TITLE PAGE

An analysis of drug-induced liver injury, which showed histological findings similar to autoimmune hepatitis

Short title: Drug-induced autoimmune hepatitis

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ABSTRACT

Background: Drug-induced liver injury (DILI) sometimes resembles autoimmune hepatitis (AIH) in its hepatic histology. However, there is lacking data of a comparison of the characteristics between such DILI and DILI without histological findings like AIH.

Methods: We enrolled 62 patients with DILI who were diagnosed using the Roussel Uclaf Causality Assessment Method, and performed a liver biopsy. These patients were classified into two groups: DILI with histology like AIH (group A, n = 23) and DILI without such histology (group B, n = 39). Sixteen patients of group A could be further classified into two groups: patients with relapse of the liver injury (group C, n = 8) and without relapse (group D, n = 8), after the recovery of the DILI. We compared the clinical and histological findings between group A and B, and group C versus D.

Results: Group A was characterized by an older age (P = 0.043), higher immunoglobulin G level (P = 0.017), positive antinuclear antibody status (P = 0.044), and a higher frequency of complementary alternative medicines and Chinese herbal medicines as the causative drug (P = 0.008). There were no significant differences between group C and D regarding the clinical data and liver histological findings.

Conclusions: The clinical characteristics of DILI which showed histological findings

similar to AIH were revealed. In such patients, the liver biopsy is recommended in order to determine the appropriate treatment strategy. In DILI with histology like AIH patients, long-term follow-up is needed to perceive the relapse.

KEYWORDS: drug-induced liver injury, liver histology, autoimmune hepatitis, complementary alternative medicines, relapsed case.

ABBREVIATIONS

DILI: drug-induced liver injury

AIH: autoimmune hepatitis

ANA: antinuclear antibody

IgG: immunoglobulin G

CAM: complementary alternative medicines

DILI with AIH-like-HF: DILI with AIH-like hepatic histological findings

RUCAM: the criteria of the Roussel Uclaf Causality Assessment Method of the Council for International Organizations of Medical Sciences [8]

JDDW-scale: the diagnostic scale of Digestive Disease Week-Japan 2004 [9]

CAM/C: CAM including dietary supplements and Chinese herbal medicines

UNL: upper normal limit; ALT: alanine aminotransferase

DILI without AIH- like-HF: DILI without AIH-like histological findings

WBC: peripheral white blood cells

ALP: alkaline phosphatase

PT: prothrombin time

G-ratio: the ratio of IgG to the UNL of IgG

HLA: Human leukocyte antigen

CYP: cytochrome P450

anti-LKM2: anti-liver/kidney microsome antibody type 2

Introduction

Some cases of drug-induced liver injury (DILI) have similar features in histology and serology to autoimmune hepatitis (AIH). Nitrofurantoin, minocycline and oxyphenisatin, a laxative that disappeared from the market, are drugs that are highly suspected of being causative of drug-induced AIH. A high level of gammaglobulin has been observed in all cases of chronic DILI associated with nitrofurantoin, with antinuclear antibody (ANA) being positive in 82% of cases [1]. In patients with oxyphenisatin-induced liver injury, a high level of gamma globulin and positive ANA status have been found in 100% and 50% of examined cases, respectively [2]. DILI associated with minocycline often presents with clinical findings that are compatible with AIH in young females. In a previously reported case of minocycline-induced fulminant hepatic failure with a high serum immunoglobulin G (IgG) level and positive ANA status, the liver histology suggested AIH [3]. Recently, there have been reports of drug-induced AIH by lipid-lowering agents [4, 5]. In patients with DILI associated with fenofibrate or simvastatin, the high level of serum IgG and positive ANA status disappeared following the withdrawal of the causative drugs [4]. In one case of atorvastatin-induced liver injury, the clinical and histological findings resembled those of AIH [5]. On the other hand, the causative drugs are not necessarily prescription drugs. In two cases of liver

injury associated with *Agaricus blazei* Murill, a type of complementary alternative medicines (CAM), the hepatic histological findings were very similar to those of AIH, and one of these patients exhibited relapse of liver damage while tapering the dose of prednisolone [6]. However, in another study, one difference in the clinical course after immunosuppressive therapy between AIH and drug-induced AIH associated with nitrofurantoin and minocycline (46% each) was that the patients with drug-induced AIH did not relapse after the withdrawal of immunosuppressive therapy [7].

First, in this study, we investigated the clinical characteristics of DILI with AIH-like hepatic histological findings (DILI with AIH-like-HF). Secondly, we investigated factors related to relapse of DILI with AIH-like-HF.

Patients and Methods

Definition

Drug-induced liver injury and causative drugs

DILI was diagnosed by using the criteria of the Roussel Uclaf Causality Assessment Method of the Council for International Organizations of Medical Sciences (RUCAM) [8] and the diagnostic scale of Digestive Disease Week-Japan 2004 (JDDW-scale) [9]. A case

of liver damage was considered to be DILI, when the RUCAM score implied a finding of "possible," "probable" or "highly probable," and when the score of the JDDW-scale was evaluated "possible" or "high possibility." Screening for the following non-drug-related causes was performed: Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Epstein-Barr virus, Cytomegalovirus, Herpes simplex virus, episodes of acute hypotension that cause shock to the liver, ultrasonography of liver and biliary tract abnormalities and alcoholic liver damage. The causative drugs were classified into two categories: CAM including dietary supplements and Chinese herbal medicines (CAM/C), and prescription drugs excluding Chinese herbal medicines.

