Developing A Third-Degree Burn Model Of Rat Using The Delphi Method

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Abstract

Background: The animal experiment plays an essential role in advances on research and treatment of burn. Currently, researchers often rely on personal experience or the literatures to complete the construction of animal disease models, which may lead to the lack of scientific rigor and wide range of animal disease models the reference value. The purposes of this study were aimed to establish a third-degree burn model of rat using a Delphi method in China to provide a reference.

Methods: Two rounds of Delphi expert consultation survey was conducted on experts (n=15) in this study, and then used the boundary value method to screen, modify and supplement the indicators. Next, determined the index weight by analytic hierarchy process, and finally the index system of the rat model of third-degree burn was established.

Results: Statistical analysis results show that the positive coefficients of the two rounds of expert consultation are 83.33% and 88.67% respectively. The expert authority coefficient values were 0.73 and 0.67 respectively, and the expert coordination coefficient test was $P < 0.001$. According to experts' suggestion, the third-degree burn model of rats with 8 first-degree indexes, 14 second-degree indexes and 46 third-degree indexes was finally established.

Conclusions: According to the characteristics and quality requirements of my country's animal models, this study constructs a rat model of third degree burns, which is expected to expressively improve the overall proficiency of burn research quality.

Introduction

Burn is caused by heat (such as boiling fluid, hot metal, flame, steam, etc.), electricity, chemicals (such as strong acid, alkali, etc.), radiation and other causes of body damage, of which the majority of thermal burns. According to the statistics of the World Health Organization (WHO), there are up to 300000 deaths caused by burns in the world every year (1). About 27 million people suffer burns in China each year, and about 130000 people die of burns (2). Burn has become a global public health issue. Burns also lead to a heavy financial burden. Studies have shown that the cost of treating 1% of total body surface area (TBSA) burns in the intensive care unit (ICU) is as much as 4600 euros (3). In addition, burns also bring serious physiological and psychological harm to patients, social impact is extensive and huge.

Due to the limitation of medical ethics, the study of partial burns is not suitable for human body. Therefore, the animal experiment with burns is of the utmost importance. Animal experiments are important bridge linking basic research and clinical research, and animal disease models are the cornerstone of scientific rigor in animal experiment. Animal model building research has greatly improved our understanding of disease mechanisms and the progress of treatment. However, in current studies on the construction of animal disease models, researchers often rely on personal experience or other researchers' literature to complete the construction of animal disease models, which may lead to the lack of scientific rigor and wide range of animal disease models the reference value.

In the early stage of our study, we conducted a systematic review of the rat burn model construction experiment (4), and found that in the process of model construction, there are various induction methods and great differences in the current rat burn model construction experiment, and the report on the model construction process is not detailed, which has a certain impact on the overall research quality. The reference value for the follow-up research is limited. In addition, our previous study found that the number of third degree burn model construction experiments in rats was more. Therefore, this study selects the rat third degree burn model construction method as the representative, and based on the evidence-based evaluation of rat third degree burn model construction and evaluation of alternative indicators, uses Delphi method to further refine and clarify the model construction items and methods, in order to standardize the rat third degree burn model construction process and evaluation indicators.

Materials & Methods

Study Design

We used a Delphi method in this study, boundary value method to determine the index of third-degree burn model of rat. The Delphi method is defined as an practical method for group consensus consultation that can find broad applications in various fields (5–6). It synthesizes the relevant data, including literature review, ideas from stakeholders and judgement of a group of anonymous experts (7), which makes the results of the Delphi survey have high credibility (8).

Building The Preliminary Indicator Pool

We designed a systematic review before the Delphi survey to collect evaluation indicators and descriptions. The literature search was implemented in MEDLINE (Ovid), EMbase (Ovid), BIOSIS Previews (Ovid), CBM, CNKI, VIP, WanFang Data databases to collect initial tertiary indicators. The following retrieval strategy was used: (((rats/) OR ((rat OR rats OR rattus).tw.)) AND ((burns/) OR ((burn OR burns).tw.))) AND ((disease models, animal/ OR models, animal/) OR ((model OR models).tw.)). The search time period is from the establishment of the database to May 2018. Some articles tentatively ruled out for not relevant to the theme according to the keywords and abstracts, 15 articles were considered at last. After evaluation of team members, a burn model of rat with 10 primary indicators, 33 secondary indicators were preliminary formed. The primary indicators were ①depth of burn, ② methods of induction, ③ burn sites, ④ induction temperature, ⑤ induction time, ⑥ anesthesia, ⑦ skin preparation, ⑧ housing post surgery, ⑨ intervention post surgery, ⑩ assessment criteria.

