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Pathophysiological mechanism of lung injury in patients with leptospirosis

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Summary

Aims: Acute lung injury (ALI) is a serious clinical problem. We investigated the pathogenetic mechanisms of ALI caused by leptospirosis.

Methods: The study included five cases of leptospirosis. We monitored the arterial pressure (AP) and heart rate (HR) and analysed the AP and HR variabilities for assessment of autonomic functions. Histopathological changes in the lung, brain, kidney, and liver were examined. In addition, we identified the expression of inducible nitric oxide synthase (iNOS) using immunohistochemical stain.

Results: Five patients associated with leptospirosis died of ALI. Before death, severe hypotension and bradycardia occurred. Spectral analysis of AP and HR variabilities indicated decreased sympathetic drive with increased parasympathetic activity. Pathological examinations revealed alveolar haemorrhage and necrotic lesions in various organs. Immunohistochemical stain disclosed iNOS activity in multiple organs. Biochemical determinations indicated hypoxia, hyperglycaemia, increased nitrite/nitrate, methyl guanidine and other factors.

Conclusions: These changes suggest that leptospirosis causes severe hypotension and bradycardia accompanied by autonomic dysfunction. Finally, multiple organ failure and damage ensued. The pathogenesis of lung and organ injury may involve iNOS and NO production.

Key words: Leptospirosis, acute respiratory distress syndrome, pulmonary oedema, inducible nitric oxide synthase.

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INTRODUCTION

In recent years, acute and fulminant pulmonary oedema (PO) or acute lung injury (ALI) has been reported in Taiwan. These rare chest cases have been demonstrated to occur in enterovirus with hand, foot, and mouth disease.[1,2] Our laboratory recently presented 18 patients with Japanese B encephalitis, breast carcinoma, fat embolism, and rupture of cerebral myocotic aneurysm, resulting in fatal PO.[3] In addition, we studied the mechanism of fulminant PO caused by enterovirus 71,[4] and revealed that viral destruction of medullary depressor areas leading to sympathetic overactivation might contribute to blood volume shift to the lung.[4] Lung parenchyma showed a marked increase in inducible nitric oxide synthase (iNOS) activity, suggesting that iNOS and nitric oxide (NO) may also play a role in the pathogenesis of PO induced by enterovirus. In the present investigation, we explored the mechanisms of fulminant PO due to leptospirosis. Acute PO complicated with leptospirosis has been reported, but the mechanisms remain unclear.

Leptospirosis is a zoonotic disease caused by spirochaetes bacteria. The diseases are transmitted by urine of vectors such as dogs, cats, and other animals. Pulmonary involvement has rarely been seen in cases of leptospirosis. Several clinical and animals studies have indicated that spirochaetes infection may result in diffuse alveolar haemorrhage and respiratory failure.[5,6] Trevejo *et al.*[7] reported an epidemic leptospirosis in Nicaragua in 1995, where 15 of 2259 residents died of pulmonary haemorrhage. Luks *et al.*[8] described one case of leptospirosis presenting diffuse alveolar haemorrhage. A recent study revealed that guinea pigs infected with isolated human leptospira demonstrated pulmonary haemorrhage with alveolar deposition of immunoglobulin and complement.[9] We encountered five cases of leptospirosis associated with acute respiratory distress syndrome (ARDS).

ARDS with leptospirosis may eventually result in respiratory failure and death. The mechanisms and aetiology of the pathogenesis of acute PO associated with this disorder remain undetermined. We obtained consent from patients with spirochaetes infection. The physiological changes, biochemical examinations and autopsy findings may provide important information with respect to the organ involvement and possible mechanisms of fulminant ARDS associated with leptospirosis.

MATERIALS AND METHODS

Patients

Five cases were farmers working in the fields who suffered mild discomfort, such as back pain, cough, myalgia, fever, sweating, and shortness of breath. Each patient was originally diagnosed in local clinics with heat stroke or common cold, but were later admitted to hospitals in Hualien and Taipei when symptoms of respiratory distress became severe.

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Progression of symptoms

Based on their history, the five farmers were thought to have been infected by spirochaetes. Although they were under intensive care, the symptom of respiratory distress became severe and blood pressure progressively declined. These patients died within 8 days following admission. On admission, chest radiography showed normal appearance. The liver and spleen were not palpable and no pitting oedema was found in the extremities. Neurological examinations showed that these patients were initially lucid, oriented, and cooperative, but later became irritable and were given tranquillisers and sedatives. Fever was up to 39°C and leukocytosis was noted. Blood culture was then performed. Empirical antibiotic usage with intravenous ceftriaxone (2000 mg) was given every 8 h for 3 days. Their body temperature was under control after the treatment (an average of 37.2°C). The Glasgow coma scale (GCS) was $E_4V_5M_4$.