AIH-like histological findings

We referred to the international criteria [10] and defined AIH-like histological findings as follows:

(A) The patient must have interface hepatitis with lymphoplasmacytic infiltration.

In addition, more than one of the following findings of ((B) or (C)) must be present on the liver histology.

(B) Rosette formation of hepatocytes

(C) Severe necro-inflammatory reaction.

We defined as “DILI with AIH-like-HF” a patient that met these two definitions of “DILI” and “AIH-like histological findings”.

Histological findings of hepatic inflammation and fibrosis

We evaluated the stage of hepatic fibrosis and grade of inflammation from 1 to 4 and from 0 to 4, respectively, referring to the classification of Ludwig [11]. Other histological findings, including the grade of plasma cell infiltration and rosette arrangement, were evaluated in four grades: “0,” “1,” “2,” and “3.” Additionally, the existence of emperipolesis was evaluated.

Relapse and non-relapse

“Relapse” was defined as an elevation of more than twice the upper normal limit (UNL) of serum alanine aminotransferase (ALT) after a normalization of liver injury, without evident cause. The UNL of ALT is 30 U/l. The number of the days from a normalization of the ALT level until verifying of a relapse was counted for the period of relapse. “Non-relapse” was defined as an ALT value that remained under the UNL for more than 3 years after the recovery of liver injury.

Patients

From 1988 to 2010, 237 patients were diagnosed with DILI by using the RUCAM [8]. In 93 patients, liver biopsies were performed with informed consent obtained in writing. Patients with liver cirrhosis, liver failure, primary biliary cirrhosis or primary sclerosing cholangitis, drinkers with a history of consuming more than 60 g of daily alcohol and patients who began receiving immunosuppressive therapy, such as steroids before the liver biopsy were excluded. All biopsy materials were reviewed by a single liver pathologist who was blinded to the clinical context of the biopsy as well as the patient's outcome. We finally enrolled 62 patients with DILI in this study. These patients were classified into two groups by hepatic histological findings, consisting of DILI with AIH-like-HF and DILI without AIH-like histological findings (DILI without AIH- like-HF).

Methods

The following characteristics were compared between the DILI with AIH-like-HF and DILI without AIH-like-HF groups: gender, age, body mass index, complications of autoimmune disease, category of causative drugs, RUCAM score [8], score of JDDW-scale [9] and clinical data. The examined data included the following onset data: peripheral white blood cells (WBC), the percentage of peripheral eosinophil, the serum

ALT level, the asparate aminotransferase level, the ratio of alkaline phosphatase (ALP) to the UNL of ALP, the gamma-glutamyl transferase level, the total bilirubin level, the percentage of prothrombin time (PT) of the standardized value, the ANA status evaluated via indirect immunofluorescence and the ratio of IgG to the UNL of IgG (G-ratio). We used ratio for evaluation of ALP and IgG. When data for the IgG levels were lacking, we used the ratio of the level of gamma globulins to the UNL of gamma globulins as the G-ratio. The following maximum values were evaluated: max ALT, max asparate aminotransferase, max ratio of ALP to the UNL of ALP, max gamma-glutamyl transferase and max total bilirubin. In addition, the minimum percentage of PT of the standardized value was compared between the two groups. The ANA status was evaluated as being either “positive” or “negative” using a cut-off index of 40 times. The exposed period by a causative drug, and the use of immunosuppressive treatment were also compared.

Furthermore, the following characteristics were compared between the relapsed and non-relapsed cases in the DILI with AIH-like-HF group: background, clinical data, score of the RUCAM [8] and the JDDW-scale [9], immunosuppressive treatment and hepatic histological findings including the stage of fibrosis, grade of inflammation and, presence of plasmacytic infiltration, rosette formation and emperipolesis. The compatibility of

AIH was evaluated using the AIH score [10] and the Simplified AIH score [12], and the scores were compared between the relapsed and non-relapsed case.

Human leukocyte antigen (HLA) typing was performed in the patients in the DILI with AIH-like-HF group, with informed consent obtained in writing.

Statistical analysis

Comparisons of two groups, i.e., "the DILI with AIH-like-HF versus the DILI without AIH-like-HF groups" and "the relapsed versus non-relapsed groups", using the Mann-Whitney and Chi-square tests in a univariable analysis. Fisher's exact test was used in place of the Chi-square test when the number of cases was small. For the multivariable analysis, a logistic regression analysis was used. Variables were considered for the multivariable models if their univariable P value was <0.05. "The use of immunosuppressive treatment" and "Exposed period by taking a causative drug" were excluded from the multivariable models because they were influenced by artificial judgment including physician's discretion. When the lower 95% confidence interval exceeded 1.0, the odds ratio was considered to be statistically significant.

Results

Comparison of the background factors associated with the existence of AIH-like histological findings

These 62 patients (45 females and 17 males) were classified into two groups, consisting of a DILI with AIH-like-HF group (n = 23) and a DILI without AIH-like-HF group (n = 39). Comparing the DILI with AIH-like-HF group and the DILI without AIH-like-HF group, the DILI with AIH-like-HF group was characterized by an older age (P = 0.002), a fewer counts of WBC (P = 0.032), a higher G-ratio (P <0.0001), a positive ANA status (P = 0.003) and a higher frequency of CAM/C as a causative drug (P<0.0001). Although the immunosuppressive treatment and exposed period involved artificial judgment, they were more frequently (P < 0.0001) and longer (P < 0.0001), respectively. Among the other subjects including score of the RUCAM [8] and the JDDW-scale [9], there were no statistical significant differences (Table 1). The results of a multivariable analysis showed an older age (P = 0.043), a high G-ratio (P = 0.017), a positive ANA status (P = 0.044) and a causative drug categorized as CAM/C (P = 0.008) to be independently correlated with DILI with AIH-like-HF (Table 2).

