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Lactic acid's role in hypertonic sodium lactate solution as a neuroprotector measured from the level of ATP, MCT-1, and necrotic areas in intracerebral hematoma rats model

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Abstract

Objective: To discover the role of hypertonic sodium lactate (HSL) as the energy source, which in turn will act as a neuroprotector, by measuring adenosine triphosphate (ATP) level, monocarboxylate transporter 1 (MCT-1) and the extent of the necrotic areas.

Design: This was an experimental study that used randomized post-test only control group design.

Setting: Experimental Animal Care Unit Universitas Gadjah Mada.

Patient and participant: 32 white mice of *Rattus norvegicus*.

Intervention: After the protocol of this study was approved by the research ethic committee,

32 rats were randomly divided into two groups HSL group (n=16) and NaCl 3% group (n=16) a the control group. Both groups were anesthetize using conversion-dose pentothal.

Results: ATP level in HSL group was higher compared to the control group (p=0.031). MCT-1 in HSL group was also higher than the control group (p=0.010). Necrotic areas were less extensive in the HSL group than the control group (p=0.000). Lactate levels at minute 30 (T30) and minute 360 (T360) increased in the HSL group, while increasing in the control group up to T30, then decreased gradually until T360.

Conclusion: Exogenous lactate in solution has effect as a neuroprotective of brain in the intracerebral hemorrhage (ICH).

Key words: ATP, extent of necrotic areas, hypertonic sodium lactate, intracerebral hematoma, neuroprotector.

Introduction

Intracerebral hemorrhage (ICH) is a bleeding in the brain parenchymal which can reach into ventricles, but rarely reaches the sub arachnoid space. This disease is a deadly stroke and anybody who sur-

vives will be disabled. (1-5) The incidence of the ICH from all stroke cases are 44% non-traumatic ICH and 15% traumatic ICH. (6) Spontaneous ICH population is higher in Asians than Europeans or Americans. (2)

ICH mortality rate is about 35-52% within 30 days after a stroke attack, whereas 38% survive in the first year, and only 20% are able to return to normal activity. (1,2,4,7) Classically, ischemia is a deficit of oxygen and glucose due to narrowing of vascular caused by various intracellular or extracellular causes, with unknown exact mechanisms. The greatest potential occurrence of ischemia in the perihematoma area is the beginning of the ICH. (8)

The critical period from decreased cerebral blood flow (CBF) is within the first six hours. During this time, early death from brain injury often occurs. (9) The deficit of oxygen and glucose due to hypoperfusion impacts on the increasing demand of

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metabolic substances, like lactate. Oxidation of glucose provides almost all the energy required by neurons to support brain activity. (10,11)

In vitro and in vivo research have shown an association between the central nervous system glial cells (astrocytes) and neurons, especially in the event of cerebral ischemia and brain injuries. (11) This begins with the release of neurotransmitter glutamate, which requires brain glucose and lactate to be produced in large quantities through glycolysis. Alternately, lactate was more favored by neurons than glucose to meet its energy needs; even lactate, not glucose, is the fuel used in the repair function of synapses after the occurrence of hypoxia in time re-oxygenation. (12,13)

In addition to the necessary process energy metabolism, ATP also plays an important role in maintaining the function of neurons and glia. The ATP levels determine the severity of brain ischemia injury; without a sufficient supply of ATP the cascade of ischemia will continue, leading to cell necrosis. (14)

Protecting the brain by administering hypertonic sodium lactate gives two therapy aspects: (1) its hyperosmolar content decreases the edema through shifting the fluid from the intracellular to the extracellular; (2) the lactate content, as an energy substrate, can produce ATP through aerobic and anaerobic glycolysis. (15-17)

Lactate is an alternative originating from endogenous substrates, but when a moderate to severe brain injury occurs it will require additional energy material from exogenous substrates like exogenous lactate.

