The effect of fractal-like mechanical ventilation on vital signs in a rat model of acute-on-chronic liver failure

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Abstract

The network of interactions between different organs is impaired in liver cirrhosis. Liver cirrhosis is associated with multi-system involvement which eventually leads to multiple organ failure. This process is accelerated by a precipitating factor such as bacterial infection which leads to respiratory distress, circulatory shock, neural dysfunction and very high mortality. Cirrhotic patients often have blunted respiratory sinus arrhythmia and impaired cardio-respiratory variability. Fractal-like mechanical ventilation is reported to enhance respiratory sinus arrhythmia and attenuate respiratory distress in experimental models. In present study we hypothesised that fractal-like mechanical ventilation may improve the outcome of cirrhotic rats with multiple organ failure. Cirrhosis was induced by chronic biliary obstruction in rats. Acute multiple organ failure was induced by intraperitoneal injection of bacterial endotoxin in cirrhotic rats. The effect of conventional mechanical ventilation (with constant tidal volume and respiratory rate) or fractal-like ventilation (with the same average but variable tidal volume and respiratory rate) were assessed on vital signs, oxygen saturation and plasma alanine aminotransferase in anaesthetised cirrhotic rats. We demonstrated that fractal-like mechanical ventilation was accompanied by improved oxygen saturation, reduced heart rate and decreased liver injury following injection of bacterial endotoxin. Moreover, variable mechanical ventilation in cirrhotic rats reduced mortality and prevented a fall in short-term heart rate variability following endotoxin challenge in comparison with rats with constant mechanical ventilation. We suggest further investigations on beneficial effects of fractal-like ventilation strategy in critically ill patients with liver failure requiring organ support and mechanical ventilation.

Key words: Cirrhosis, Endotoxin, Fractal, Liver failure, Mechanical ventilation, Network physiology
1. Introduction

Liver cirrhosis is a major cause of mortality worldwide with a rate of ~2 million deaths per year (Byass 2014). In addition to specific liver-related complications, cirrhosis is associated with multisystem involvement which leads to impaired cardiovascular, respiratory, renal, neural and immune function. The process of multiple organ failure in cirrhosis can be accelerated by a precipitating factor such as bacterial infection (Jalan et al 2012). In fact, bacterial infection results in acute-on-chronic liver dysfunction and accelerated failure of other organs (Mücke et al 2017). Cirrhotic patients with multiple organ failure often have poor prognosis and require intensive care unit admission (O ‘brien et al 2012).

The complex interaction between different organs system in cirrhosis makes it an interesting topic for investigation within the context of Network Physiology. The mechanism of progressive organs failure is not well understood in patients with acute-on-chronic liver failure. It appears that the network of interactions between different organs is impaired in cirrhosis. For examples, the interaction between respiratory cycles and heart rhythm is blunted in cirrhosis (i.e. reduced respiratory sinus arrhythmia). Respiratory sinus arrhythmia is crucial for optimum tissue oxygenation and cardiac function (Ben-Tal et al 2012). Patients with cirrhosis often have reduced heart rate variability (HRV) which carries a poor prognosis in this patient population (Mani et al 2009, Bhogal et al 2018). Respiratory cycles in healthy individuals is associated with fractal-like fluctuations in rate and volume (Raoufy et al 2016). Although such complex fluctuations enhances tissue oxygenation (Mutch et al 2005, Raoufy et al 2017), most conventional mechanical ventilators are designed to deliver constant rhythm and do not mimic physiological breath-to-breath fractal-like variations in the respiratory rate and volume. Mutch et al., reported that mechanical ventilation of anesthetised pigs with a fractal-like rhythm improves respiratory sinus arrhythmia (Mutch et al 2005). Other reports indicate that the outcome in animals model of acute respiratory distress syndrome and asthma is improved following variable mechanical ventilation (Ilka et al 2018, Boker et al 2002).

The outcome of cirrhotic patients requiring mechanical ventilation in the intensive care unit is very poor and still no specific therapy is available to improve their survival (Levesque et al 2014). We hypothesise that variable mechanical ventilation may improve the outcome in such critically ill patients. This study was aimed to test this hypothesis in a rat model of acute-on-chronic liver failure.

2. Method

2.1. Ethics statement: All animal procedures were approved by the Ethics committee of Tarbiat Modares University. All animal procedures were in accordance with recommendations of NIH guidelines on animal experimentation (publication no. 85-23). 6-8 rats were used in each experimental group.