The causative drugs for DILI with AIH-like-HF are shown in Table 3. Twelve cases of "CAM/C" and five cases of "Prescription drugs excluding Chinese herbal medicines",

involved single agents. In three cases of DILI associated with *Agaricus blazei* Murill, the serum zinc sulfate turbidity test which reflects gamma globulin, reaching 6.7, 3.4 and 2.2 times of the base line, respectively, when the causative drug was stopped. In one of these cases, ANA was negative at the start of the causative drugs, and became positive at the onset of liver damage. In addition, the serum IgG level increased from 1720 mg/dl to 2420 mg/dl during the same period.

Clinical Course

The causative drugs were discontinued in all 62 patients. Hepatoprotective therapy (i.e., ursodeoxycholic acid and intravenous injection of glycyrrhizin) and the administration of steroids were carried out. These therapies were administered as single or combination therapies. The administration of steroids was performed in 14 cases of the 23 DILI with AIH-like-HF cases (60.9%) and four of the 39 DILI without AIH-like-HF cases (10.3%). Steroid therapy was more frequently used in the patients with DILI with AIH-like-HF ($p<0.0001$). However, the decision to administer steroids was made by each physician in reference to the liver histology. Hepatoprotective therapy without steroid was administered in nine DILI with AIH-like-HF cases and 23 DILI without AIH-like-HF cases. Remained 12 DILI without AIH-like-HF cases was the

only withdrawal of the causative drugs.

In addition, the serum IgG level or gamma globulin was observed after discontinuing the causative drugs in 20 of the 23 (87.0%) cases of DILI with AIH-like-HF. These values decreased in all cases. The ANA status was followed in 14 DILI with AIH-like-HF cases (positive versus negative = 8 versus 6), and became negative after the withdrawal of the drugs in five of the eight positive cases (62.5%). None of the six negative ANA cases changed to a positive status during the observation periods.

Relapsed and non-relapsed cases of DILI with AIH-like-HF

The ALT levels of all patients with DILI with AIH-like-HF normalized due to the therapies, excluding one patient who died before normalization of ALT levels could be achieved. Sixteen of the 23 patients (69.6%) with DILI with AIH-like-HF were judged regarding whether they developed relapse of liver damage. Two patients who died due to pulmonary aspergillosis under immunosuppressive therapy, and five patients whose liver injury recovered but did not satisfy the observation period requirements were excluded from the comparison of the relapsed and non-relapsed cases. There were eight relapsed and non-relapsed cases each. The median observation period for the

non-relapsed cases was 2,290 days (range: 1295-3294 days). In seven non-relapsed patients who were administered steroids, the observation periods were calculated from the withdrawal of the steroids, while in one patient, steroids was not administered, the observation period was calculated from the time that a normalization of the ALT level was observed. Eight cases relapsed, consisting of four patients treated without steroids, two patients who had previously received steroids and two patients undergoing a tapering of the steroid dosage. The median period until relapse was 283 days (range: 47-1090 days) (Table 4). The serum IgG level was evaluated at relapse in the six of these eight patients, and it increased after the recovery of the first liver injury in all patients (Table 4).

The AIH scores [10] and Simplified AIH scores [12] at the first liver injury of the relapsed cases were 11.0±2.1 (mean±SD) (range, 9-16) (one definite case, six probable cases) and 5.5±1.7 (3-8) (two definite cases, two probable cases), respectively. The scores of the non-relapsed cases were 11.5±1.4 (9-13) (no definite cases, seven probable cases) and 5.5±1.6 (3-7) (two definite cases, two probable cases), respectively. Therefore, there were no significant differences in the scores between the relapsed and non-relapsed cases (Table 5). There were also no significant differences in the comparison between

the two groups regarding the clinical data, the score of the RUCAM [8] and the JDDW-scale [9], liver histological findings, and use of immunosuppressive therapy (Table 5). The stage of fibrosis and the inflammatory grade at the first liver injury of the relapsed versus non-relapsed cases were 1.0±0.5 (range, 1-2) versus 1.0±0.5 (1-2) and 6.0±1.4 (4-8) versus 6.0±1.3 (4-8), respectively. We additionally presented the hepatic histology of two relapsed (Fig. 1) and two non-relapsed cases (Fig. 2); the findings were very similar.

Phenotypes of HLA typing in the DILI with AIH-like-HF group

Seven of the 23 DILI with AIH-like-HF patients were assessed for HLA typing. These seven cases included three relapsed cases, three non-relapsed cases and one case that was excluded from assessment of relapse. Five of the patients were positive for DR4 (71.4%), including three relapsed and two non-relapsed cases.

Discussion

In this study, 23 of 62 cases (37.1%) of DILI showed histological findings that were very similar to those of AIH. This rate which is approximately one-third, is not low. However, this ratio is considered to be actually lower since the liver biopsy is not usually done in

mild DILI patients. In consideration of this, elucidating the characteristics of such patients' conditions is thus considered to be important. In addition, these results suggested that there are two disease states after the withdrawal of the causative drugs: namely relapsed and non-relapsed cases.

