EXPERIMENTAL STUDY

A new experimental burn model with an infrared heater

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ABSTRACT

BACKGROUND: This study was undertaken to develop new experimental burn injury model using conventional infrared heaters.

METHODS: 21 Sprague-Dawley rats were divided into 3 groups. Portion of dorsal area was exposed to infrared radiation from distance of 50 cm to create burn injury. Length of exposure to heat for Group 1 was 5 minutes; Group 2 was exposed for 7½ minutes, and Group 3 was exposed for 10 minutes. Macroscopic and histopathological evaluations were utilized to demonstrate depth and characteristics of injury.

RESULTS: There was no burn injury in first group. Group 2 developed partial thickness burn, and result was full thickness burn injury in Group 3. In Groups 2 and 3 there was statistically significant difference in dermal collagen denaturation. Dermal injury depth was statistically significantly higher in Group 3 compared to Group 2.

CONCLUSION: New experimental burn injury model is described using conventional infrared heaters. Standard variables pertaining to model were defined to produce burn injuries at predictable depth: 10 minutes of exposure from 50 cm distance for full thickness burn, and 7½ minutes of exposure from the same distance for partial thickness injury.

Keywords: Burn; infrared heater; rat model.

INTRODUCTION

Though a better understanding of burn trauma has improved survival of burn patients in recent years, there are still many points to be researched that may lead to discovery of new treatment modalities.[1–3] Rat experimental models are most-preferred option, and various models (e.g., scalding, direct contact with heated metal, or application of electrical current or chemical agent) have been used since the 1960s.[2,4–6] However, all of these models have disadvantageous, such as optimization. Therefore, defining a reliable experimental model is still very important for research purposes.

Infrared heaters are popular, commonly available devices that transfer heat to another object through electromagnetic radiation without increasing nearby air temperature. Infrared spectrum is wavelength from 0.7 to 300 μm.[7] Heat energy is released when molecules in object with lower temperature become excited and vibrate upon contact with infrared wave. Absorption of heat released as result of exothermic reaction can be sufficient to cause burn trauma.[8,9]

The present study researched a new, practical, and standardized rat burn model using infrared heaters based on previously reported papers.[10,11] Primary target for 8.25 μm radiation emitted by heater is dermal collagen.

MATERIALS AND METHODS

Approval for the study was obtained from Gülhane Military Medicine Academy animal research ethics committee.

In order to achieve uniform burn areas, protective shied with 4x5 cm aperture in the middle was prepared and covered

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with aluminum foil to reflect heat and protect adjacent tissue. A standard, popular, and easily available model of infrared heater was selected: 220–230 v, 50-60 Hz, 2300–2500 watt heating unit with dimensions of 86x19x10 cm (UFO-L23; UFO Heating Systems, Istanbul, Turkey).

Distance between heater and rat, and length of time exposed to heat were the variables were taken into consideration to determine experimental model. A preliminary study was conducted to identify optimal distance. First rat was placed 25 cm from heater and after 5 minutes, third-degree burn injury appeared. No damage occurred at distance of 1 m, which was the minimum safe distance according to manufacturer's instruction manual, even after 30 minutes. Air temperature of area behind protective shied was measured, as it could affect burn depth at measurement over 40°C. After 5 minutes at a distance of 25 cm, surrounding area temperature was 43°C and 42.5°C at 40 cm. However, at a distance of 50 cm, it was below 40°C even after 10 minutes and observance of full thickness burn injury. Therefore, 50 cm was selected as optimal distance between heater and rats for model (Fig. 1).

21 Sprague-Dawley rats weighing between 250 and 275g were randomly divided into 3 groups of 7. Anesthesia was performed with combination 5 mg/kg ketamine, 2 mg/kg xylazine administered intraperitoneally. Following anesthesia, dorsal skin location was shaved as target area. Protective shield was positioned parallel to heater. In the first group, rats were exposed to heat from 50 cm distance for 5 minutes; second and third groups were placed at same distance for 7½ and 10 minutes, respectively (Table 1, Fig. 2).

