SCIENTIFIC ARTICLE

Infrared image monitoring of local anesthetic poisoning in rats

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Monitoring; Local anesthetics; Acute intoxication; Ropivacaine; Infrared imaging; Rats

Abstract
Background and objectives: To evaluate the thermographic predictive value of local anesthetic poisoning in rats that indicates the early recognition of thermal signs of intoxication and enable the immediate start of advanced life support.
Methods: Wistar rats underwent intraperitoneal injection of saline and ropivacaine; they were allocated into pairs, and experiments performed at baseline and experimental times. For thermography, central and peripheral compartment were analyzed, checking the maximum and average differences of temperatures between groups. Thermographic and clinical observations were performed for each experiment, and the times in which the signs of intoxication occurred were recorded. In the thermal analysis, the thermograms corresponding to the times of interest were sought and relevant data sheets extracted for statistical analysis.
Results: Basal and experimental: the display of the thermal images at times was possible. It was possible to calculate the heat transfer rate in all cases. At baseline it was possible to see the physiology of microcirculation, characterized by thermal distribution in the cranio-caudal direction. It was possible to visualize the pathophysiological changes or thermal dysautonomias caused by intoxication before clinical signs occur, characterized by areas of hyper-radiation, translating autonomic nervous system pathophysiological disorders. In animals poisoned by ropivacaine, there was no statistically significant difference in heat transfer rate at the experimental time.
Conclusions: The maximum temperature, medium temperature, and heat transfer rate were different from the statistical point of view between groups at the experimental time, thus confirming the systemic thermographic predictive value.

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Introduction

Assessment of the systemic thermographic predictive value is very important for increasing safety in anesthesia and surgical procedures. The observation of accidents in clinical practice of anesthesia and their potentially fatal effects suggest the development of a supplementary perioperative monitoring method to predict cases of intoxication by local anesthetics (LA). This method can provide early recognition of the intoxication signs and the immediate start of advanced life support in these critical situations.

The direct intraperitoneal LA instillation started to be used in clinical practice, with confirmed efficiency and decreased morphine use for postoperative (PO) analgesia. An important study in the UK showed results of decreased immediate postoperative pain complaints, particularly in the first hours and when LA was used intraperitoneal at the beginning of surgery. The study concluded that LA use is safe and provides significant pain reduction in the early postoperative period. Another study of intraperitoneal LA nebulization for pain management was performed. The authors highlighted the importance of this technique, but stressed the need for further studies to evaluate the safety of intraperitoneal anesthetic administration. This important observation demonstrates the relevance of the present study regarding the pathophysiology of acute intoxication induced by intraperitoneal LA.

Studies of intraperitoneal LA for PO analgesia in laparoscopic procedures have yielded conflicting results. LA distribution and inadequate absorption along the peritoneal surface were considered as one of the factors contributing to such results. New forms of intraperitoneal LA administration were tested to provide analgesia and better distribution and peritoneal absorption, such as intraperitoneal aerosolization. The development of new devices that deliver LA combine with the pneumoperitoneum insufflation gas is also highlighted.

Bupivacaine has often been used in anesthesia, particularly in long procedures, and provides excellent sensory and motor anesthesia. However, some unexpected accidents with its use encouraged the search for safer options regarding cardiovascular complications, as well as central nervous system toxicity. Because of these complications, ropivacaine was developed. However, more studies are needed to assess the behavior of thermal changes and the pathophysiology involved in its intraperitoneal administration and clinical implications.

Assessing the systemic thermographic predictive value in LA acute intoxication is of paramount importance to improve surgical safety, as neurotoxic and cardiotoxic complications are related to changes in microcirculation and vasomotor state and increased rate of intercompartmental heat transfer.

Therefore, it becomes scientific imperative the proposition of an experimental model consisting of a qualitative and quantitative predictive method for early recognition of poisoning signs and symptoms, understanding the involved pathophysiology, in order to improve the advanced life...
support management and surgery monitoring. The use of perioperative infrared (IR) monitoring may contribute to increase safety in anesthetic procedures and improve surgical clinic management, as it is a noninvasive complementary method and safe for recognition and prevention of anesthetic complications.