Characteristic features of DILI with AIH-like-HF

Four characteristics of DILI with AIH-like-HF were revealed by multivariable analysis. The patients with DILI with AIH-like-HF were older. In general, exposure to drugs and the metabolites increases with age because the metabolism of drugs is reduced in elderly patients. This phenomenon is related to an increased risk of an immune reaction against a drug. A high G-ratio and a positive ANA status suggest that various immune mechanisms result in clinical histological findings similar to those of AIH. One of these mechanisms is that immune reactions to neoantigens, which are produced by combining the reactive metabolites of the drug and the proteins of hepatocytes, trigger immune activation. For example, in some drug-metabolites mediated by cytochrome P450 (CYP), metabolite-CYP adducts that activate the immune system, are produced and become neoantigens [13, 14]. Another pathogenic mechanism is that of DILI associated with tienilic acid, which resembles type 2 AIH in its

serological findings. This type of DILI is caused by immune reactions between antibodies for alkylated CYP under drug metabolism and antigens appearing on hepatocytes [15]. The appearance of anti-liver/kidney microsome autoantibody type 2 (anti-LKM2) in DILI associated with tienilic acid, has been reported [16-18]. Anti-LKM2 is a specific marker of this DILI. A molecular target of anti-LKM2 is CYP2C9, the primary metabolic enzyme for tienilic acid [19, 20]. In the same way, anti-liver microsomal antibody, a molecular target for CYP1A2, has been detected in the serum of patients with DILI by dihydralazine [15, 21, 22]. Anti-LKM2 disappeared after the recovery of DILI associated with tienilic acid [16, 17]. In this study, a positive ANA status was frequently observed in patients who had DILI with AIH-like-HF, and the ANA disappeared when the liver damage recovered, similar to that observed for anti-LKM2 in patients with DILI associated with tienilic acid [16, 17]. Because there are some common features between the existence of ANA and anti-LKM2, immune activation with the appearance of autoantibodies under the progression of drug-metabolism may trigger DILI with AIH-like-HF. Additionally, the decreasing levels of serum IgG and gamma globulin following the recovery of DILI with AIH-like-HF suggest an immune reaction caused by drugs or drug-metabolites. On the other hand, the pathogenesis with cases of a negative ANA status in DILI with

AIH-like-HF cannot be clearly explained. However, the results suggest that a resemblance between a patient's own proteins and extraneous proteins leads to the formation of self-reactive cytotoxic T lymphocytes. It is inferred that so-called molecular mimicry is involved in the reaction.

The fourth characteristic of DILI with AIH-like-HF is that a significantly high proportion of causative drugs were CAM/C. This proportion is higher than that of a national investigation conducted between 1997 and 2006 in Japan. It showed that dietary supplements and Chinese herbal medicines accounted for 10.1% and 7.1% of the causative drugs, respectively [23]. We do not have clear explanation for this high frequency in this study. However, some few consumers recognize CAM as drugs. This is the reason for the delayed diagnosis of DILI caused by CAM in some patients. This time lag results in severe liver injury needing to admit into a hospital. In general, diagnosing DILI associated with CAM at an early stage is more difficult than diagnosing DILI caused by prescription drugs. When examining patients with liver injury, it is most important not to miss any signs of DILI and to ask the patient about their use of drugs containing CAM. If a patient is diagnosed with DILI, the prompt discontinuation of the causative drug is most effective for preventing chronicity and the progression of disease

in severity.

Genetic factors of DILI with AIH-like-HF

It is known that the onset of AIH is related to genetic factors involving specific HLA. DR3 and DR4 are frequently detected in patients with AIH [24, 25]. In one case of drug-induced lupus syndrome with findings of AIH associated with minocycline, HLA-DR4 was positive [26]. In another patient with the same syndrome induced by atorvastatin, HLA-DR3 and DR4 were observed [27]. Additionally, in patients with minocycline-induced fulminant hepatitis, HLA-DR3 has been documented [3], Japanese patients with AIH frequently have DR4 [28-30]. In this study, HLA-DR4 is positive in 71.4% of patients with DILI with AIH-like-HF, and this rate is higher than that observed in healthy Japanese DR4-positive subjects (38.6%) [29]. There is a possibility that genetic factors are involved in the pathogenesis of DILI with AIH-like-HF.

Type of DILI with AIH-like-HF: relapsed and non-relapsed cases

In our study, DILI with AIH-like-HF was classified into two types according to the clinical courses. In non-relapsed cases, a decreased serum IgG level and negative conversion of ANA after stopping the causative drug were observed. It was considered

that these non-relapsed cases might be the drug-induced AIH [7]. Because no relapse of liver injury is one of the characteristics of drug-induced AIH [7]. The other type is the "relapsed cases". Mackay, who proposed the concept of AIH, described AIH as occurring in patients with genetic factors when some additional causes, such as viruses or drugs, are involved [31]. A previous case report has described drug-triggered AIH by statin [32]. In this report, the clinical course and serological findings were similar to our relapsed cases, and the hepatic histology at the relapse was compatible with AIH. This case report suggested that the relapsed cases in our study might be drug-triggered AIH, although we did not confirm the liver histology at the relapse. We speculate that the relapsed cases in this study obtained autoimmune reactivity, which may easily occur by the first DILI.

In one case of DILI with AIH-like-HF associated with *A. blazei* Murill and fucoidan, the liver damage relapsed at 255 days after a recovery under treatment with prednisolone at a dose of 2 mg per day [6]. In other relapsed cases, the serum IgG level also increased again when the liver injury relapsed. This suggests that such patients had some predisposing factors for the development of AIH. However, there were no significant differences in the comparison between the relapsed and non-relapsed cases

regarding the clinical data, the score of the RUCAM [8] and the JDDW-scale [9], the AIH scores [10] and Simplified AIH scores [12], liver histological findings of inflammatory grade and stage of fibrosis, and use of immunosuppressive therapy in this study. Further investigations are needed because our study contained a small number of subjects. As it took 1090 days from the normalization of ALT for relapse to occur in one relapsed case, observation for more than 3 year is required to make a judgment of the existence of relapse in a patient with DILI with AIH-like-HF.