Using the Delphi method to build a third-degree burn model of rat

The expert Delphi survey included three parts: basic information of a group of experts, assessment of their familiarity with model building indicators evaluation, and evaluation of the constructed index system, along with comments. The evaluation focused on the suitability and reliability of each indicator, score on a scale from 1 to 10. The familiarity and judgement of indicators were also an important part,

which score on a scale from 1 to 5 by these experts, these scores based on theoretical analysis, work experience, understanding of domestic and foreign peer, and perception.

we conducted two rounds of the Delphi survey and data analysis, the results were carried out by 15 experts via email or questionnaire survey. Explains the purpose, significance, content, requirements and precautions of the study in detail, and invites experts to evaluate and provide opinions on various indicators. The SPSS 20.0 statistical software was used to calculate the positive coefficient, authority and coordination coefficient of participants to prove the effectiveness of the two rounds of Delphi expert consultation. According to the second round of expert feedback, we calculated the effectiveness of the Delphi process, and based on discussions with experts, used the boundary value method to modify or delete the unqualified indicators. In the end, we can form the final model indicator system according to the indicators which are calculated based on the results of the questionnaire.

Data analysis

Expert positive coefficient

The positive coefficient of experts is expressed by the questionnaire recovery rate, reflecting the active input of experts; a recovery rate greater than 70% is considered good (9). A total of 18 copies were distributed in the first round of Delphi expert survey in this study, and 15 copies were recovered, with an effective recovery rate of 83.33%. Delphi experts distributed 15 copies in the second round of investigation and recovered 13 copies. The effective recovery rate was 86.67%, indicating that the positive coefficient of the two rounds of experts was good.

Expert authority coefficient

The expert authority coefficient (Cr) is the arithmetic mean of judgment coefficient (Ca) and familiarity coefficient (Cs), namely: $Cr = (Ca + Cs) / 2$, where $Cr \geq 0.7$ indicates acceptable reliability. Ca represents the evidence at the time of expert judgment, and Cs represents the expert's familiarity with the problem. (10) Ca is calculated based on expert judgment and the degree of influence of each index. In this research, experts used terms such as "practical experience", "theoretical analysis", "understanding of peers" and "insight" as judgments. The evaluation criteria (11) are shown in Table 1. Then add the evaluation criteria to the Ca of each indicator. When $Ca = 1$, the judgment has a greater influence on the expert; when $Ca = 0.5$, the influence on the expert's judgment is moderate; when $Ca = 0$, it has no obvious influence on the expert's judgment. (12) Cs refers to the expert's familiarity with the problem. This study uses a scale method to calculate the expert's familiarity with the problem from 0 to 1 (1 = very familiar, 0.8 = familiar, 0.6 = relatively familiar, 0.4 = generally familiar, 0.2 = not very familiar, 0 = unfamiliar) and calculated the average statistical familiarity of the consultant. The concentration of experts' opinions is described by M_j ,

„ $M_j = \frac{1}{m_j} \sum_{i=1}^m C_{ij}$ in the formula, means the average scores of j indicator by experts, means the number of experts participating in j indicator evaluation, means the specific score value of expert i on the importance of j indicator. Expert coordination is an important indicator for judging the consistency of indicators

between experts, including Kendall's W coordination coefficient and the coefficient of variation of each indicator. The coefficient of variation is an important basis for indicator deletion (13). Kendall's W coordination coefficient test was used to judge the degree of expert coordination, and $P < 0.05$ was considered statistically significant. The larger the Kendall's W coefficient, the higher the degree of expert coordination and the higher the consistency of expert opinions. When Kendall's W value > 0.4 , the research coordination is better, and when Kendall's W value > 0.5 , consulting work can be completed. In this study, Kendall's W test was performed on the expert coordination coefficient of each indicator.