Method

The experimental study was performed in the Experimental Surgery Laboratory of the Graduate Program in Surgery, Federal University of Paraná (UFPR), and approved by the UFPR Ethics Committee. Experimental procedures were conducted in accordance with the ethical principles of the Brazilian College of Animal Experimentation (COBEA) and the Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care) requirements.

A total of 24 male Wistar rats (Rattus norvegicus albinos, Rodentia mammalia) with 70 days of life and weight of 330–400 g were used. The animals were placed in cages, each with five animals. It was observed a circadian rhythm of 12h/12h. Preoperatively, rats type Cr-1 received standardized food and free access to water up to 12 h before the procedure. Environmental variables (temperature, humidity, and air velocity) were recorded. There was minimal thermal variation, with ambient temperature maintained within the limits of 22°C by programming the previous environment climate and relative humidity at 50%, both checked using a thermo-hygrometer, so that the animal was in thermal comfort at the beginning of the trial, with no clinical signs of sweat or thermogenic shivering.

The animals were allocated into two groups of 12 rats each (n=24). Group S (saline solution) received intraperitoneal injection of saline solution in the same volume used in Group R; Group R (ropivacaine) received intraperitoneal injection of 1% ropivacaine at a dose of 300 mg kg⁻¹. Twelve paired experiments were performed in two specific time points (baseline and experimental) in a controlled environment. The analysis of animals in their physiological state without comitant injection was considered as baseline. The analysis after intraperitoneal injection of saline or ropivacaine was considered as experimental.

A trial box was assembled with the bottom isolated by polystyrene to minimize thermal variations, and two cameras on fixed tripods at an approximate angle of 30° were placed on each side of the box, one meter away from the study subject. For thermographic observation, a radiometer of the UFPR Graduate Program in Surgery, with 640 × 480 pixels image resolution and 307,000 absolute temperature calibrated points per frame and its computer programs, was used. After baseline measurements, the animals were carefully picked up by the dorsal cervical region by a research assistant and submitted to antisepsis and local infiltration of the application point on the right hypogastric region with the respective injection assigned to each group.

Clinical and thermographic evaluations of the animals were performed, and the times in which clinical signs of intoxication occurred were recorded. Subsequently, the thermograms corresponding to the times of interest were sought in the thermographic images and worksheets containing numeric data of temperatures were extracted for statistical analysis. The animals were observed until they showed signs of LA poisoning, defined by the onset time of ataxia, aimless executive movements, arched tail and neck, seizures (graduated by +, ++ or +++, according to severity), color of skin and mucous membranes, breathing difficulty, and recovery time or death. That is, the time required for clinical presentation of LA intoxication signs, adapted from experimental model.

It was considered an emissivity of 0.95; that is, 95% of the radiation emitted to the environment suffered no reflection to the surface itself. The interface representation of digital numeric spreadsheets for conversion into thermogram was prepared by the computer program, generated file extension, and the respective images were converted into AVI extension video clips. Thermograms were prepared and standardized with temperature range, temperature level, and isotherms (a continuous colorimetric scale with lines connecting points of equal temperature) in white, red, yellow, green, light blue, dark blue and black colors. These colors represented, respectively, a decreasing scale of temperature areas, equally distributed from hotter to colder. The colorimetric scale was maintained until the end of the experiment.

Data for the animals’ weight, environmental temperature and humidity, the Gaussian attributes, independence and homogeneity of variances were tested. Previous tests met the proposed criteria in the univariate analysis (ANOVA). Values for Group S heat transfer rate were compared at baseline and experimental time points (intergroup analysis), in order to test the hypothesis of whether there was or was not a difference in the use of intraperitoneal saline injection (surgical stress). For intragroup analysis of Group R, the heat transfer rates were also compared at baseline and experimental time points to test the hypothesis of whether there was or was not a difference in the use of intraperitoneal saline injection. The baseline and experimental values of both groups average temperature were tested using the parametric method ANOVA/MANOVA (intergroup analysis) in order to determine whether there was difference between the stress caused by saline injection and intoxication caused by local anesthetic. A significance level of 0.05 or 5% (p<0.05) was set for all tests. Areas and points with numerical information yielded from a thermogram or digital photo underwent tests for difference between means or t-test (post hoc) for independent variables.