In conclusion, the characteristics of DILI with AIH-like-HF include a positive ANA status, a high level of serum IgG or gamma globulin, an older age and a causative factor of CAM or Chinese herbal medicines. Therefore, when examining patients with DILI who have such clinical characteristics, a liver biopsy is recommended in order to determine the optimal treatment strategy because such DILI cases may be very similar to AIH in regard to the liver histology. In addition, the clinical course of DILI with AIH-like-HF can be classified into relapsed and non-relapsed types. These relapsed types might therefore be drug-triggered AIH. Strict and long-term follow-up is highly recommended in DILI with AIH-like-HF patients to perceive the relapse.

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Complaiance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Legends

Fig. 1. Histology of the liver of patients with relapse

a, **b** A relapsed case of the drug-induced liver injury associated with *Agaricus blazei* Murill extract, and fucoidan (case No. 2 from Ref. [6]).

a The portal tract shows fibrosis, moderate interface hepatitis and infiltration of inflammatory cells. The bile ducts are intact. Rosette formation of the ballooning hepatocytes is shown. Hematoxylin and eosin (H&E) stain (×100).

b Marked lymphoplasmacytic infiltration and swollen hepatocytes are observed in the hepatic lobule. H&E stain (×400).

c, **d** A relapsed case of the drug-induced liver injury associated with Chinese herbal tea.

c Severe infiltration of inflammatory cells are shown in the hepatic lobule and the portal area, and marked interface hepatitis is shown. H&E stain (×100).

d Severe interface hepatitis and infiltration of inflammatory cells in the portal tract. Bile duct has no significant change. Rosette arrangement of ballooning hepatocytes is found in the hepatic lobule. H&E stain (×200).

Fig. 2. Histology of the liver of patients without relapse

a, b A non-relapsed case of the DILI associated with *Serenoa repens*, and turmeric extract

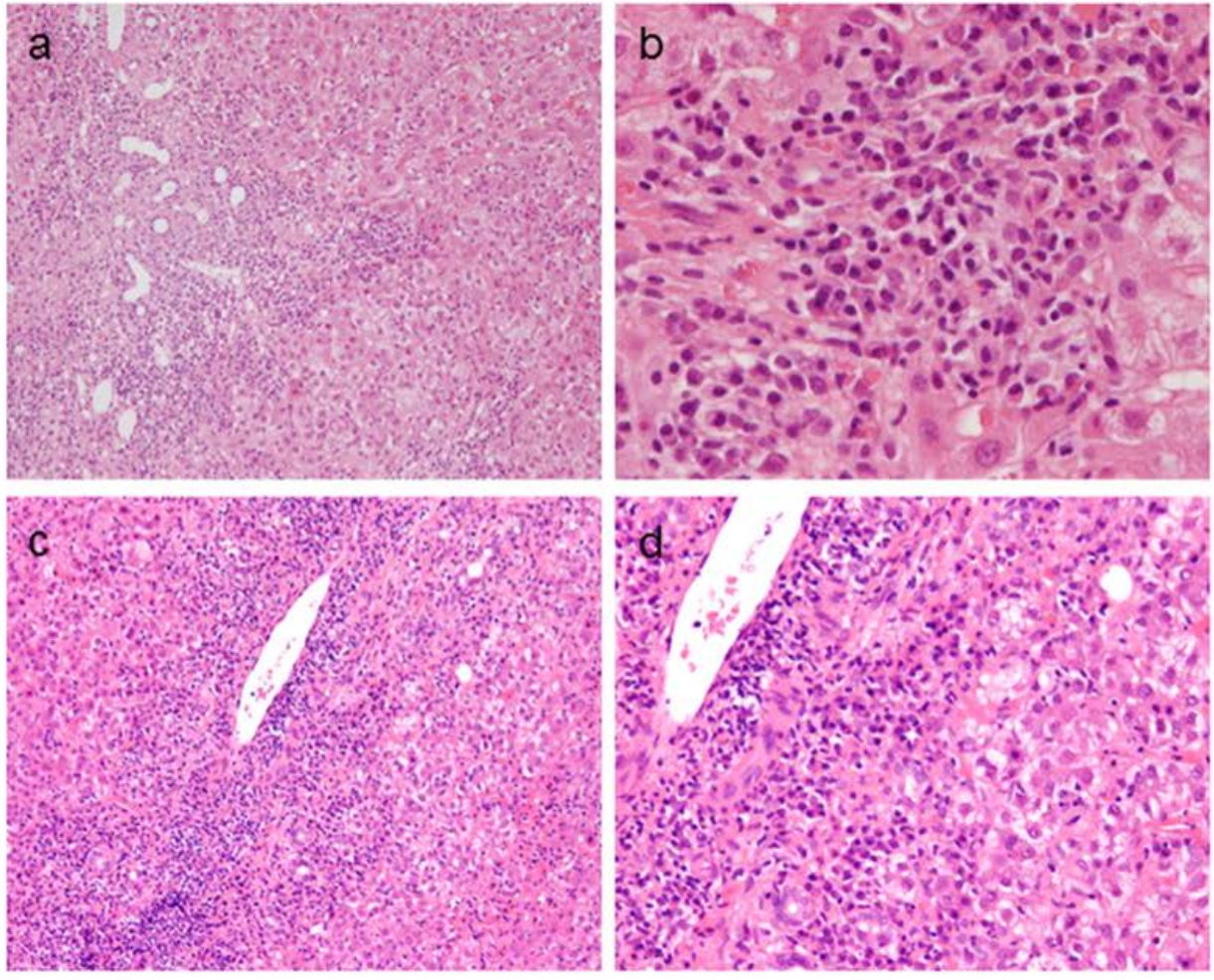
a The portal tract shows moderate interface hepatitis and infiltration of inflammatory cells. Expansion of the portal area is shown. The bile ducts are intact. Rosette formation of ballooning hepatocytes is shown. H&E stain (×100).