Table 1
Judgement basis and the degree of influence

Judgment scores	Judgment criterion			
	Practical experience	Theoretical analysis	Understanding of peers	Insight
0~1	0.3	0.1	0.1	0.1
2~3	0.4	0.2	0.1	0.1
4~5	0.5	0.3	0.1	0.1

Statistical analysis

The databases were established using Excel 2016, and statistical analyses were performed using SPSS 20.0. The test indicates the statistical significance of the correlation coefficient. $P < 0.05$ was considered statistically significant.

Ethics approval

Because this is only a study on the method of animal model construction and does not involve specific animal experiment, so this study protocol was granted a waiver of ethics approval by the West China Hospital Research Ethics Board, and informed consent was waived, all methods were performed in accordance with the Declaration of Helsinki.

Results

Basic information of experts

A total of 15 experts were selected for this consultation study, including 7 clinical medical experts (46.67%) and 8 animal research experts (53.33%). There were 5 experts (33.33%) with working years less than 15 years, 8 experts (53.33%) with working years 15–30 years and 2 experts (13.33%) with working years more than 30 years (Table 2).

Table 2
Characteristics of the expert panel

Characteristic	Round1, n (%)	Round2, n (%)
Professional field		
Clinical medicine	7(46.67)	5(38.46)
Animal research	8(53.33)	8(61.54)
Experience		
Less than 15 years	5(33.33)	5(38.46)
Between 15 and 30 years	8(53.33)	6(46.15)
More than 30 years	2(13.33)	2(15.38)
Title		
Associate professor	10(66.67)	9(69.23)
Professor	5(33.33)	4(30.77)

Authority coefficient and coordination coefficient

In the two-phase Delphi process, the expert authority coefficient values were 0.73 and 0.67. In the first round, Kendall's W value was 0.435. In order to make the results more reasonable and scientific, we carried out the second round of expert consultation on the basis of the first round, and the Kendall's W value was 0.530, so the consultation work was ended.

Delphi process

In the first round of expert consultation, 15 experts evaluated the importance evaluation results of 8 primary indicators, 16 secondary indicators and 54 tertiary indicators (Table 3). According to the results of the first round of expert consultation, the boundary value method was used to analyze and modify the alternative evaluation indicators. A total of 11 indicators were deleted, including 2 secondary indicators which were local anesthesia and analgesia. Nine tertiary indicators were deleted, including more than 200°C, Ketamine, lidocaine, bupivacaine, vaccination, analgin, lappaconitine, dexmedetomidine and model construction time consumption. Add a new indicator which was ionizing radiation. There were one indicator updated, the wound was pale. After referring to experts' opinions, a third-degree burn model of rat with 8 primary indicators, 13 secondary indicators and 46 tertiary indicators was finally formed (Table 4).

Table 3
The first round of index system based on Delphi method

Primary indicators	Secondary indicators	Tertiary indicators	M_j
Methods of induction	High temperature solid	Electric scald instrument	7.80
		Hydrothermal flask	5.00
		Water bath hot steel bar	5.73
	High temperature liquid	Hot-water bath	7.33
		Water spray injury cup	5.13
		Water bath high temperature gauze	5.27
	Contact combustion	Skin application fuel	4.67
	Thermal radiation	Infrared heater	4.67
Burn sites	Back		9.07
	Abdomen		5.00
	Buttock		3.93
Induction temperature	Less than 80°C		4.93
	Between 80 and 100°C		8.40
	More than 200°C		3.40
Anesthesia	General anesthesia	Pentobarbital sodium	7.67
		Chloral hydrate	6.20
		Ketamine	4.13
		Isoflurane	5.73
		Serazine	2.87
		Uratán	3.73
		Diethyl ether	5.40
	Local anesthesia	Lidocaine	3.87
		Bupivacaine	3.27
Skin preparation	Chemical method	Barium sulfide	4.67
		Sodium sulfide	6.80
	Physical method	Razor	4.73