For this model, the first law of thermodynamics for a closed system on a permanent basis was applied. The system volume control was defined as the region between the central and peripheral portions of the animal; that is, from internal organs to the outer skin surface.

The rat’s heat loss to the external environment (Q) was measured, considering the mass and specific heat of the animal (3.8 kJ kg⁻¹·C⁻¹) divided by the total experiment time:

\[ Q = \frac{m \cdot c \cdot \Delta T}{\Delta t} \]
where: $Q$ – heat loss [W]; $m$ – animal mass [kg]; $c$ – animal specific heat [J kg$^{-1}$ C$^{-1}$]; $\Delta T$ – difference between initial and final temperatures [C]; $\Delta t$ – time interval of each experiment [s].

For analysis purposes, it was assumed that the animal specific heat is constant (3.8 kJ kg$^{-1}$ C$^{-1}$), the experiment time interval, as determined by the baseline and experimental time points and the fact that the tests are paired; that is, each animal paired with his own control.

After the experiments, the animals were killed by deep inhalation anesthesia, subjected to autopsy performed by mentopubic incision, and chondrosternal plastron was removed for access to thoracoabdominal organs. A macroscopic study of the viscera was performed.

**Results**

Eleven paired experiments were considered as valid cases (22 experimental animals analyzed). A pair of animals was excluded due to lower limb blockade after LA injection, possibly by injection site error, which prevented the animal from walking in the testing area.

The environmental conditions of temperature and humidity remained constant throughout the experiment. Temperature remained steady, maintained at 22 C, and during the study no animal showed signs of heat stress, and thermogenic sweating or tremors were not observed.

There was no statistically significant difference between both groups regarding age and sex, as well as weight ($p = 0.930902$). There was no mortality in Group S and nine animals died in Group R. The remaining two animals evolved with signs of cyanosis in mucous membranes and extremitities and signs of respiratory failure, and were subsequently sacrificed by deep inhalation anesthesia.

Experiments were analyzed and experimental time points were observed. The respective thermograms performed in each experiment and their corresponding data sheets were derived from the thermal database. Thermographic study included the selection of areas of interest (head, body, and tail) by selecting computer graphics ellipses resources that surrounded the highlighted area in the image. Subsequently, the selected areas were automatically converted into numerical spreadsheets in CSV file format, which allowed obtaining various data, such as maximum temperature, average temperature, standard deviation, colorimetric scale, sum of temperature areas, emissivity, distance, hot and cold spots, location, size, and pixels.

For thermodynamic study, the values of maximum and average temperatures of the animal’s head and body were recorded for both groups at baseline and experimental time points.

Data used for obtaining the results presented were collected from the qualitative analysis (clinical) and quantitative analysis (thermal).

**Baseline observation**

Figure 1 shows the animal in its physiological state and the autonomic nervous system (ANS) preserved.

**Experimental observation**

In Group R there were signs of thermal dysautonomia (TD) predictive of clinical signs of intoxication. At the initial experimental time, immediately after receiving the LA injections, signs of morphological thermal changes could be seen in the animal’s dorsum (peripheral compartment), as shown in the thermograms (Figs. 2 and 3).

In the poisoned animal, it was observed that the head (central compartment) and dorsum (peripheral compartment) maximum temperatures were similar (centrifugal effect). Microcirculatory and thermal changes showed signs of blood congestion and increased temperature in the animal’s tail, subsequently confirmed by autopsy. During the maximum intensification of poisoning signs, the observed pathophysiological modes showed TD signs in central and peripheral compartments (Figs. 4–6).

Thermography identified signs of thermal dysautonomia in the poisoned animals’ tails. Clinical observation corroborated these findings, as it evidenced purplish tails with signs of congestion due to vasodilation. A subsequent autopsy confirmed the blood congestion in the histological sections.
Results of intragroup statistical analysis: Group S

The result of intragroup analysis showed no difference in maximum and average $\Delta T$ in Group S at the experimental time point compared to baseline (Figs. 7 and 8).