b Marked lymphoplasmacytic infiltration in the portal tract, and swollen hepatocytes are observed in the hepatic lobule. H&E stain (×400).

c, d A non-relapsed case of the DILI associated with Amlodipine besilate

c Severe infiltration of inflammatory cells are shown in the both hepatic lobule and the portal area. Marked interface hepatitis is shown. H&E stain (×100).

d Severe interface hepatitis and lymphoplasmacytic infiltration in the portal tract. Bile duct is intact. Rosette formation of swollen hepatocytes is found in the hepatic lobule. H&E stain (×200).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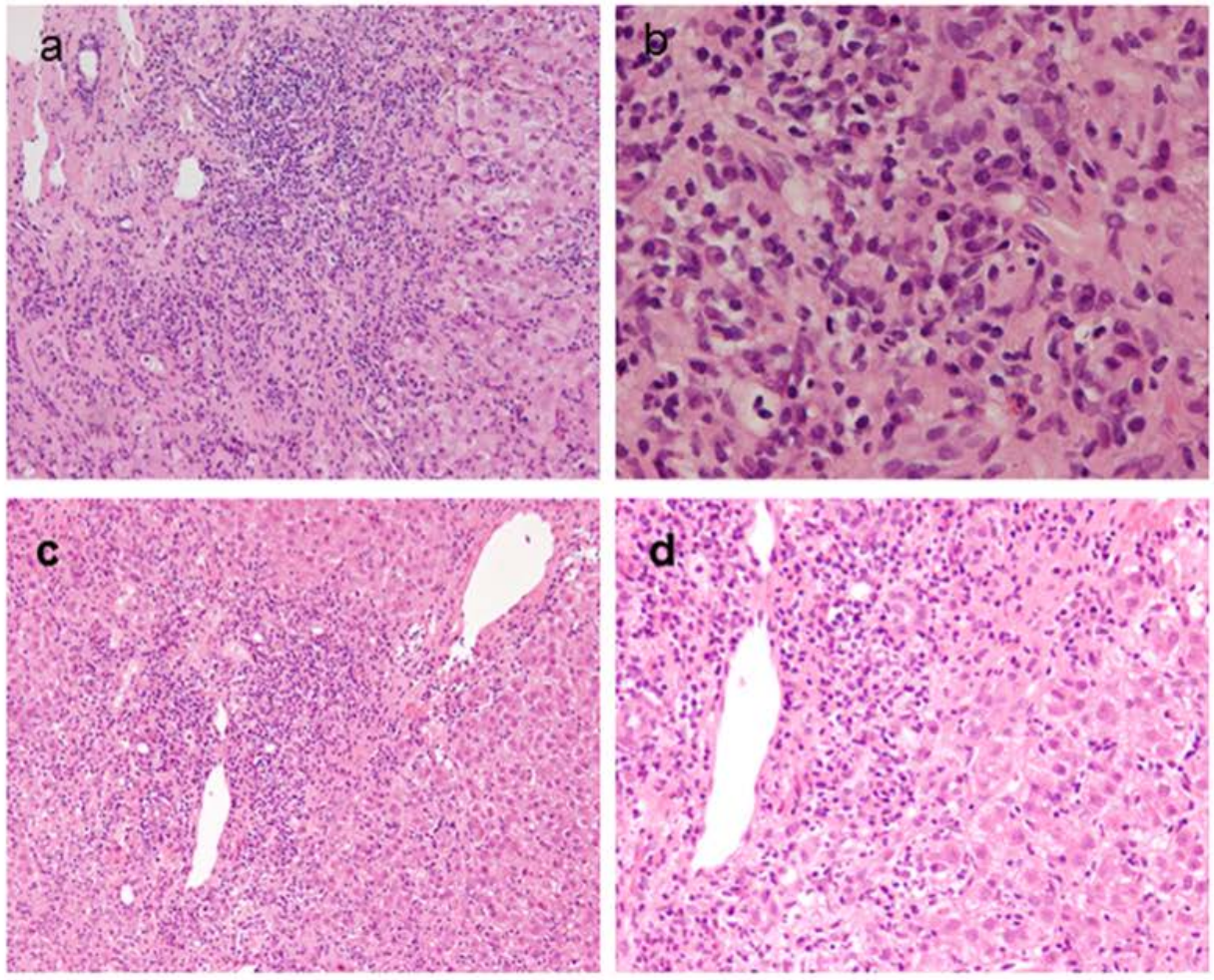
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Table 1. Comparison between DILI with AIH-like-HF and DILI without AIH-like-HF