Primary indicators	Secondary indicators	Tertiary indicators	M_j
		Push shear	6.80
Housing post surgery	Rearing environment	Rearing temperature	8.13
		Rearing humidity	8.07
		Environmental ventilation	7.87
		Ambient light	7.20
		Rearing density	8.07
		Selection of bedding material	7.53
	Rearing food	Feed	6.73
		Drinking water	7.13
Intervention post surgery	Prevention of shock	Lactate Ringer's solution	6.87
		Hyperoxia compound sodium chloride	4.53
		Disinfection of animal living environment	7.07
	Prevention of infection	Vaccination	2.07
		Penicillin, generation I cephalosporin	5.00
		Analgin	3.40
	Analgesia	Lappaconitine	2.73
		Dexmedetomidine	2.80
Assessment criteria	Macroscopic results	Wound color	8.73
		Blister formation	7.67
		Eschar formation	8.53
	Microscopic results	Epidermis injury	7.53
		Dermal injury	8.13
		Subcutaneous injury	8.67
	Other comments	Success rate of model construction	8.47
		Model construction time consumption	7.33
		Model building resource consumption	6.87
		Mortality of rats	8.07

Primary indicators	Secondary indicators	Tertiary indicators	M_j
		Incidence of complications	7.93

Table 4
The second round of index system based on Delphi method

Primary indicators	Secondary indicators	Tertiary indicators	M_j
Methods of induction	High temperature solid	Electric scald instrument	8.08
		Hydrothermal flask	6.38
		Water bath hot steel bar	5.77
	High temperature liquid	Hot-water bath	7.38
		Water spray injury cup	5.38
		Water bath high temperature gauze	5.54
	Contact combustion	Skin application fuel	5.15
	Thermal radiation	Infrared heater	4.85
		Ionizing radiation	4.15
Burn sites	Back		9.15
	Abdomen		5.08
	Buttock		5.92
Induction temperature	Less than 80°C		8.62
	Between 80 and 100°C		4.85
Anesthesia	General anesthesia	Pentobarbital sodium	8
		Chloral hydrate	7.38
		Isoflurane	5.23
		Diethyl ether	5.46
		Uratane	4.23
		Serazine	3.08
Skin preparation	Chemical method	Barium sulfide	7.69
		Sodium sulfide	6.08
	Physical method	Razor	7.08
		Push shear	6.15
Housing post surgery	Rearing environment	Rearing temperature	8.08
		Rearing humidity	8

Primary indicators	Secondary indicators	Tertiary indicators	M_j
		Environmental ventilation	7.85
		Rearing density	7.46
		Selection of bedding material	7.85
		Ambient light	7.54
	Rearing food	Feed	7.46
		Drinking water	7.46
Intervention post surgery	Prevention of shock	Lactate Ringer's solution	7.62
		Hyperoxia compound sodium chloride	5.85
	Prevention of infection	Disinfection of animal living environment	7.08
		Penicillin, generation I cephalosporin	4.62
Assessment criteria	Macroscopic results(visual observation)	Wound was pale	8.85
		Eschar formation	8.77
		Blister formation	7.46
	Microscopic results(pathological section observation)	Subcutaneous injury	9.15
		Dermal injury	8.85
		Epidermis injury	8.85
	Other comments	Success rate of model construction	8.69
		Incidence of complications	8.23
		Mortality of rats	8.31
		Model building resource consumption	7.08

Discussion

Animal experiment is the basis of promoting human experiment, and animal model of disease is the prerequisite to ensure the authenticity and stability of animal experiment. Therefore, the construction of animal model of disease is the most basic and important link in the whole biomedical research chain. In

the web of science core collection database, "search filter of animal studies" (14–15) and "burn" were used for subject retrieval. It was found that the research on burn related animal experiments was on the rise. About 53% of the research were published from 2008 to 2018, and 28% of them were published in the past five years. It suggests that burn animal experiments have attracted more and more attention and become an important part of exploring burn treatment. However, a systematic review of heparin in the treatment of burn has shown that the quality of experimental evidence of heparin in the treatment of burn is not high, which is mainly manifested in the inconsistent experimental modeling. In the process of modeling, there are mainly the following problems in burn animal experiments, ① early studies did not adopt the random grouping method, which may lead to selective bias, ② the risk of bias of some subjective evaluation indexes may increase if the blind method is not set, ③ the sample size is not estimated, and some of the experimental samples are small, so the results may not be able to accurately infer the overall parameters, ④ most of the domestic researches lack the statement of animal ethics, ⑤ No potential conflict of interest was reported. At present, there is no research report on the construction method and standard evaluation system of burn animal model at home and abroad, and most studies only focus on the systematic review of a certain animal model (16–20).