2.2. Animal model of acute-on-chronic liver failure: Liver cirrhosis was induced by surgical ligation of bile duct under general anaesthesia as described (Mani et al 2006). Bile duct ligated (BDL) and sham-operated (SHAM) control rats had access to food and water ad libitum and were used for the next step of experiments four weeks after the operation. Injection of bacterial lipopolysaccharide (LPS) in cirrhotic rats induces rapid deterioration of liver function and precipitates multiple organ failure (Wright et al 2007, Harry et al 1999). Thus, acute-on-chronic liver failure was induced by intraperitoneal injection of a single dose of LPS (1 mg/kg, extracted from Salmonella typhimurium, Sigma, UK) (Haddadian et al 2013).

2.3. Experimental design and mechanical ventilation: Four weeks after bile duct ligation or sham surgery, animals were mechanically ventilated under general anaesthesia as described (Ilka et al
2018). In brief, the trachea was cannulated and connected to a volume controlled ventilator (Harvard Apparatus, Holliston, MA). Animals were randomly assigned to have either constant or fractal-like mechanical ventilation. In the constant ventilation group, a non-variable tidal volume (1.7 ml) and respiratory rate (77 breath/min) were given with a positive end-expiratory pressure (PEEP) of 5 cm H2O. The fractal-like ventilation groups received mechanical ventilation with the same average but a variable tidal volume and respiratory rate to obtain a minute ventilation equal to that during constant ventilation (Ilka et al 2018). PEEP was kept at 5 cm H2O in both groups. We used an application programming interface which communicates with the ventilator through a RS232C serial interface. This interface allows to control the rate and volume of each cycle of mechanical ventilation directly from the MATLAB command line. 1000 cycles of respiratory rate and tidal volume obtained from the pattern of physiological healthy breathing were continuously looped itself until the end of the experiments (Ilka et al 2018). In fractal-like ventilation group: animals were ventilated with a variable tidal volume (mean=1.7 ml, coefficient of variation [CV]=7.4%) and respiratory rate (mean=77 breaths/min, CV=7.1%). Rats were placed on a heated pad to maintain their body temperature throughout mechanical ventilation. Fractal-like behaviour of inter-breath interval variations were assessed using detrended fluctuation analysis (DFA) (Peng et al 1995, Ma Qianli D Y, Bartsch et al 2010, Chen et al 2005, 2002). ECG, systolic blood pressure and oxygen saturation (SpO2) were recorded using a Powerlab system (ADInstrument, Australia). One hour after the commence of mechanical ventilation, LPS (1 mg/kg) or saline were injected intraperitoneally and animals were followed for 4 hours. In a separate group of animals, blood samples were taken 2 hours after LPS or saline injection for measurement of plasma alanine aminotransferase (ALT) using a routine biochemical assay.

2.4. Heart rate variability analysis: ECG signals were recorded at the sampling rate of 10 kHz using a Powerlab system. The R peaks were detected and the R-R interval series was generated using an ad hoc computer program. The standard deviation of the R-R intervals (SDNN) was calculated as a measure of total HRV in 5 min ECG recordings. Short-term and long-term HRV were measured by calculating SD1 and SD2 using the Poincare’ plot respectively (Haddadian et al 2013).

2.5. Statistical analysis: Data are expressed as mean ± SEM. Two-way analysis of variance was used for statistical analysis followed by post-hoc Tukey’s test. P<0.05 was considered statistically significant.

3. Results

BDL rats showed clinical manifestations biliary cirrhosis such as development of ascites, jaundice and elevation of plasma liver enzymes (e.g. ALT). Post-mortem analysis also showed a significant increase in spleen weight (in index of portal hypertension) as well as liver stiffness (data not shown). Detrended fluctuation analysis of inter-breath intervals in group of rats with variable mechanical ventilation demonstrates a linear correlation between the log(scale) and log(fluctuations) as shown in figure 1. This indicated that variable mechanical ventilation has a fractal-like dynamic similar to healthy individuals (Raoufy et al 2016). Similar fractal-like pattern of fluctuation was observed in tidal volume fluctuations in the variable mechanical ventilation group as expected (data not shown).

Figure 2 shows plasma level of ALT in four experimental groups. Two-way ANOVA showed a significant interaction between type of mechanical ventilation and type of surgery (SHAM versus BDL) (P=0.0061). This indicates that fractal-like ventilation has different effect on plasma liver enzyme level in SHAM versus BDL group. There was a statistically significant difference in plasma ALT
levels between BDL rats which had constant or fractal-like mechanical ventilation (P<0.001, Tukey’s post-test).