Endogenous and exogenous lactate can enter the cell or move to neighboring neurons with the help of conveyors known as monocarboxylate transporter (MCT). Fourteen MCT have been identified and 3 different MCTs are known in the brain (MCT-1, MCT-2, and MCT-4). (18-20) The transport power of MCT-1 and MCT-4 for lactate is greater than that of MCT-2. (19-21) In studying ischemic mice models, MCT-1 expression increases in the injured cerebral cortex. (12)

Lactate endogenous and exogenous form ATP through glycolysis. Some literatures suggest that the study of glucose metabolism in the brain via the glycolytic pathway begins with the phosphorylation of glucose and lactate, finishing with the formation of astrocyte-neuron lactate shuttle hypothesis (ANLSH). This explains that the brain is in a state of rest or active, glycolysis is always moving in a state of aerobic and anaerobic towards the final stages of the lactate anaerobic through the reaction aid lactate dehydrogenase (LDH). Lactate

and pyruvate are the substrate for the oxidative cycle tricarboxylic acid (TCA) cycle as the consequence of this hypothesis. (13,16,22)

An increasing interest of ANLSH has been shown by many studies, but until now there are no pharmacological interventions and surgical therapies that appropriately address the entire intracerebral hematoma problem. Experimental research studies in animal models are required to obtain new information about the role of exogenous lactate in the brain tissue damage due to intracerebral hematoma. (23)

Material and methods

The research object was white mice of *Rattus norvegicus* obtained from the Experimental Animal Care Unit Universitas Gadjah Mada. The sample size was 32 rats. This research was a real experimental study (true experimental) that used randomized post-test only control group design.

Pentothal dose given for anesthesia was 5-8 mg/kg, after being multiplied by a human dose conversion factor to the mice based on the Laurence Bacharach dose conversion table. The preparation method of a 25 mg/ml pentotal solution was that pentotal was diluted with aqua injection into a 500 mg/20 ml solution in a bottle, then obtaining 1 ml solution that contained 25 mg pentotal.

The free two-sample t-test was used to ATP examination, while Mann-Whitney test was used to MCT-1 examination and areas of necrosis. The basis data was tested with Kolmogorov-Smirnov test to examine the normal distribution. If the data was not normally distributed, then the non-parametric Mann Whitney will be conducted; and while the distribution was normal, then performing the t-test.

Results

The general characteristics of sample

Statistical analysis using two independent samples t-test of both the ICH group and control group (NaCl 3%) that obtained $p > 0.05$ showed there was no significant difference between the two groups before the treatment. Thus, the object of the research was homogeneous and worthy to be compared (Table 1)

In the research object 30 minutes after treatment, statistical analysis was performed on the independent variables such as systolic pressure, diastolic pressure, mean arterial pressure (MAP), blood glucose levels, pH, PaO₂, HCO₃, SaO₂, hemoglobin in the HSL group and the control group (NaCl 3%), we obtained $p < 0.05$, meaning there was a sig-

nificant difference at T30 after treatment (**Table 1**).

In the research object 360 minutes after treatment, statistical analysis was performed on the independent variables such as systolic pressure, diastolic pressure, MAP, blood glucose levels, lactate levels, pH, PaO₂, HCO₃, SaO₂, BE, hemoglobin in the HSL group and the control group (NaCl 3%), we obtained p<0.05, suggesting there was a significant difference at T360 after treatment (**Table 1**).

Analysis of variance (ANOVA) tested the variables of systolic, diastolic, MAP, pulse, rectal temperature, glucose concentration, lactate levels, pH, PaCO₂, PaO₂, HCO₃, SaO₂ (%), BE, hemoglobin in the control group. We obtained p<0.05, meaning there were significant differences between the observations of T0, T30, and T360.

Table 1 showed the mean score and standard deviation of research object at T0, T30, and T360 observation. ANOVA was conducted to test the variables of systolic, diastolic, MAP, pulse, glucose, lactate levels, pH, PaCO₂, PaO₂, HCO₃, SaO₂ (%), and hemoglobin in the HSL group. We obtained p<0.05, showing significant differences between the T0, T30, and T360.