In this study, Delphi expert consultation method was used to further refine and clarify the items and methods of model construction based on the establishment of rat third-degree burn model and evaluation of alternative indicators. After two rounds of Delphi expert consultation, 8 primary indicators, 14 secondary indicators and 46 tertiary indicators were finally included, according to the importance of the indicators. Experts' opinions were described by average scores, so the importance of each index is ranked according to the average scores.

In terms of methods of induction, the top three interventions are electric scald instrument, hot-water bath and hydrothermal flask. This may be mainly because these three kinds of equipment have better temperature control, which can provide a more stable and constant scald temperature and will not affect the formation of wound due to temperature fluctuation. In terms of burn sites, back is considered the most suitable part. The main reason is that the area of skin on the back is larger than that on buttock. In addition, the skin on the back is thicker than that on the abdomen. The sensitivity of rats to trauma is lower than that on the abdomen. Therefore, the interference factors of the experiment are reduced, which is conducive to the development of the experiment. In terms of burn induction temperature, experts believe that lower temperature is more conducive to the control of wound formation, on the other hand, the tolerance of rats is also relatively better, so as to further reduce the experimental interference factors. In terms of anesthesia and skin preparation, pentobarbital sodium and barium sulfide were considered to be a more appropriate intervention. In terms of housing post-surgery, combined with experts' opinions, it is suggested to adopt normal SPF animal room environment (barrier system), the temperature is 20–25 °C, the relative humidity is 40–70%, the ventilation frequency is 10–15 times per hour, the lighting time is 12h every day, the feed is SPF special, and the drinking water is disinfected. It is best to conduct only one experiment in each room to avoid cross infection. In terms of intervention post surgery, experts believe that the use of Lactated Ringer's solution is better in reducing postoperative shock, so as to reduce the confounding factors of the experiment and reduce the impact on the experimental results as much as

possible. In terms of model evaluation, the evaluation of experimental results should be combined with microscopic and macroscopic results, and the success rate of model construction, mortality of rats, incidence of complications and resource consumption of model construction should be described.

Therefore, it is of great significance to explore the construction methods and evaluation criteria of animal models of diseases for ensuring and promoting the strict implementation of animal experiments. The standardized animal model construction method and evaluation system can help to ensure the internal authenticity and stability of animal experiments, help peer researchers to carry out in-depth research on the basis of the same baseline, and promote the smooth and safe progress of preclinical research to clinical research. Therefore, it is particularly important to use scientific and rigorous methods to build animal burn models, so as to improve the quality and repeatability of research.

However, in the practical application of Delphi method, experts have confirmatory bias, that is, once experts form wrong opinions or hypotheses at the beginning, in the subsequent decision-making process, they often only pay attention to obtain the information that supports their original opinions or hypotheses, and ignore other information, so that the wrong opinions, expectations or hypotheses continue, the research methods need to be further studied.

Conclusions

Our study demonstrated that when constructing the third degree burn model of rats, the electric scald apparatus should be used to carry out the experiment on the back of rats under the condition of less than 80 °C. Barium sulfide should be used for skin preparation. Pentobarbital sodium should be used for anesthesia. At the same time, attention should be paid to the postoperative feeding environment of rats. Lactate Ringer's solution should be used for postoperative intervention. The success rate of model construction, mortality of rats, incidence of complications and model building resource consumption were evaluated.

Declarations

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AUTHOR CONTRIBUTIONS

FT: Developing design, literature search, manuscript writing. ZC and YZ: Developing design, literature search, manuscript writing, and analysis of results. All authors read and approved the final manuscript.

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None.

DATA AVAILABILITY STATEMENT

The dataset will be available upon request unless there are legal or ethical reasons for not doing so.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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