The effect of fractal-like mechanical ventilation on heart rate, systolic blood pressure and SpO₂ is demonstrated in figure 3. In general, BDL rats exhibited lower heart rate and systolic blood pressure in comparison with SHAM groups, a phenomenon which goes along with previous reports (Mani et al 2006, Gaskari et al 2015, 2002). BDL-fractal group showed even lower heart rate and blood pressure in comparison with BDL rats with constant ventilation. BDL rats with constant mechanical ventilation exhibited a steep drop in oxygen saturation (SpO₂) 3 hours post LPS injection which was statistically significant compared to SHAM rats as well as BDL rats given fractal-like mechanical ventilation (figure 3C).

The effect of fractal-like ventilation on HRV indexes is shown in figure 4. We did not observe a significant difference in SDNN between experimental groups. Poincare’ plot was used to compute short-term and long-term variability separately. Short-term HRV (SD1) dropped significantly in BDL rats with constant mechanical ventilation after endotoxin challenge in comparison with BDL-fractal group as well as SHAM rats. There was no significant difference in long-term HRV (SD2) between the experimental groups.

The effect of variable mechanical ventilation on survival is depicted in figure 5. Mantel–Cox test indicated a significant difference in survival between the groups (Chi-square = 6.26, P= 0.036). Most SHAM rats survived the endotoxin challenge within 4 hours. There was high mortality in BDL group within 4 hours of LPS injection despite mechanical ventilation. Four-hour survival rate was higher in BDL rats given fractal-like rather than constant mechanical ventilation.

4. Discussion

Chronic liver failure is a multisystem disease and eventually leads to multiple organ failure, a process with is often accelerated by bacterial infection/sepsis. Cirrhotic patients with sepsis are often admitted to intensive care units and may require mechanical ventilation (Gustot et al 2009). However, the survival rate of cirrhotic patients requiring mechanical ventilation is lower than patients without underlying liver disease (Rabe et al 2004). Although the mechanism of endotoxin-induced acute respiratory distress syndrome has been studied in cirrhotic rats in the past (Chang and Ohara 1994), still no specific therapy is available. In present study we hypothesised that fractal-like mechanical ventilation may improve the outcome in such critically ill patients and tested this hypothesis in a rat model of acute-on-chronic liver failure. Our results showed that fractal-like ventilation in cirrhotic rats was associated with less elevation of plasma transaminase (ALT) which indicates less severity of acute-on-chronic liver injury following LPS injection in the fractal-like group. In addition, we did not observe a steep drop in SpO₂ in cirrhotic rats with fractal-like mechanical ventilation in comparison with cirrhotic rats with constant mechanical ventilation. This observation goes along with less severity of respiratory distress and corroborates with previous reports showing that acute respiratory distress syndrome is attenuated following fractal-like mechanical ventilation in a pig model (Boker et al 2002).

Although these results are promising, the exact mechanism is not investigated in the present study. However, one may speculate that variable mechanical ventilation my directly affect the airways (Ilka et al 2018) or indirectly affect the autonomic control system through neural feedback mechanisms (Mutch et al 2005). Mutch and colleagues reported that fractal ventilation enhances respiratory sinus arrhythmia (Mutch et al 2005). It is well known that vagus nerve is an important component of
respiratory sinus arrhythmia (Katona and Jih 1975). The vagus nerve also plays a pivotal role in prevention of organ failure during systemic inflammation (Tracey 2007, Rosas-Ballina et al 2011). Tracey and colleagues, demonstrated the existence of a neuronal circuit termed “the cholinergic anti-inflammatory pathway” in which the major component is the descending branch of the vagus nerve (Tracey 2007). Based on this model, action potentials coming from the vagus nerve inhibit the production of pro-inflammatory cytokines through activation of alpha7 nicotinic acetylcholine receptors (Tracey 2007, Mazloom et al 2013). The mechanism of such immune modulation is not precisely known, but experimental evidence suggests that vagus nerve stimulation leads to activation of a subset of T lymphocytes that express choline acetyltransferase (Rosas-Ballina et al 2011). These T cells migrate to the site of inflammation and release acetylcholine that inhibits macrophage function (Rosas-Ballina et al 2011). The discovery of this neural anti-inflammatory pathway is important within the context of cirrhosis for two reasons: Firstly, this model explains why there is an inverse correlation between plasma inflammatory cytokines (e.g. interleukin-6) and indexes of vagal activity in patients with cirrhosis (Mani et al 2009). Secondly, it introduces the possibility of vagus nerve stimulation as a potential therapy for management of inflammatory conditions such as acute-on-chronic liver failure where an exaggerated immune response may cause harm (Eftekhar et al 2014, Hajasgharzadeh and Baradaran 2017). The effect of fractal-like ventilation in rats with acute-on-chronic liver disease is in line with the latter. Our results showed that fractal-like ventilation decreases heart rate and blood pressure and prevent a drop in short-term HRV (an index of respiratory sinus arrhythmia). We have previously shown that liver cells express alpha-7 nicotinic acetylcholine receptors (Hajasgharzadeh et al 2014) and central neural mechanisms can modulate the severity of liver injury (Eftekhar et al 2014). Therefore, our observations corroborate with the effect of fractal ventilation on modulation of vagus nerve activity, although further studies are required to prove this explanation.