The results of t-test on ATP levels in the HSL group obtained p<0.05, showing a significant difference between the HSL group and the control group (NaCl 3%) (**Table 2**).

Mann-Whitney test was used to examine MCT-1 levels in the control group. We obtained p<0.05, depicting significant differences in the levels of MCT-1 in the treatment group (HSL) and the control group (NaCl 3%) (**Table 2**).

The statistical analysis by Mann-Whitney test of the areas of necrosis in the treatment group (HSL) and the control group (NaCl 3%) obtained p<0.05, showcasing the vast difference in the areas of necrosis in both groups (**Table 2**).

The correlation between the levels of lactate in solution of the HSL with ATP, MCT-1, and the areas of necrosis

Correlation of lactate in 360 minutes with ATP, the areas of necrosis, and MCT-1

Lactate 360 minutes to necrotic areas obtained a correlation of r=-0.761, p=0.000, showing a significant correlation between lactate 360 minutes to the necrotic areas. Increased levels of lactate in 360 minutes followed by a decrease in the areas of necrosis. The regression test between lactate levels and necrotic areas resulted in y=11.01-1.88x with p=0.000, which meant the areas of necrosis would decrease as lactate levels increased (**Table 3**).

Moreover, a significant correlation was also found

between lactate 360 minutes and ATP with r=0.420 and p=0.017. Thus, the regression test between lactate levels with ATP resulted in y=1.06+0.26x with p=0.017. Increase in lactate levels 360 minutes followed by increase of ATP.

The correlation test between lactate 360 minutes by MCT-1 also found a significant correlation with r=0.350 and p=0.049 - an increase in lactate levels followed by increase of 360 minutes MCT-1. Result of regression test between lactate levels with MCT-1 was y=0.82+0.26x with p=0.007.

Multivariate correlation lactate levels of 360 minutes, ATP, and MCT-1 with total areas of necrosis by the linear regression statistical test of linear correlation (R²=0.606) were significant (p=0.000).

Statistical analysis using linear regression with enters method showed that not all variables related to the necrotic areas. Subsequent analysis using linear regression of the stepwise method, showed only 360 minutes lactate levels associated with the areas of necrosis (R²=0.632) were significant (p=0.000).

Monitoring blood glucose, lactate, and hemodynamics

The statistical analysis of two independent samples was the t-test. At baseline, blood sugar levels were homogenous and not significant (p>0.05) between the two groups (**Table 1**). Meanwhile, observations at minute 30 (T30) insignificantly increased the blood sugar levels in the two groups (p>0.05); at minute 360 (T360) HSL group increased while the control group declined, obtained p<0.05, showing a significant difference between the treatment groups (HSL) and the control group (NaCl 3%).

Based on **Table 1**, by using a statistical analysis of two independent t-test samples lactate levels at baseline (T0) homogeneous with no significant difference at 30-minute observations (T30) as lactate levels in the two groups increased, but p>0.05, meant no significant difference, while in the 360-minute observation lactate levels increased in the treatment group while in the control group decreased, obtaining p<0.05, meaning there was a significant difference between the treatment groups (HSL) and the control group (NaCl 3%).

Glucose levels at baseline obtained p>0.05, meaning that there was no significant difference between the treatment groups (HSL) and the control group (NaCl 3%). Observations at minute 30 and minute 360 obtained p<0.05, showing significant differences between the treatment groups (HSL) and the control group (NaCl 3%). However, the statistical analysis of two independent samples

t-test lactate levels at the beginning of the study and observation of minute 30 obtained $p>0.05$, so no significant differences between the treatment groups (HSL) and the control group (NaCl 3%). The observation of minute 360 obtained $p<0.05$, showing a significant difference between the treatment groups (HSL) and the control group (NaCl 3%).