Interventions

Sudden onset of dyspnoea developed 4–8 days after admission. Endotracheal intubation was performed. The respiration was subsequently supported by mechanical ventilation. An intra-arterial line and ECG were installed to record the arterial pressure (AP) and heart rate (HR). The mean AP was decreased to 60–70 mmHg. Dopamine was given. Lumbar puncture was performed on the same day. Examinations of cerebral spinal fluid did not reveal abnormal findings. Blood culture was also negative.

Chest radiography was taken again because of persistent dyspnoea and hypoxia. Marked infiltration with multiple small patches in the lung was found. Moxifloxacin (400 g) and ribavirin (1000 mg) per day, as well as rifampicin (300 mg) 12 hourly were prescribed.

Blood pressure dropped further even though dopamine and norepinephrine were given. The GSC declined to $E_2V_1M_2$ or $E_1V_2M_2$. These patients died within 8 days of admission. Consent for pathological examination was obtained from their relatives.

Analysis of AP and HR variabilities

The AP and HR variabilities were assessed to evaluate the changes in sympathetic and parasympathetic activities before and after the onset of ARDS.[10–12]

Examination of plasma contents and biochemical factors

When ARDS occurred, blood was taken for determination of haematocrit, white blood cells, platelets, blood pH, PaO_2 and bicarbonate. Biochemical factors such as glucose, nitrite/nitrate, methyl guanidine, creatinine, blood urea nitrogen, creatinine phosphokinase, amylase, and glutamic oxaloacetic transaminase were measured using an autoanalyser (Vitro 750; Johnson & Johnson, USA) and a nitrite/nitrate detector (ENO-20; AD Instruments, Japan).

Pathological and immunochemical examinations

At autopsy, the lung, heart, liver, kidney, and brain were examined. The tissue sections were stained with H&E. Antigen retrieval immunochemical staining was used to demonstrate the presence of iNOS in the pulmonary, cardiac, hepatic and renal tissues.[13] We also employed Warthin-Starry method for the detection of spirochetes bacteria in the lung.[14]

Statistical analysis

The plasma elements and biochemical factors were compared with the normal range. χ^2 test was used for comparison of a small sample size ($n=5$ for leptospirosis) with a large sample (normal values, assuming $n=1000$). A p value <0.01 was considered to be statistically significant.

RESULTS

Acute episode of ARDS

Symptoms of acute respiratory distress worsened, with all patients eventually suffering from haemoptysis and respiratory failure within 8 days of admission. Laboratory examination revealed anaemia, leukocytosis, thrombocytopaenia, acidosis, and increased bicarbonate (Table 1).

Biochemical changes

Biochemical determinations revealed hyperglycaemia, as well as increases in nitrite/nitrate, methyl guanidine, creatinine, blood urea nitrogen, creatinine phosphokinase, amylase, and glutamic oxaloacetic transaminase. The data suggest that hyperglycaemia is a risk factor. Concomitantly, plasma nitric oxide and free radicals (methyl guanine) increased. The changes in biochemical factors also imply that spirochaetes bacterial infections affected various organ functions such as liver, kidney, pancreas, and possibly other organs (Table 2).

Changes in AP, HR and variability

AP and HR gradually declined. Before death, the AP was reduced to below 25 mmHg, and the HR to less than 30 beats/min (R-R interval 1800 ms) (Fig. 1). Spectral analysis of AP and HR variabilities revealed that sympathetic activity (low frequency component of the power spectrum) declined with the progression of ARDS. Parasympathetic activity (high frequency component of the power spectrum) became dominant (Fig. 1). At this time, the AP and HR progressively dropped even though vasoconstrictors were provided.

TABLE 1 Basic data and blood contents

	Patients with leptospirosis ($n=5$)	Normal range
Age range, years	42–63	–
Sex	3M / 2F	–
Haematocrit, %	30 ± 5*	40–47
White blood cells, cells $\times 10^3/\mu L$	19406 ± 384*	4000–11000
Platelets, cells $\times 10^3/\mu L$	114 ± 16*	200–500
pH	6.12 ± 0.22*	7.35–7.45
PaO_2, mmHg	62 ± 6*	70–100
Bicarbonate, meg/L	19 ± 6*	10–14

Values are means ± SEM in five cases of leptospirosis.
*$p<0.01$ compared with the corresponding values of normal range.

TABLE 2 Biochemical factors

	Patients with leptospirosis ($n=5$)	Normal range
Glucose, mg/dL	198 ± 18*	70–100
Nitrite/nitrate, p mol	28 ± 6*	10–12
Methyl guanidine, mg/dL	9.22 ± 2.21*	1.58–2.55
Creatinine, mg/dL	4.6 ± 0.8*	0.3–1.3
BUN, mg/dL	54 ± 8*	8–20
CPK, unit/L	647 ± 32*	26–308
Amylase, unit/L	312 ± 12*	53–123
GOT, unit/L	78 ± 8*	5–35

Values are means ± SEM in five cases of leptospirosis.
*$p<0.01$ compared with the corresponding values of normal range.
BUN, blood urea nitrogen; CPK, creatinine phosphokinase; GOT, glutamic oxaloacetic transaminase.