We demonstrated that fractal-like mechanical ventilation is accompanied by a decrease in short-term mortality, improved oxygen saturation and decreased liver injury following injection of bacterial endotoxin. Future investigation can pave the way to understand the mechanism of these effects by using an electrophysiologic (direct vagus nerve recording) or pharmacologic approach. Development of new analytical methods in Network Physiology will also help to uncover the mechanism of multiple organ failure in cirrhosis form a different perspective (Bartsch et al 2015, Xiong et al 2017, Asada et al 2016, Ivanov et al 2016, Kanter et al 2015). For example, multiscale network construction (Shashikumar et al 2017) can be employed for quantification of cardiovascular and respiratory interaction within the context of sepsis in patients with liver failure. Such novel approaches are crucial as classical medicine currently lacks a good pathophysiological model to explain the mechanism of multiple organ failure in acute-on-chronic liver failure.

In the present study we used analytical techniques that were previously developed for analysis of physiological fluctuations in health and disease. Our aim was to introduce a method to ameliorate acute-on-chronic liver failure by alteration of respiratory pattern in an animal model of multiorgan failure. The association between respiratory pattern and liver function has not been studied previously and a Network approach can potentially explain the complex interaction between multiple organs during systemic inflammation. The major limitation of our study is that we have not developed a novel computational tool to assess interaction between different organs in liver failure. However, our study introduces a new phenomenon which is suitable for analysis using techniques that are being developed in the emerging field of Network Physiology (Bashan et al 2012, Bartsch et al 2015, Ivanov et al 2016, Kanter et al 2015). In recent past decades hepatology has benefited enormously from collaboration with other scientific communities (Shirazi et al 2016). A multidisciplinary approach is more likely to introduce alternative models and uncover the mechanism of experimental therapies such as fractal-like ventilation in patients with liver failure.
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Figure 1. Detrended fluctuation analysis for inter-breath interval time-series in a representative fractal-like mechanical ventilation group. The scaling exponents ($\alpha=0.8$) in this graph is within the range of the scaling exponent of respiratory fluctuations in healthy individuals (Raoufy et al 2016).
Figure 2. The effect of fractal-like mechanical ventilation on plasma transaminase (ALT) levels in control (SHAM) and cirrhotic (BDL) rats after 2 hours challenge with endotoxin (LPS). Fractal-like breathing in cirrhotic rats was associated with lower ALT levels in comparison with rats given constant ventilation. *** P<0.001.
Figure 3. The effect of fractal-like mechanical ventilation on mean heart rate (A), systolic blood pressure (B) and SpO$_2$ (C). a: P<0.0001 BDL fractal vs. BDL constant. b: BDL constant ventilation vs. SHAM constant P<0.0001. *: cirrhotic constant vs. SHAM constant (Tukey’s post-test) P<0.05. #: cirrhotic fractal vs. cirrhotic constant (Tukey’s post-test) P<0.05.
Figure 4. The effect of fractal-like mechanical ventilation on total HRV (SDNN, A), short-term HRV (SD1, B) and long-term HRV (SD2, C). a: P<0.05 BDL fractal vs. BDL constant. b: BDL constant ventilation vs. SHAM constant P<0.05.
Figure 5. Kaplan–Meier survival curve in cirrhotic (BDL) or sham-operated (SHAM) control rats after acute endotoxin challenge. The rats were given either constant or fractal-like mechanical ventilation under general anaesthesia. A log-rank (Mantel–Cox) test showed a significant difference in mortality (P<0.05).