Systolic pressure

Statistical calculation of the mean systolic blood pressure over times obtained $p<0.05$, showing significant differences in the control and treatment groups (**Figure 1**).

Diastolic pressure

The mean diastolic pressure over times obtained $p<0.05$, showing differences between the control and treatment groups (**Figure 1**).

Average arterial pressure

The results of statistical calculations mean MAP at times obtained $p<0.05$, a significant difference in the control group (NaCl 3%) and treatment (HSL).

Pulse frequency

The results of statistical calculations of the mean pulse frequency at times obtained $p>0.05$, no significant difference in the control and treatment groups.

Temperature

Statistical calculations obtained the mean rectal temperature over times of $p>0.05$, no significant difference in the control group (NaCl 3%) and treatment (HSL). From **Table 1**, the temperature of variables in observation at 0 minute, 30 minutes, and 360 minutes had no significant difference either to the treatment or control groups.

Discussion

General description

This study aimed to determine the neuroprotective effects caused by HSL solution. As a reference, the solution was 3% of NaCl. HSL solution is relatively new and still being developed. The research was conducted on the mice models, not directly on humans. The reference solution used was NaCl 3%, with its osmolarity (1026 mOsm/L) that almost matches the HSL solution (1020 mOsm/L).

ATP levels

ATP is vital for the brain. In addition to needing energy for the metabolic process, ATP also plays a key role in maintaining the function of neurons and

glial cells. In this study, ATP levels rose significantly compared to the control group. Arguably, the lactate as an energy substrate was able to reach the cells and be metabolized through the TCA cycle and be used by neurons. The ATP increased in group HSL was caused by lactic, where it provided hemodynamic stability, improving cerebral blood flow, and thereby reinvigorating the oxidative metabolic. Lactate group proved to give more meaningful results than NaCl 3% group.

This proved that the improvement of perfusion not the only factor of osmotherapy exists in both groups. Another proof was that although HSL has a higher osmolarity of plasma, its role was not too prominent by the nature of its tonicity, which after administration by intravenous soon changed after mixing with blood plasma. Tonicity solution HSL was declining, therefore anion lactate was immediately transported through the cell membrane and quickly metabolized. Thus the factor of osmolarity contained in the HSL solution affecting oxidative metabolism was difficult to prove, despite having a minimal effect. (24,25)

Theoretically, this has also proved capable of protecting ischemia, which could damage the brain tissue by cutting the upstream path of tissue damage due to the intracerebral hematoma (hematoma-deficit O₂ and glucose--cascade of metabolic suppression of ischemia-necrosis), supported by examination results of brain tissue material that was experiencing ICH, obtaining more extensive necrotic areas that decreased in HSL group compared to the NaCl 3% group, and statistically concluded that there were significant differences.

MCT-1 levels

The increase of lactate spurred MCT-1 formation and the state of ischemia has also been demonstrated in increasing the expression of MCT-1 in mice (rats). (26) In this study, the percentage of MCT-1 was mostly found in brain tissue mice model of ICH HSL group than the control group. It was suggested that the HSL group has a content of lactic exogenous able to stimulate or increase the expression of MCT-1 because of the ability of lactic to facilitate MCT-1 when entering or diffusing into the cell membrane. Arguably, the presence of MCT-1 were exposed on the outward appearance of mice brain tissue model of the ICH, transporting lactate.

Necrotic areas

The administration of exogenous lactate was considered a substrate energy shown to decrease the incidence of cell necrosis in the observation group

getting HSL or exogenous lactate compared to the control group. The statistical results showed that there were significant differences at $p < 0.05$. Then, ischemia happened due to compression of the mechanical hematoma decreasing the cerebral blood flow. This was aggravated by factors of pathology at the ICH potentially causing the catecholamine release in response to injury (vasoconstriction of blood vessels of the brain), deficienting O₂ and glucose towards death cell pathways necrosis. In this study, a decrease in necrosis was more significant in the control group compared to HSL group, concluding that the intervention group HSL with exogenous lactate solution could reduce the damage to cell necrosis pathways. However, some areas of necrosis in some study samples had a magnitude of as much as 5%, proving that the role of lactate in HSL solution was not maximized to overcoming necrosis due to the intracerebral hematoma. Therefore, the multimodal therapeutic approach could be considered to complete all pathology that occurs in ICH.