Assessment Methods

Skin elasticity, turgor, and tonus were evaluated in all specimens by single researcher in macroscopic evaluation.

Table 1. Experiment protocol

Group	Distance (cm)	Time (min)	Number of rats (n)
Group 1	50	5	7
Group 2	50	7½	7
Group 3	50	10	7

Figure 1. The setup of burn model.

Figure 2. Group 1 (right), Group 2 (middle), and Group 3 (left) rats after thermal injury.

Rats were euthanized following macroscopic assessment with large dose of anesthetic. Full thickness tissue samples were collected, as well as 1 cm healthy tissue sample for comparison, taken from area adjacent to burn. Biopsy materials were stained with hematoxylin and eosin (H&E) and evaluated under 20x amplification light microscope by single-blind pathologist. Dermal collagen denaturation, dermal injury depth and injuries to skin appendices were evaluated semi-quantitatively using scoring system (Table 2).

Data obtained from histopathological evaluation were evaluated statistically using chi-square test; $p<0.05$ was considered significant.

Table 2. Histological scoring system

Dermal collagen denaturation	Dermal injury depth	Skin appendices injury	Histological score
None	None	None	0
Mild	Partial thickness	Exist	1
Moderate	Full thickness		2
Severe			3

RESULTS

Macroscopic assessment revealed decreased skin elasticity and reduced turgor pressure and tonus in Group 3 (Fig. 3, 4).

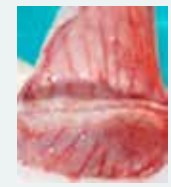
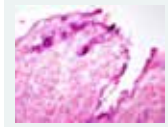
Histological examination indicated homogeneous burn injury in all parts of burn area. Although some inflammatory changes in adjacent healthy tissue were detected, they were not severe and burn margins were clear.

Only subepithelial neutrophils and edema formation were observed in first group. Mean histological score was zero for criteria of dermal collagen denaturation, dermal injury depth, and skin appendices injuries. In second group, exposed to heat for a longer period, denatured collagen bundles were seen, but limited to upper half of dermis. Although epidermal change (edema) was present in this group, there was no injury to skin appendices. Histological scores of second group were 1, 1, and 0, respectively, for the 3 criteria evaluated. In third group, denatured collagen bands were observed throughout dermis layer, as well as epithelial changes, degenerated skin extensions, and muscle edema. Group's mean histological scores were 2, 2, and 1. Histological differences indicated full thickness burn injury in Group 3 and partial thickness burn in Group 2 (Table 3).

Figure 3. Turgor pressure and skin tonus of a subject in Group 1.

Figure 4. Turgor pressure and skin tonus of a subject in Group 3.

Table 3. Macroscopic and histological pictures of all groups

Group 1 (50 cm 5 min)	Group 2 (50 cm 7 1/2 min)	Group 3 (50 cm 10 min)
No injury	Partial thickness injury	Full thickness injury
		
		

Statistically significant difference in dermal collagen denaturation ($p<0.05$) was found in the second and third groups compared to first group. Statistically significant difference ($p<0.05$) in dermal injury depth was detected between second and third groups. Skin appendices assessment revealed no injury in first 2 groups, and finding was statistically significant ($p<0.05$). These results confirmed full thickness burn injury in Group 3 and partial thickness burn injury in Group 2.

DISCUSSION

Although burn injuries are now better understood, additional research is still needed. To study burn physiopathology and to test treatment options, various experimental burn models have been developed and used, such as scalding, direct contact with heated metal, and application of electrical current or chemical agent.

The present study used a conventional infrared heater to formulate a new experimental burn model. This model is extremely easy to implement, repeatable, and yields burn injuries of consistent depth. Uniform partial thickness or full thickness burn injuries can be induced using this method.

Ideal experimental model has features such as low cost and safety for the researcher; however, the most important characteristic is standardization and replicability. Among disadvantages of burn models using heated metal plates or similar devices is difficulty of maintaining uniform pressure and precise positioning of instrument. In addition, laboratory accidents that can injure the researcher may occur with heated metal and water-related burn models.