Results of intragroup statistical analysis: Group R

There was no difference in maximum $\Delta T$ in Group R at the experimental time point compared to baseline (Fig. 9).

In Group R, there was difference in the average $\Delta T$ at experimental time point compared to baseline and statistically significant difference regarding the animals' average temperature of head and dorsum ($p = 0.004266^*$) (Fig. 10).

Results of intergroup statistical analysis at baseline

The result of intergroup analysis showed no difference in maximum and average $\Delta T$ between groups at baseline. The average difference was 3.6524°C in Group S and 3.6647°C in Group R ($p = 0.984040$) (Figs. 11 and 12).
Results of intergroup statistical analysis at experimental time

There was a difference in maximum and average $\Delta T$ between S and R groups at the initial experimental time compared to baseline.

The analysis results showed that there was statistical significance between the differences in the head and dorsum maximum and average temperatures of the animals’ in Group R compared to animals’ in Group S, at experimental time. Maximum $\Delta T$ ($p = 0.040232$) and average $\Delta T$ ($p = 0.021741$) (Figs. 13 and 14).

Autopsy results

Macroscopic evaluation showed no signs of visceral damage by puncture, and it was found that all injections were intraperitoneal. In poisoned animals, there were widespread signs of blood congestion, mainly in the liver. In Group S, no visceral signs of blood congestion were found in the animals autopsied.

Discussion

Temperature range measurements allow the assessment of blood supply in a vascular territory. Therefore, knowledge
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Figure 14  Average $\Delta T$ (Group S x Group R): experimental.

of vasomotor state is essential to understand the physiology and pathophysiology of drug interactions and acute LA poisoning. This understanding contributes to improve clinical management of accidental cases with these drugs and obtain further information as additional parameters for decision-making in critical moments of advanced life support.

When the thermal behavior of an animal is studied, the basic technical criteria for using high precision equipment should be considered, the correct and current nomenclature should be adopt, proper preparation and management of experimental animals should be developed, and adequate control of thermal variables and atmospheric phenomena in the research laboratory should be performed. All these criteria were observed and applied strictly.

The relationship between the causes and effects of severe acute cases of intraperitoneal LA poisoning was demonstrated in this experimental study, as of the 11 animals in Group R, nine died immediately after receiving the drug injection and the other two that had brief survival developed severe signs of respiratory failure and underwent euthanasia.

IR view provided the image recognition of the physiological state and also of pathophysiological changes caused by poisoning. As a result of this complication, characteristic changes were registered in the thermal image, consisting of anomalies compared to the animal’s baseline thermal image. These thermal changes or signs of thermal dysautonomia could be seen before the occurrence of clinical signs. To mathematically confirm these visual morphological changes in thermal imaging, calculations were performed. As described in the thermodynamic model, the heat transfer rate is determined by the animal’s mass product, multiplied by the specific heat, multiplied by the initial and final temperature difference, divided by the experiment time interval. For analysis purposes, it was assumed that the animal specific heat is constant, the experiment time interval, as determined by the baseline and experimental times and the fact that the tests are paired; that is, each animal paired with his own control. There were no statistically significant differences in the weight of the animals between the groups. Thus, the heat transfer rate can be determined by the difference of initial and final temperatures between the compartments of interest for the study. For objective demonstration purposes, the differences in maximum and average temperatures of the head and body were assessed.

Intragroup comparison at each time point (baseline and experimental) was performed, each case served as its own referential control. Subsequently, intergroup comparison at the baseline time point was performed. Afterwards, intergroup comparison was performed at the experimental time point, comparing the microcirculatory and thermal changes in poisoned animals with those that received the same volume of saline, to mitigate the analysis of differences regarding the surgical stress caused in experimental animals.

The results of baseline assessments showed that there were no difference in maximum and average temperatures and in any of the cases; all animals showed physiological thermal distribution and no thermal change to justify exclusion from the experiment. To minimize the influence of the surgical trauma and the endocrine-metabolic responses associated, it was decided to submit the animals in control to saline solution injection. The analysis of the group subjected to saline injection showed no difference between the maximum and average temperatures of the head and body.