Glucose

Blood sugar levels at T0 in the two groups were normal and homogeneous. Minute 30 (T30) or reaching the first 30 minutes after the injury of ICH was associated with an increase in blood sugar levels in both groups caused by the stress of the metabolic response to injury and catecholamine release. It was supported by other researchers. (26,27) Thus, the increase in blood sugar levels at T30 in the two groups were caused by the metabolic response to injury (ICH is a moderate to severe brain injury) and factors of mobilization reserves of glucose. Because the brain inability to form adequate glycogen storage for a long term, mobilization another source of energy from liver and other metabolic pathway is necessary. However, until now, the line has no evidence, so lactate was an alternative energy substrate to immediately meet the energy deficit oxidative happening at the cellular level.

In the control group, the declined of blood glucose level after T30 showed that the glucose and glucose reserves (glycogen) was used as an energy source in the brain that suffered from metabolic suppression. On the other hand, in the treatment group, the inclined of glucose levels in T30 and T360 correlated to lactate level, because exogenous lactate was taken into the body to be used directly by the brain cells (astrocytes) through the help of MCT-1. The remaining lactate was changed into pyruvate in the cytosol by cytosolic LDH, and then stored. The stored pyruvate would

undergo the same pathway when needed (gluconeogenesis).

Increased levels of glucose in both groups did not meet the criteria for hyperglycemia (normal rat glucose level 50-135 mg/dl) so the clinical significance did not affect the dependent variable, although the statistical significance occurred meaningful changes.

Lactate levels

The results of this study found an increase in lactate levels in the observation T0 to T30 (examination of blood sugar levels at T30) in both groups, but due to different causes. In the treatment group the increased levels of lactate was caused by exogenous and endogenous lactate, while in the control group only by endogenous lactate.

At T30 and T360, lactate levels in HSL group tended to increase while in the control group decreased. In the control group, there was a breakdown of lactate by the use of brain tissue, which could be explained by the idea that at the time of the mice suffering a hematoma would be moderately to severely injured, requiring greater energy to address areas experiencing an energy deficit (penumbra); no supply of exogenous glucose and glycogen brain as a backup meant it was only able to be supplied within 3 minutes.

The infusion of exogenous lactate bolus and maintenance for 360 minutes increased levels of ATP and MCT-1, proving that exogenous lactate entered through cell membranes, occurring in the metabolism in mitochondrial brain tissue. As it is known theoretically, lactate as a result of metabolism in astrocytes will be used by neurons to ensure its function and for the restoration of damaged neurons or protecting the ongoing damage. The real proof of the administration of exogenous lactate was the result of histological examination of brain tissue turned out to be lesser in the areas of necrosis in HSL group than in the control group. Statistically significant differences found a decrease in the areas of necrosis between HSL and NaCl 3% groups.

Systolic and diastolic pressure, and MAP

The addition of the initial load measured in this study was the MAP, hemoglobin and haematokrit, but this parameter simplistically described that the hemodynamic situation was ideal. The results showed a mean change in systolic blood pressure from minute 0 to 360, and based on the results of statistical calculations, mean systolic blood pressure over times obtained $p < 0.05$, showing a significant difference in control and treatment groups.

Figure 1 shows changes in mean diastolic pressure from minute 0 to 360. Mean systolic and diastolic pressures over times obtained $p < 0.05$, showing a significant difference in the control and treatment groups.