The experimental model defined in this study offers standardized results because neither device nor specimen must

be manipulated while inducing burn. Thus, method is extremely safe way for researchers to achieve standardized burn depth compared to other methods using boiling water and contact burns. Though it does require more time to perform burn, the present model is also less traumatic for researcher.

Use of infrared heater also provides flexibility. In the present study, with 4x5 cm opening in the shield, a burn area of less then 20% of total body surface area (BSA) was created. No resuscitation was required. Change in size of opening would allow researchers to create conditions for study of numerous topics related to burn trauma, such as burn physiopathology in different burn percentages, varied treatment modalities, early treatment, or resuscitation.

In experimental studies, various animals are used as test subjects.[12–22] When defining present model, rats were selected because they are easily acquired, inexpensive, and easy to manipulate compared to larger animals. Due to need for only conventional infrared heater and rats, model offers genuinely low cost alternative for burn studies.

During the study process it was observed that epidermal structures are affected only after longer periods of exposure to heat source and primary target of infrared radiation is dermal collagen. Therefore, it is foreseen that infection would be rare with this model as epidermal layer would be partially healthy. Further studies are recommended based on these initial findings.

Even among experienced researchers, correct burn depth estimation ranges between 64 and 76%. Optimal method for estimation of burn depth is histological evaluation. H&E staining has been used to determine partial and full thickness burns, normal and denatured collagen fibers, and patent vascular structure for many years.[23] In present study, biopsy samples were embedded in paraffin blocks, samples 5 µm thick were taken, stained with H&E, and assessed by single pathologist in order to objectively determine ideal length of exposure to heat and distance between subject and device to achieve standardized burn injury.

Conclusion

In this study, a new experimental burn model using a conventional infrared heater is described. Full thickness burn was achieved by positioning rats 50 cm from infrared heater for 10 minutes, and partial thickness burn damage resulted after 7½ minutes at same distance. Histopathological changes resulting from infrared heater burn injury are described. Model is practical, replicable, yet flexible method for burn injury research.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

İnfrared ısıtıcı kullanarak oluşturulan yeni bir deneysel yanık modeli

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AMAÇ: Bu çalışma konvansiyonel infrared ısıtıcılar kullanılarak yeni bir yanık modeli tanımlanması amacıyla gerçekleştirildi.

GEREÇ VE YÖNTEM: Yirmi bir adet Sprague-Dawley sıçan üç gruba ayrıldı. Sıçanların sırt derisi zaman ve mesafe değişkenleri doğrultusunda; 50 cm 5 dakika (Grup 1), 50 cm 7.5 dakika (Grup 2) ve 50 cm 10 dakika (Grup 3) olacak şekilde infrared ısıtıcıya maruz bırakıldı. Yanığın derecesi ve karakterinin belirlenmesi amacıyla makroskobik ve histopatolojik değerlendirme yapıldı.

BULGULAR: Birinci grupta yanık oluşumu izlenmezken, ikinci grupta parsiyel kalınlıkta, üçüncü grupta ise tam kat yanık oluşumu izlendi. İki ve üçüncü gruplar arasında dermal kollajen denatürasonu açısından istatistiksel olarak anlamlı farklılıklar gözlemlendi. Dermal hasarlanma üçüncü grupta ikinci gruba kıyasla daha derin olduğu gözlemlendi.

TARTIŞMA: Konvansiyonel infrared ısıtıcılar kullanılarak yeni bir yanık modeli tanımlanırken model içerisinde standart değişkenlerde ortaya koyuldu. Buna göre 50 cm mesafeden 10 dakika infrared ısıtıcıya maruz kalınmasıyla tam kat, aynı mesafeden 7.5 dakika maruz kalınmasıyla kısmi kalınlıkta yanık modelleri oluşturuldu.

Anahtar sözcükler: İnfrared ısıtıcı; sıçan modeli; yanık.

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