Subjects	DILI with AIH-like-HF (23 cases)	DILI without AIH-like-HF (39 cases)	*p-value*
Gender (female : male)	18 : 5	27 : 12	0.561
Age (mean±SD)	59.0±14.4	47.0±15.1	0.002*
Body mass index (kg/m^2) (mean±SD)	23.1±2.3	21.8±5.1	0.131
Complication of allergy (yes : no)	5 : 18	8 : 31	1.000
Complication of autoimmune disease (yes : no)	4 : 19	6 : 33	1.000
Category of causative drug (CAM/C : Others)	16 : 7	8 : 31	0.000**
RUCAM score[8)] (mean±SD)	6.0±1.1	6.0±1.4	0.612
JDDW score [9)] (mean±SD)	6.9±1.2	7.0±1.4	0.746
WBC (μ/l) (mean±SD)	4500±1691	5300±2960	0.032*
Peripheral eosinophil (%) (mean±SD)	3.0±4.0	3.0±4.2	0.827
Platelet (×10000μ/l) (mean±SD)	19.6±7.3	21.8±7.3	0.112
Onset AST (U/l) (mean±SD)	253±524	466±2279	0.387
Onset ALT (U/l) (mean±SD)	438±813	523±1496	0.517
Onset ALP[a] (mean±SD)	1.04±1.19	1.74±1.43	0.054

Subjects	DILI with AIH-like-HF (23 cases)	DILI without AIH-like-HF (39 cases)	P-value
Onset GGT (U/l) (mean±SD)	146±266	229±282	0.237
Onset T.Bil (mg/dl) (mean±SD)	1.1±4.9	2.4±3.9	0.916
Onset prothrombin time (%) (mean±SD)	84.0±18.8	93.0±23.1	0.423
Ratio of (IgG/upper normal limit) (mean±SD)	1.07±0.51	0.69±0.28	0.000**
ANA status (positive : negative)	12 : 11	5 : 29	0.003*
Max AST (U/l) (mean±SD)	524±784	518±2505	0.833
Max ALT (U/l) (mean±SD)	603±942	622±1904	0.890
Max GGT (U/l) (mean±SD)	251±285	245±287	0.939
Max ALP[a] (mean±SD)	1.38±1.19	1.83±1.51	0.135
Max T.Bil (mg/dl) (mean±SD)	2.7±9.3	3.9±8.9	0.864
Min prothrombin time (%) (mean±SD)	75.0±21.6	83.0±26.0	0.224
Exposed period by causative drug (days) [b] (mean±SD)	143±188	32±120	0.000**
Immunosuppressive treatment (yes : no)	14 : 9	4 : 35	0.000**

* p<0.05; ** p<0.0001

[a]Ratio of (ALP/upper normal limit of ALP), [b]Days from starting to stopping a causative drug

Abbreviations; AIH: autoimmune hepatitis, DILI: drug-induced liver injury, AIH-like-HF: AIH-like hepatic histological findings, CAM/C: complementary alternative medicines including dietary supplements and Chinese herbal medicines, Others: prescription drugs excluding Chinese herbal medicines, RUCAM score: score of the Roussel Uclaf Causality Assessment Method, JDDW score: score of the diagnostic scale of Digestive Disease Week-Japan 2004, WBC: white blood cells, AST: asparate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, T.Bil; total bilirubin, IgG: immunoglobulin G, ANA: antinuclear antibody.

Table 2. Multivariable analysis between drug-induced liver injury with AIH-like-HF and without AIH-like-HF

Subjects	OR	95% CI	*p*
Age	1.096	1.003-1.198	0.043*
WBC	1.000	0.999-1.001	0.898
Ratio of (IgG/upper normal limit)	8989.890	5.128-15759448	0.017*
ANA status	104.994	1.133-9733.902	0.044*
Category of causative drug (CAM/C)	251.300	4.290-14721.432	0.008*

* $p<0.05$

Abbreviations; AIH: autoimmune hepatitis, AIH-like-HF: AIH-like hepatic histological findings, OR: odds ratio, CI: confidence interval,

WBC: white blood cells, IgG: immunoglobulin G, ANA: antinuclear antibody,

CAM/C: complementary alternative medicines including dietary supplements and Chinese herbal medicines

Table 3. Causative drugs of drug-induced liver injury with AIH-like-HF

Category	Causative drug
CAM/C (16 cases)	*Agaricus blazei* Murill
	Agaricus blazei Murill, and *Ganoderma lucidum*
	Agaricus blazei Murill, and Fucoidan
	Apple vinegar, and Supplement containing protein
	Chinese herbal medicines (Bo-fu-tsu-sho-san)
	Chinese herbal medicines (Ho-chu-ekki-to)
	Chinese herbal medicines (Sai-rei-to) (two cases)
	Chinese herbal medicines (Syo-ken-chu-to)
	Chinese herbal tea
	Chinese dietary supplement (two cases)
	Supplement containing noni and berry
	Monascus purpureus
	Turmeric extract, and *Serenoa repens*
	Turmeric extract
Others (seven cases)	Amlodipine besilate
	Azithromycin hydrate, and ivermectin

Category	Causative drug
	Ambroxol hydrochloride, and clarithromycin
	Indomethacin
	Mosapride
	NSAID containing salicylamide and phenacetin
	Sarpogrelate

Abbreviations; AIH: autoimmune hepatitis, AIH-like-HF: AIH-like hepatic histological findings, CAM/C: complementary alternative medicines including dietary supplements and Chinese herbal medicines,