By observing for 360 minutes in the treatment and the control groups (**Figure 1**), the MAP changed from minute 0 to 360. The mean systolic blood pressure over times was $p < 0.05$, showing a significant difference in the control and treatment groups. By monitoring the pulse for 360 minutes in both groups, (**Figure 1**) the mean changes from minute 0 to 360 minutes. The mean pulse over times was $p > 0.05$, showing no significant difference in both groups.

PaO₂, SaO₂, PaCO₂, and hemoglobin

The results showed significant increase in the levels of PaCO₂ in both groups, by factors of hypertonic solutions in the control group, and the lactate and hypertonic factors in HSL.

PaCO₂ concentration in the blood was very significant. In the cerebral injury it increased (PaCO₂ > 46) in intracranial pressure and a decrease in extreme (PaCO₂ < 30) will occur. The vasomotor effect was closely related to ventilation or breathing patterns, so that head injuries were particularly necessary in monitoring his case. Observation of T₀, T₃₀, T₃₆₀ had statistically and significantly different meanings, but the range of the value was normal (PaCO₂ 35-45 mmHg) so the clinical significance did not influence the dependent variable.

pH, BE, HCO₃

The testing in the treatment group (HSL) showed a meaningful increase in pH, HCO₃, and BE and clinical significance occurred with mild metabolic alkalosis, whereas in the control group they rose significantly, but the little clinical significance did not cause alkalosis.

In addition, exogenous lactate transferred into plasma was not a form of lactic acid, but the salt compound derived from the strong acid such as HSL solution. Thus, the effect of this altered strong ion difference (SID) slightly. This situation was understandable because lactate in the solution HSL will soon undergo change by metabolism, while Na⁺ will improve SID, causing the condition of metabolic alkalosis. Based on the above mention, alkalization was important to the process of glycolysis, because acidosis will affect or hinder the process of glycolysis, causing barriers to the formation of ATP.

Conclusion

The provision of lactate solution in HSL with bolus and continuous infusion for 360 minutes post brain injury acts as a neuroprotective of the brain by increasing levels of ATP, increasing the MCT-1 in the brain tissue, and reducing the areas of necrosis in the mice model of intracerebral hematoma. There is a correlation between lactate levels of ATP, MCT-1, and the areas of necrosis in the provision of lactate solution in HSL with bolus and continuous infusion over 360 minutes post brain injury in a mice model of intracerebral hematoma.

Table 1. Mean score and standard deviation of sample general characteristics at 0, 30, and 360 minutes observation in control (NaCl 3%) and HSL groups

