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A comparative study of the mortality rate of rats receiving a half lethal dose of fat intravenously: Under general anaesthesia versus under spinal anaesthesia☆

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ABSTRACT

Background: There is no data that demonstrates what anaesthesia is suitable for patients who have a high risk of fat embolism syndrome (FES). We investigated the mortality rates of rats that received a half lethal dose (LD_{50}) of fat by intravenous injection after induction of general or spinal anaesthesia.
Methods: An LD_{50} of fat for rats was determined by using a toxicological method. Three hundred and seventy five rats were randomly assigned to receive general anaesthesia (group GA, $n = 125$), or spinal block (group SA, $n = 125$), or no anaesthesia (group C, $n = 125$). The rats were injected with the LD_{50} of fat at 20 min after anaesthesia induction. The mortality rates were recorded at 2, 8, 12, and 24 h after fat injection.
Results: The LD_{50} of fat was 0.706 ml/kg and its 95% CI was 0.622 ml/kg–0.801 ml/kg. The mortality rate was lower in the group GA than in the group SA ($p < 0.01$), whilst there was no statistical difference between the group SA and the group C ($p = 0.442$).
Conclusion: It is feasible to assess the efficacy of various treatments for FES by comparing the mortality rates of animals after injection of an LD_{50} of fat. The mortality rate of rats was lower when FES was induced under general anaesthesia than under spinal anaesthesia which implies that general anaesthesia is superior to spinal anaesthesia for patients who have a high risk of FES.

Introduction

Clinically, fat embolism syndrome (FES) rarely exists independently, it usually occurs as a complication of a trauma or surgery. It is difficult to evaluate the efficacy of various treatments for clinical FES because of different primary traumas and the wide range of severity within the syndrome.[1] In experimental fat embolism, the efficacy of a treatment is usually deduced from the results of studies that employed pathological tests and some organic functional parameters as primary parameters after non-fatal doses of fat were administered.[2,3] These parameters are of great variation and therefore cannot precisely reflect the prognosis and outcome of FES.[4]

Almost all organs of the body are involved in FES, and its precise mechanisms remain unclear. In physics, a "black box method" is usually used to explore an unclear and complicated process. Ignoring the pathological procedure of FES, we consider it reasonable to employ mortality rate as the only parameter by which to evaluate the efficacy of various treatments after a half lethal dose (LD_{50}) of fat has been intravenously injected.

It has been reported that neuraxial blockade is superior to general anaesthesia for patients who have a high risk of pulmonary thromboembolism.[5] But to date, no data demonstrate what anaesthesia is suitable for patients who have a high risk of fat embolism. Studies using rabbit models have reported that providing high oxygen after fat injection reduced the mortality rate whilst haemorrhagic and tourniquet shock elevated it.[6,7] Considering that perfect oxygenation under general anaesthesia and sympathetic block under spinal block, we proposed a hypothesis that general anaesthesia was superior to spinal anaesthesia for patients who have a high risk of FES.

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In this study, we determined an LD_{50} of fat for rats using a toxicological method. Then we investigated the mortality rates of rats that received an LD_{50} of fat by intravenous injection after induction of general or spinal anaesthesia.

Part one: determining the LD_{50} of fat for rats

Material and methods

Animals and fat

This study was reviewed and approved by the Animal Research Committee of Shanghai Jiaotong University. Male Sprague–Dawley rats weighing 280–300 g were used in the study.

Allogeneic perirenal fat was obtained from the rats that were left over from our pre-experiments.[6] When a rat died after the pre-experiment, the fat was collected within 2 h after its death. If a rat was still alive after the experiment, overdose pentobarbital was given in order to obtain the fat. The fat was homogenized by an ultrasonic cell breaker after having been cut into fragments. The homogenate was centrifuged at 4000 rpm at 4 °C for 10 min. The supernatant, which was a clear, straw-coloured liquid fat, was stored at −20 °C in a refrigerator. The frozen fat was put in a thermostatic water container at 37 °C in advance of being used.