The baseline infrared analysis represents the physiology of microcirculation and associated vasomotor phenomena, in addition to muscle, organs and tissue production, which are autonomically controlled by the central nervous system (CNS). Baseline thermogram showed the intact, unchanged autonomic nervous system image, performing the homeothermic control by maintaining the vasomotor tone constriction. This important function performed by the ANS enables the thermal distribution maintenance in body compartments, a redistribution phenomenon of body heat that has thermal gradient in descending order, from warmer to cooler, from the body’s center to periphery. In baseline thermogram it was also observed a cranio-caudal physiological pattern of thermal distribution from warmer to cooler, from the head to the tail. In the thermographic analysis, it was possible to compare using the isothermal method (lines of constant temperature), which also confirmed the thermal distribution morphology in descending order from the head to the body. So, this thermal distribution theory in the cranio-caudal direction was followed.

The thermograms analysis results showed that, immediately after LA injection (Group R), at the beginning of the experimental time, it was possible to determine that there was statistically significant difference in average temperature between the animal’s head and body. Immediately after drug injection, it was possible to calculate mathematical differences between the physiological and changed states, which demonstrated objectively and predictively the clinical signs of the drug acute poisoning. These differences were observed qualitatively and morphologically by infrared, through the perception of thermal changes manifested by hyper-radiation areas and quantitatively confirmed by stratification of statistical mathematical results.

Thermographic view at experimental time showed morphological changes common to all intoxicated animals in Group R, characterized by areas of hyper-radiation (heat) in the paws and tail, peripheral and central compartments. The experimental thermogram showed a changed
ANS image with obvious morphological differences from baseline thermogram of the same animal. Clinical observation corroborated these findings, evidencing purple paws and tails with signs of congestion due to vasodilation. The subsequent autopsy confirmed blood congestion.

The thermal changes were described in this study as thermal dysautonomia (TD), characterized by areas of hyper-radiation caused by microcirculation disorders manifested by vasomotor effect (vasodilatation), resulting from ANS disorders caused by neurotoxicity and, having as consequences, changes in heat transfer rate between body compartments.

Neurotoxicity is the first manifestation of LA poisoning; thus, it is expected that the use of IR monitoring associated with the use of anesthetic drugs may contribute to the predictive recognition of TD signs and prevent complications resulting from drug poisoning and its deleterious consequences. Such “thermal aberrations” may be explained by the direct neurotoxic action and effects of LA cerebral circulation after the drug systemic absorption, which intraperitoneally takes place in the portocaval system.

Possible drug interactions or cases of LA CNS intoxication may cause thermal changes resulting from disturbances in the vasomotor state microcirculation and impaired autonomic control of temperature. Therefore, the mathematical results obtained from the IR images have confirmed the existence of morphological changes observed in the thermal imager and predictively demonstrated the occurrence of thermal dysautonomia resulting from the anesthetic intoxication.

Current thermographic systems feature easy handling and several autocomplete features, automatic auxiliary modes that reduce error margins with several variables, such as distance, emissivity, humidity, and temperature. IR monitoring has no practical and theoretical limitations, as it is a practical noninvasive method, with low cost compared to supplementary monitoring, which will result in increased safety in surgery and technological innovations in pain control management and postoperative recovery. It can be used in clinical practice for baseline IR monitoring of patients at rest before the procedure and then compare it with the monitoring during surgery and post-anesthesia care unit (PACU) stay.

Surgical monitoring techniques greatly evolved; in the last decades, monitoring devices such as the bispectral index (BIS), capnography, electronic gas analyzers, and neuromuscular transmission monitoring have been developed. Several important accessory parameters that allowed improved management, better differential diagnosis, and increased safety in surgery. BIS monitoring is a neuropsychological monitoring system that constantly analyzes electroencephalogram to determine the level of awareness of patients undergoing general anesthesia. The present study proposition found convergence with the mathematical model proposed by the computerized system, which converts the EEG signals into a number from 0 to 100 in BIS monitor. Considering that, as several studies using the IR sought the formulation of thermographic scores for normalization of temperature readings, the predictive value analysis also offers the possibility of developing a score or dimensionless calculation to determine thermal monitoring parameters.