Variable	Group	Observation			p value (ANOVA)
		0 minute	30 minutes	360 minutes	
Systolic (mmHg)	Control	123.9 (4.4) ^a	122.8 (3.9) ^a	116.9 (2.1) ^b	0.000
	HSL	124.2 (4.0) ^a	132.3 (2.6) ^b	130 (3.6) ^b	0.000
Diastolic (mmHg)	Control	90.6 (1.8) ^a	90.7 (3.0) ^a	86.8 (2.3) ^b	0.000
	HSL	90.5 (2.3) ^a	92.8 (1.8) ^b	90.8 (0.9) ^a	0.001
MAP	Control	101.8 (2.5) ^a	101.4 (2.8) ^a	96.9 (2.1) ^b	0.000
	HSL	101.7 (2.1) ^a	105.9 (1.8) ^b	104.1 (1.4) ^c	0.000
Pulse (beats/min)	Control	329.7 (4.5) ^a	317.1 (1.3) ^b	325.0 (2.8) ^a	0.000
	HSL	328.0 (0.0) ^a	317.0 (0.0) ^b	327.5 (8.0) ^a	0.000
Rectal temperature (°C)	Control	36.8 (0.1) ^a	36.9 (0.3) ^a	36.9 (0.1) ^a	0.688
	HSL	36.8 (0.1) ^a	36.9 (0.2) ^a	36.9 (0.2) ^a	0.138
Glucose (mg%)	Control	102.7 (4.2) ^a	133.5 (4.1) ^b	98.1 (6.3) ^c	0.000
	HSL	105.7 (4.4) ^a	124.4 (3.1) ^b	130.3 (3.6) ^c	0.000
Lactate levels (mmol/L)	Control	1.01 (0.09) ^a	1.93 (0.31) ^b	1.21 (0.24) ^c	0.000
	HSL	1.02 (0.09) ^a	1.81 (0.47) ^b	4.35 (0.35) ^c	0.000
pH	Control	7.39 (0.01) ^a	7.45 (0.01) ^b	7.43 (0.01) ^c	0.000
	HSL	7.37 (0.03) ^a	7.38 (0.03) ^a	7.42 (0.02) ^b	0.001
PaCO ₂	Control	39.9 (1.1) ^a	44.8 (0.7) ^b	41.5 (2.0) ^c	0.000
	HSL	38.3 (3.2) ^a	44.9 (0.7) ^b	41.5 (2.2) ^c	0.000
PAO ₂	Control	88.6 (1.6) ^a	86.2 (2.1) ^b	84.2 (2.2) ^c	0.000
	HSL	88.1 (1.9) ^a	90.0 (1.3) ^b	87.3 (2.4) ^a	0.001
HCO ₃	Control	23.9 (0.9) ^a	24.4 (0.7) ^a	22.9 (0.8) ^b	0.000
	HSL	24.3 (0.7) ^a	23.4 (1.4) ^b	24.4 (1.2) ^a	0.032
SaO ₂ (%)	Control	97.1 (0.9) ^a	96.5 (0.5) ^b	95.5 (0.9) ^c	0.000
	HSL	97.2 (0.8) ^a	97.8 (0.6) ^b	97.1 (0.6) ^a	0.025
BE	Control	1.23 (0.34) ^a	1.39 (0.47) ^a	1.14 (0.43) ^a	0.222
	HSL	1.33 (0.27) ^a	1.64 (0.40) ^b	2.23 (0.44) ^c	0.000
Hemoglobin	Control	12.3 (0.3) ^a	12.60 (0.4) ^b	11.7 (0.3) ^c	0.000
	HSL	12.2 (0.3) ^a	12.2 (0.3) ^a	11.2 (0.1) ^b	0.000

Legend: HSL=hypertonic sodium lactate; ANOVA=analysis of variance; MAP=mean arterial pressure.

Table 2. Levels of ATP, MCT-1, and necrotic areas in both groups

Variables	Group		p value
	HSL (n=16)	NaCl 3% (n=16)	
ATP levels, mmol/L (SD)	2.19 (1.26)	1.40 (0.47)	0.031
MCT-1 levels			0.010
• - (%)	0 (0.0)	5 (31.2)	
• +/- (%)	4 (25.0)	4 (25.0)	
• +1 (%)	8 (50.0)	7 (43.8)	
• +2 (%)	4 (25.0)	0 (0.0)	
Necrotic areas			0.000
• 0% (%)	7 (43.8)	0 (0.0)	
• 5% (%)	9 (56.2)	4 (25.0)	
• 10% (%)	0 (0.0)	12 (75.0)	