Others: prescription drugs excluding Chinese herbal medicines,

NSAID: non-steroidal anti-inflammatory drug

Table 4. Clinical course of the relapsed cases

		At the first liver injury				At the recovery		At the relapse			
sex (age)	Causative drug	max ALT (U/L)	IgG (mg/dL) AIH score [10][a]	ANA	steroids	IgG (mg/dL)	ANA	time to relapse (days)	ALT (U/L)	IgG (mg/dL)	ANA
F (56)	NSAID containing salicylamide and phenacetin	483	1907 12	+ (1:40)	no	1400	+ (1:40)	358	382	2180	+ (1:640)
M (85)	Turmeric extract	355	2490 11	+ (1:40)	yes	1270	-	47[b]	64	2560	-
M (46)	Sai-rei-to	603	1724 9	-	no	1627	NP	1090	162	NP	NP
F (73)	Indomethacin	239	4818 16	+ (1:320)	yes	1924	NP	193[†]	193	2240	NP
F (46)	Chinese herbal tea	1269	2438 11	-	yes	1042	-	234[‡]	314	1182	-
M (75)	*Agaricus blazei* Murill/, fucoidan	584	3051 10	-	yes	1180	-	255[c]	98	2342	-
F (49)	Supplement containing noni and berry	1993	1561 11	+ (1:80)	no	1478	-	310	1815	1813	-
F (58)	Clarithromycin, ambroxol hydrochloride	1365	1334 10	+ (1:40)	no	1286	-	365	144	NP	NP

Abbreviations; M: male, F: female, ALT: alanine aminotransferase, IgG: immunoglobulin G, AIH: autoimmune hepatitis, AIH score: score of the International criteria for diagnosis of AIH in 1999, ANA: antinuclear antibody, NSAID; non-steroidal anti-inflammatory drug, NP: not performed.

[a] Pretreatment score

[b] After the withdrawal of administration of steroids

[c] Under the tapering of steroids.

Table 5. Comparison between the relapsed and non-relapsed cases

Subjects	Relapsed case	Non-relapsed case	*p-value*
Gender (female : male)	5 : 3	6 : 2	1.000
Age (mean±SD)	57±15	61±12	0.674
Body mass index (kg/m^2) (mean±SD)	21.3±1.5	24.0±2.3	0.059
Complication of allergy (yes : no)	1 : 7	2 : 6	1.000
Complication of autoimmune disease (yes : no)	2 : 6	1 : 7	1.000
Category of suspected drug (CAM/C : Others)	5 : 3	6 : 2	1.000
WBC(μ/l) (mean±SD)	4250±1952	4640±1764	0.636
Peripheral eosinophil (%) (mean±SD)	2.0±3.3	5.4±2.5	0.131
Platelet (×10000μ/l) (mean±SD)	17.9±7.5	18.7±4.1	0.834
Onset AST (U/l) (mean±SD)	253±531	159±657	0.908
Onset ALT (U/l) (mean±SD)	369±669	402±861	0.674
Onset ALP [a] (mean±SD)	1.35±0.83	0.93±0.61	0.600
Onset GGT (U/l) (mean±SD)	171±408	158±94	0.834
Onset T.Bil (mg/dl) (mean±SD)	1.0±6.2	1.0±3.2	0.954
Onset prothrombin time (%) (mean±SD)	87.0±13.6	90.0±26.7	0.878
Ratio of (IgG/upper normal limit) (mean±SD)	1.16±0.69	0.98±0.45	0.294

Subjects	Relapsed case	Non-relapsed case	p-value
ANA status (positive : negative)	5 : 3	3 : 5	0.619
Max AST (U/l) (mean±SD)	414±512	509±1160	0.372
Max ALT (U/l) (mean±SD)	594±613	570±1280	0.834
Max GGT (U/l) (mean±SD)	363±435	247±95	0.248
Max ALP [a](mean±SD)	1.46±1.01	1.38±0.53	0.563
Max T.Bil (mg/dl) (mean±SD)	2.0±8.2	2.8±9.6	0.834
Min prothrombin time (%)(mean±SD)	79.5±17.1	67.5±26.0	0.382
RUCAM score [8] (mean±SD)	5.8±1.2	6.0±1.0	0.584
JDDW score [9] (mean±SD)	6.8±1.4	7.0±1.4	0.583
Exposed period by causative drug (days) (mean±SD)	108±124	147±272	0.345
AIH score (pretreatment) [10] (mean±SD)	11.0±2.1	11.5±1.4	0.707
Simplified AIH score [12] (mean±SD)	5.5±1.7	5.5±1.6	0.556
Steroid treatment (yes : no)	4 : 4	6 : 2	0.608
Stage of fibrosis (mean±SD)	1.0±0.5	1.0±0.5	0.602
Grade of inflammatory activity (mean±SD)	6.0±1.4	6.0±1.3	0.706
Portal	3.0±0.6	3.0±0.7	0.680

Subjects	Relapsed case	Non-relapsed case	p-value
Lobular	3.0±0.8	3.0±0.7	0.340
Interface hepatitis (mean±SD)	2.0±0.8	3.0±1.0	0.689
Infiltration of plasma cell (mean±SD)	2.5±0.9	1.5±0.9	0.105
Rosette formation (mean±SD)	0.5±1.3	0.5±1.0	0.819
Emperipolesis (yes : no)	6 : 2	6 : 2	1.000

[a] Ratio of (ALP/upper normal limit of ALP)

Abbreviations; CAM/C: complimentary alternative medicines including dietary supplements and Chinese herbal medicines, Others: prescription drugs excluding Chinese herbal medicines, WBC: white blood cells, AST: asparate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, T.Bil; total bilirubin, IgG: immunoglobulin G, ANA: antinuclear antibody, RUCAM score: score of the Roussel Uclaf Causality Assessment Method. JDDW score: score of the diagnostic scale of Digestive Disease Week-Japan 2004, AIH: autoimmune hepatitis, AIH score: score of the International criteria for diagnosis of AIH in 1999. Simplified AIH score: score of the Simplified criteria for the diagnosis of AIH in 2008.