Determination of the minimal dose of fat that can cause 100% of the rats to die (LD_{100}) and the maximum dose that will not cause any of the rats to die (LD_0)

The sequential method was used to determine the LD_{100} and the LD_0.[8] Six rats composed a group. Each group received various doses of fat via the vena caudalis at a speed of 0.04 ml/min without anaesthesia. According to previous literature and our preliminary test, the dose given to the rats in the first group for determining LD_{100} was 1.5 ml/kg. After injection, the rats were put back into their cages under routine conditions. The number of rats that died within 24 h was observed and recorded. If all of the rats in the group were dead, the dose would be reduced 0.1 ml/kg (1.4 ml/kg) for the rats in the second group; if two or more rats survived, the dose would be increased 0.1 ml/kg (1.6 ml/kg) for the rats in the second group. This continued for subsequent groups until a dose was found that led to only one surviving rat in a group. LD_{100} was set at the next adjacent high-dose. Using a similar method, the first dose for estimating LD_0 was 0.2 ml/kg. Within 24 h, if all the rats survived, the dose would be raised 0.1 ml/kg; if more than one rat died, the dose would be reduced to 0.1 ml/kg. This continued for subsequent groups until a dose was reached that led to only one death in a group. LD_0 was set at the next adjacent low-dose. The LD_{100} and LD_0 were determined to be 1.3 ml/kg and 0.3 ml/kg, respectively (four groups and three groups of rats were used, respectively).

Grouping and treatments for determining LD_{50}

In accordance with Karber's method,[9] seventy Sprague–Dawley rats were randomly assigned to one of seven groups. Each group contained ten rats and received various doses of fat. The doses of each group were determined according to the formula:

$$R^6 = \frac{D_7}{D_1} \tag{1}$$

$$D_n = D_{n-1} \times R \tag{2}$$

R represented the common ratio between the two adjacent groups and D_n represented the doses for each group. D_1 was LD_{100} (1.3 ml/kg) and D_7 was LD_0 (0.3 ml/kg), which had been ascertained using the procedure above. So the value of R was 0.78 according to formula (1), and the doses of fat in various groups were determined to be 1.300, 1.018, 0.797, 0.624, 0.489, 0.383 and 0.300 ml/kg, respectively, according to formula (2).

The rats fasted but could drink water during the 12 h before the procedure. The scheduled doses of fat were respectively given to the rats in the corresponding groups via the vena caudalis at a rate of 0.04 ml/min. After injection, the rats were allowed to resume normal feeding in their cages.

Death or survival was observed and recorded at 0.5, 2, 8 and 24 h after fat injection. The lungs, brains and kidneys were obtained after death for the dead rats or after 24 h for the surviving rats, which were sacrificed by injection of an overdose of pentobarbital. Oil red "O" stain was used for the tissue examination.

Karber's formula was used to calculate LD_{50} and its 95% CI. The formula is as follows:

$$\mathrm{LD_{50}} = \log^{-1}[\mathrm{Xm} - I\sum P - 0.5] \tag{3}$$

$$95\%\mathrm{CI} = \mathrm{LD_{50}} \pm 4.5\mathrm{LD_{50}SX_{50}} \tag{4}$$

$$\mathrm{SX_{50}} = I\sqrt{\frac{(\sum P - \sum P^2)}{n-1}} \tag{5}$$

(Xm is the logarithm of the maximum dose; I is the logarithm of the ratio of the adjacent doses; P is the mortality of each group; ΣP is the sum of the mortalities of each group; SX_{50} is the standard error for the LD_{50}, n is the number of animals in each group; P^2 is the square of the mortality rate in each group; ΣP^2 is the sum of each value of P^2).

Results

The number of dead rats in each group are listed in Table 1. LD_{50} was 0.706 ml/kg and its 95% CI was 0.622–0.801 ml/kg.

After the injection, the high-dose group showed a high mortality rate with most deaths occurring within half an hour, whilst mortality rates were relatively lower in the low-dose groups with most deaths happening during 8–24 h after fat injection. Tachypnea, dyspnoea, cyanosis, weakness and sluggishness were common symptoms. Various degrees of pulmonary oedema and haemorrhage could be seen by the naked eye. Many orange drops, which were fat globules, could be seen in the frozen sections of lung, brain and kidney tissues that were stained with red oil "O".

Table 1
The number of dead rats and mortality rates in all groups for calculating LD_{50} and its 95% CI.

Group (n = 10)	Dose (ml/kg)	0–0.5 h	0.6–2 h	3–8 h	9–24 h	Total death	Mortality rate (%)
1	1.300	9	1	0	0	10	100
2	1.018	3	4	2	0	9	90
3	0.797	0	1	4	2	7	70
4	0.624	0	0	0	3	3	30
5	0.489	0	0	0	1	1	10
6	0.383	0	0	0	0	0	0
7	0.300	0	0	0	0	0	0