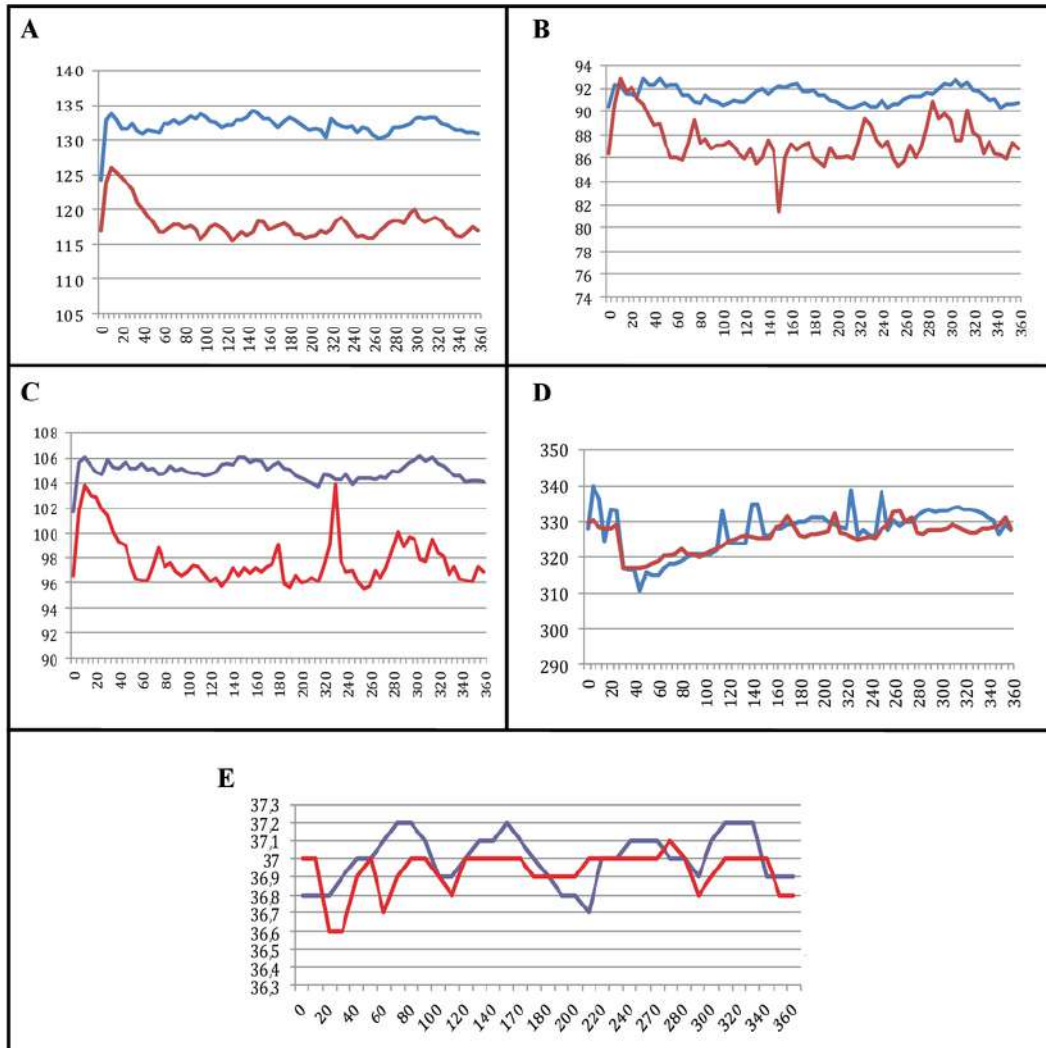
Legend: ATP=...; MCT-1=monocarboxylate transporter 1; MCT-1 (-)=figure outward approximately 10%, (+/-)=figure outward weak appearance of 10%-25%, (+1)= figure strong staining of 25%-50%, (+2)=figure strong outward once more than 50%; HSL=hypertonic sodium lactate; SD=standard deviation; necrotic areas (0%)=negative staining necrotic areas, (5%)=5% staining areas of necrosis, (10%)=outward appearance areas of necrosis 10%.

Table 3. Correlation between lactate at minute 360 with ATP, local areas necrosis, and MCT-1

Lactate at minute 360	r	p value
Regions of extensive necrosis	-0.761	0.000
ATP	0.420	0.017
MCT-1	0.350	0.049

Legend: Spearman rho correlation test; ATP=...; MCT-1=monocarboxylate transporter 1.

Figure 1. Monitoring over 360 minutes



Legend: Monitoring over 360 minutes in the hypertonic sodium lactate (HSL) group (blue) and control group (red); A=systolic pressure; B=diastolic pressure; C=mean arterial pressure (MAP); D=pulse; E=rectal temperature.

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