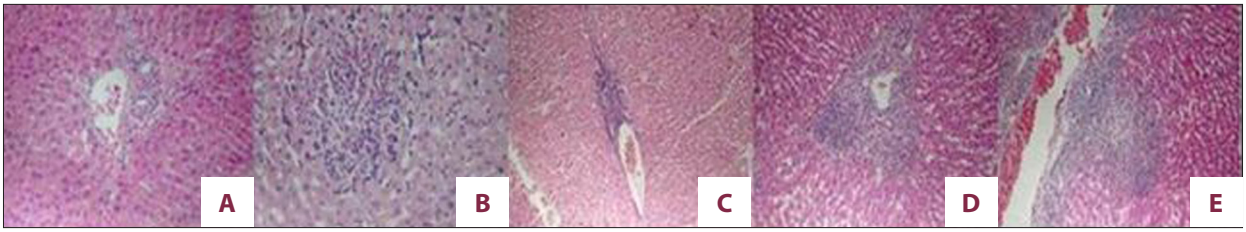

Figure 1. (**A**) Native portal area [no lymphocytic infiltration] (×200, H&E). (**B**) Hepatic tissue with focal necrosis (×200, H&E). (**C**) Moderate lymphocytic infiltration in portal area (×100, H&E). (**D**) Intensive lymphocytic infiltration in portal area (×100, H&E). (**E**) Severe Piecemeal necrosis (×100, H&E).

Table 2. Comparison of obtained scores in each group.

Groups	IL-8 group (n=6) Mean ±SD (min.–max)	Control group (n=6) Mean ±SD (min.–max)
Total score	5.67±1.51 (3–7)	2.67±0.82 (2–4)
Portal inflammation	2.67±1.03 (1–4)	1.17±0.41 (1–2)
Focal necrosis	2.00±0.63 (1–3)	1.17±0.41 (1–2)
Piecemeal necrosis	1.00±0.00 (1–1)	0.33±0.52 (0–1)

SD – Standard Deviation.

Histopathological analysis

The harvested specimens were fixed in 10% formaldehyde solution for 48 hours, routinely processed, and embedded in paraffin. Slices of 4 ìm thickness were obtained from paraffin blocks and put onto polylysine-coated slides. One of the slices was stained with hematoxylin-eosin (H&E) and the other was stained with Masson's Trichrome. Counting procedure was conducted by 2 blinded pathologists at different times. These pathologists examined slides under a light microscope (higher magnification – 400×; Nikon E400). Microscopically, regional tissues were scored according to portal inflammation, focal necrosis, piecemeal necrosis, and total impact. For every experimental animal, median values of the scores generated by 2 pathologists were calculated (decimal numbers were rounded up). The histopathologic scoring criteria are listed in Table 1. The native histopathologic microscopic view of the portal area and varied scores of damaged hepatic areas are provided in Figure 1.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 10.0 for Windows). All differences associated with a chance probability of 0.05 or less were considered statistically significant. The percentages of necrosis score values for the 3 groups were compared using Pearson's correlation test. Two-sided p values were considered statistically significant at $p \leq 0.05$.

Results

The obtained scores of portal inflammation, focal necrosis, piecemeal necrosis, and total impact were presented as mean ± standard deviation (SD) in each group.

Total impact score, portal inflammation, focal necrosis, and piecemeal necrosis were the histopathologic changes present in a higher incidence in the IL-8 group compared with the control group. The obtained scores are summarized in Table 2. The total impact scores (5.67±1.51 *vs.* 2.67±0.82), portal inflammation scores (2.67±1.03 *vs.* 1.17±0.41), focal necrosis scores (2.00±0.63 *vs.* 1.17±0.41), and piecemeal necrosis scores (1.00±0.00 *vs.* 0.33±0.52) were markedly higher in the IL-8 group.

The differences were significant when the groups were compared according to total impact score, portal inflammation, focal necrosis, and piecemeal necrosis according to Pearson's correlation ($p<0.05$). The most significant differences were detected at the total impact scores ($p=0.002$) and the portal inflammation scores ($p=0.008$). The statistical findings are presented in Table 3.

Discussion

IL-8, a member of the chemokine family, has been identified as a neutrophil chemotactic polypeptide in the conditioned media of lipopolysaccharide-stimulated peripheral blood monocytes. The protein, consisting of 72 amino acids in its mature form, is

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Table 3. Statistical syllogism of IL-8 and control groups.

		Total	Portal	Focal	Piecemeal	Groups
Total	Pearson Correlation	1.000	0.956	0.897	0.632	0.805
	Sig. (2-tailed)	0.000	0.000	0.000	0.027	0.002
Portal	Pearson Correlation	0.956	1.000	0.826	0.625	0.723
	Sig. (2-tailed)	0.000	0.000	0.001	0.030	0.008
Focal	Pearson Correlation	0.897	0.826	1.000	0.644	0.651
	Sig. (2-tailed)	0.000	0.001	0.000	0.024	0.022
Piecemeal	Pearson Correlation	0.632	0.625	0.644	1.000	0.707
	Sig. (2-tailed)	0.027	0.030	0.024	0.000	0.010
Groups	Pearson Correlation	0.805	0.723	0.651	0.707	1.000
	Sig. (2-tailed)	0.002	0.008	0.022	0.010	0.000

identified as a basic and heparin-binding protein [7,9,11,12]. IL-8 also exhibits chemotactic activities against T lymphocytes and basophils, as well as neutrophils *in vitro* [9,10]. IL-8 production has also been observed *in vitro* in a wide variety of cells, including monocytes, T lymphocytes, neutrophils, vascular endothelial cells, dermal fibroblasts, keratinocytes, hepatocytes, and human gastric cancer cells [13].