Due to its noninvasive analysis capacity, thermographic systems are presented as important tools for a wide range of application areas in researches and industrial development due to its reliability and accuracy. IR is used in areas such as microelectronics, automotive and aerospace industries, mechanical wear testing, plant and biology researches, and materials evaluation.

In the medical field, the use of IR as a monitoring method is still meager. The use of this technology presents questioning of interference in temperature measurement due to the influence exerted by environmental conditions and the production of heat by the individual organism and interference caused by the equipment operator. Over time, various theories have been established in an attempt to neutralize such interference in the results, particularly the development of a thermographic index for IR quantification (and this method was subsequently used in several studies). Other related theories were proposed for IR quantification, such as the measurement of heat transfer rate through the correlation of normal and abnormal skin areas. Other authors have developed normalization methods to quantify IR by comparing the points of difference between normal and abnormal tissue temperatures, the thermal information of each point were compared with the surrounding areas. The method of determining the heat transfer rate used in this research has found convergence with the method used by these authors, as the analysis of temperature differences was performed.

Vargas et al. designed an important method for IR normalization by determining the dimensionless temperature. They followed for 587 days the treatment evolution of a 50-year-old male patient, with leprosy for many years and hepatitis C. The evaluated the concomitant medical treatment of both diseases. The standardized IR results showed success in the treatment of leprosy from day 87, while the skin pigmentation occurred only at day 182, subsequently confirmed by biopsy on day 390. The leprosy treatment evaluation via IR was able to determine signs of skin injury healing 95 days before the clinical possibility of seen this evolution. Similar to the present study, thermographic signs of poisoning were also observed prior to its clinical manifestations. Therefore, both studies showed convergences regarding the thermographic predictive value. The predictive identification of LA poisoning signs may enable early treatment approaches, such as oxygen administration, maintenance of airway patency, appropriate ventilatory support, and use of benzodiazepines. IR monitoring may also assist in the diagnosis of the dreaded and unexpected cases of malignant hyperthermia.

Several image processing studies have been performed. Other studies have been conducted to develop a thermographic index to increase diagnostic accuracy. The most current methods of image processing are the automated target recognition (ATR), threshold algorithms, and artificial neural networks (ANN), among others. Thermographic evaluation of thermal asymmetry can be done with image segmentation (threshold algorithms), feature extraction (ANN), and pattern recognition techniques (ATR). ATR was developed for military use in rocket launch and target location. But it also has peaceful applications, including a security system that uses radar signals to identify people.
or objects that fell on the subway tracks. The drawback of this method for IR normalization, as in LA poisoning assessment, is the requirement of a reliable. A thermogram database of cases of acute poisoning, as the images are processed based on the image database files compared to the new assessed image. This aspect may hinder its use as the sole method of identifying thermographic changed patterns. In contrast, the artificial neural networks use a set of data to produce the pattern recognition.

It is noteworthy that all these features associated with IR can contribute significantly to mitigate the technical influence exerted by equipment operators experience and improve diagnostic accuracy. Therefore, it is concluded that IR application in acute LA poisoning monitoring demonstrated its predictive value. In clinical practice, frequent IR monitoring may enable the physician the “recognition familiarity of normal thermographic image” and its “differentiation from morphologically altered images”, which can be confirmed by the results of mathematical calculations of measured temperature readings.

The development of a thermographic predictive index for LA acute poisoning is presented as a new horizon for further studies. It can enable the development of “thermal warnings” scheduled from the analysis of temperature differences by isothermal feature (lines of equal temperature) and decrease the influence of equipment handling by the operator, which facilitates practical application of infrared monitoring in surgery.

Conflicts of interest

The authors declare no conflicts of interest.

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References

10. Covino BG. One hundred years plus two of regional anesthesia: annual scientific meeting of the American Society of Regional Anesthesia; 1986. p. 11.