High IL-8 levels in the liver and the circulation have been found in patients with acute liver injury, such as alcoholic hepatitis or ischemia-reperfusion injury [3,5,6,10]. These conditions are clearly associated with neutrophil-mediated tissue damage, but less is known about the involvement of IL-8 and/or neutrophils in chronic liver injury.

Reports in the literature have indicated that IL-8 may have many harmful impacts. In particular, it was claimed that IL-8 has an important role in many hepatic disorders induced by varied agents. IL-8 had been functionally linked to hepatic neutrophil infiltration and liver inflammation, and to activation of profibrogenic, collagen-producing hepatic stellate cells [14–18]. Nobili et al suggested that IL-8 was responsible for portal inflammation in patients with chronic liver disease and found that serum IL-8 levels were elevated in patients with neonatal hepatitis [14]. Zimmermann et al reported that IL-8 has a novel role in recruitment and activation of hepatic macrophages in chronic liver disease [15]. Taïeb et al. declared that alcoholic hepatitis involved elevated tumor necrosis factor alpha and IL-8 plasma and tissue levels [16]. Additionally, Purohit et al noted that mortality was related with the serum concentrations of certain cytokines, including tumor necrosis factor alpha, IL-6, and IL-8, in hospitalized patients with alcoholic hepatitis [17]. Therefore, Tachibana et al. investigated the effect of IL-8 in patients with chronic hepatitis C and reported that increased IL-8 production may be related with malignant transformation of hepatocytes [18]. Another trial, investigating carbon tetrachloride induced hepatotoxicity, reported that "carbon tetrachloride causes a rapid increase in IL-8 mRNA expression in labeled poly A+ RNA from cultured human hepatoma cells and this increase correlates with a later and significant increase in the levels of IL-8 protein" [1]. Additionally, James et al claimed that elevated IL-8 level was predictive of hepatocellular injury caused by acetaminophen hepatotoxicity in children and adults [3]. Similar published studies have found many more cytokines in hepatotoxicity by separate or combined impact [19,20]. However, there was not sufficient data to establish the isolated effect of IL-8 in hepatic tissues. In the present study, we aimed to reveal the hepatic impacts of isolated IL-8 injection in a rabbit model. Our results demonstrated that the portal area is highly affected by IL-8 injection.

The Knodell histology activity index (HAI), published in 1981, was the first system of its type and is widely regarded as the benchmark for objective, semiquantitative, reproducible description of the various morphological lesions of chronic hepatitis [21]. Since publication of the Knodell HAI, systems for grading and staging incorporate the view that necroinflammation is not only a measure of severity but also of ongoing disease activity and is the parameter most potentially responsive to therapy. This is referred to as "grade". The lesions of fibrosis and parenchymal or vascular remodeling are referred to as "stage" and indicate long-term disease progression. Grade may fluctuate with disease activity or therapeutic intervention; stage is considered

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relatively constant. All systems report grade and stage, although they may arrive at a score using different criteria. The differences are subtle but potentially important when comparing clinical studies that have used 2 different systems. Ishak's 1994 review promotes the use of descriptive terminology for activity and fibrosis, rating the different elements of activity as either present or absent; when present, a degree of severity is stated [22]. Finally, a recent modification of the Knodell HAI, commonly referred to as the Ishak system, provides consecutive scores for well-defined lesions within 4 separate categories, which are added together for the activity grade [23] (Table 1). In the present study, portal inflammation showed higher statistical significance between IL-8 injected rabbits and the control group (p=0.008). Moreover, focal necrosis (p=0.022), piecemeal necrosis (p=0.010), and total impact (p=0.002) were significantly higher in intramuscular IL-8 – injected rabbits. These findings suggested that IL-8 alone caused hepatic disruption in all hepatic areas.

There are 2 limitations that need to be acknowledged and addressed regarding the present study. The first limitation concerns use of an animal model in the study. The results should be supported with other *in vivo* and *in vitro* experimental models. The second limitation concerns the dosage of IL-8 injection. It can be assumed that the dose of IL-8 injection used in the study is toxic. However, IL-8 injection is not a currently used method. We conducted one of the few studies in the literature that focused on investigating the angioneogenic effect of IL-8 administered intramuscularly in rats, and concluded that daily administration at a dose of around 1 mcg/kg caused local tissue necrosis [9]. However, duration of repeat doses and safety dosage ranges must be clarified by further investigations.

Conclusions

The inflammatory action of hepatotoxic agents is already known. The current treatment strategies were focused on preventing these harmful hepatic inflammatory responses. However, the therapeutic target has not yet been defined. Our results show that IL-8 may damage hepatocytes. This can be the defined target for new therapeutic strategies. Further trials should be designed to obtain definitive results.

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