Intestinal tract and parenteral multi-organ sequential pathological injury caused by necrotizing enterocolitis

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Research article

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Abstract

Background: To explore the relationship between the pathological changes of colon, terminal ileum, lung, liver and kidney, and the changes of Bax, PCNA and PAF in a rat model of NEC.

Methods: 140 neonatal SD rats were randomly divided into 2 groups. NEC rat model is the experimental group. From the 1st day to the 7th day,10 rats were sampled in each group for pathological examination of colon, terminal ileum, lung, liver and kidney tissue. The levels of Bax, PCNA and PAF were investigated by immunohistochemistry.

Results: On the 1st day, the colon, terminal ileum, lung, liver and kidney showed inflammatory damage. On the 5th day, inflammatory injury was decreased. The inflammatory injury was nearly gone by the 7th day. There were differences in the time of apoptosis in intestine. In the intestine, the proliferation of weak to strong. Bax in liver and kidney showed marked apoptosis and apoptosis time increased in lung. PCNA was elevated in lung, liver and kidney, and the expression of PAF in lung and liver was increased.

Conclusions: NEC can lead to secondary injury of different degrees in colon, terminal ileum, lung, liver and kidney, and the degree and time of injury and repair were different. In general, organ repair played a leading role on the 4th day after modeling.

Background

Neonatal necrotizing enterocolitis is a serious life-threatening gastrointestinal emergency in the neonatal stage. It is one of the most destructive diseases in neonates [1]. Research on the pathogenesis and specific treatment of NEC has become an important subject in pediatrics. At present, the study of NEC mainly focuses on the etiology and pathogenesis, as well as the protection and prevention of injured intestinal organs. However, there is a lack of systematic research and understanding on the pathological and functional changes of various organs in the whole body after the occurrence of NEC. We established an animal model of NEC to study pathological changes the of colon, terminal ileum, lung, liver and kidney after induced NEC, and then detected the expression of Bax, PCNA and PAF in these organs [2].

Bax is an important apoptosis promoting gene, and its rise indicates that the cell starts the apoptosis process. PCNA plays an important role in the initiation of cell proliferation and is a good indicator of cell proliferation. PAF is a phospholipid medium induced by endotoxin and cytokines. It can improve vascular permeability, promote platelet aggregation, enhance the release of arachidonic acid and have a negative inotropic effect on the heart. In this study, the above three cytokines were detectedin SD rats. Human beings, like SD rats, produce these cytokines when they are exposed to NEC.We hope to study the pathological progress and the change rule of the outcome of NEC from three aspects of apoptosis, proliferation and inflammatory damage.

Methods

Materials

The study protocol was approved by the Medical Animal Care and Welfare Committee of Shantou University Medical College. SD rats (grade: SPF) were from the Shantou Univercity Medical College Lavoratory Animal Center. The rats were aged 6-8 weeks(weight: 205-298g), which male and female 1:2 caged. They were provided with clean drinking water and full price feed. All behaviors of sampling or causing pain to the rats were anesthetized first. The rats were killed by CO_2 suffocation after the experiment. The newborn rats born from these SD rats were used as experimental animals (weight: 6-7g) all of the rats are healthy.

One hundred and forty rats were randomly divided into an observation group and a control group,70 rats in each. Their average weight is 6.7g, and their gender is unlimited. A rat model for necrotizing enterocolitis was established by artificial feeding of dairy substitutes, hypoxia and cold stimulation (100% nitrogen hypoxia 90 seconds, 4℃ cold stimulation 10 minutes, twice a day for 3 consecutive days) [3-5]. In the observation group, the induction of NEC was initiated on the 3nd day after birth. The control group was given the number of days after birth of the corresponding control group.

Sampling

On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day after modeling, 10 animals were randomly euthanized by cervical dislocation. The abdominal cavity was opened with hemostatic forceps and surgical scissors to separate the colon, terminal ileum, lung, liver and kidney. Images were taken with digital camera (MVC-FD 91, Sony).

Pathological section preparation and HE staining

The above specimens were fixed in 10% formalin for 48 hours after rinsing with normal saline, then embedded in paraffin. Tissue sections of 4-6micron thickness were prepared and stained with HE. Criteria: after HE staining, the morphological changes of intestinal tissue were observed under light microscopy. A histological score (>2) was defined as NEC.

Immunohistochemical staining

Daily, from the 1st through 7th day after modeling, the expression of Bax, PCNA and PAF in colon, terminal ileum, lung, liver and kidney of the observation and control groups were observed. Judgment criteria: the staining reaction was observed under an optical microscope, and positive staining was defined as a brownish yellow particle deposition in the nucleus or cytoplasm. Image-Pro Plus v5.1 image analysis software was used for analysis.

Statistical Analysis

Statistical SPSS 16.0 software was used for analysis and processing. LSD test was used for comparison between groups. Dunnet'T3 test was used for uneven variance. Measurement data were expressed as

mean (+standard deviation). The difference was statistically significant with $P<0.05$.

Results

Pathological changes of colonic, ileal, lung, liver and kidney injury induced by NEC

On the 1st day after the establishment of the NEC model, we observed intestinal villi falling off, structure disappearance caused by necrosis, submucosal and muscular edema, intestinal wall congestion, hemorrhage, and necrosis accompanied by infiltration by many inflammatory cells, mainly neutrophils. Histological scores centered at 3 and 4 points on Day 1, but decreased to 2 points by the 3rd to 5th day, and became <1 on the 7th day.

In the observation group, on the first day after modeling, pulmonary epithelium, pulmonary interstitial and renal interstitial edema was accompanied with inflammatory cell infiltration, and inflammatory exudates were seen in the alveolar cavities and bronchi, vacuolar degeneration of hepatocytes, infiltration of inflammatory cells around necrotic foci, ischemic changes of glomeruli and obvious edema of the proximal convoluted tubule cells. On the third day, alveolar walls continued to thicken, interstitial edema was obvious, vacuolar degeneration of the liver was alleviated, necrosis of the liver was reduced, the cytoplasm was still loose, glomerular congestion was obvious, and tubular cells were still edematous. On the fifth day, pulmonary edema and interstitial thickening were significantly alleviated, and by the 7th day, alveolar inflammatory exudation and absorption were more obvious, hepatic inflammatory cell infiltration was reduced, and glomerular congestion and tubular edema were significantly alleviated.

Bax expression in colonic, ileal, lung, liver and kidney

Bax was expressed in intestinal villi epithelial cells, bronchial epithelial cells, inflammatory cells in the pulmonary interstitium and the alveolar area, hepatocytes, renal corpuscles, tubules and medulla. The expression of Bax in the intestinal tract of the NEC group was stronger than that in the control group. Bax expression gradually decreased with time and reached a minimum on the 7th day. The expression of Bax in lung, liver and kidney in the observation group was higher than that in the control group ($P<0.05$). Among them, the expression of Bax in the lung of the observation group showed a gradually increasing trend to a higher level up to the 5th day after modeling, and then gradually decreased on the 1st day, and then stabilized after the 5th day. In the kidney, decreased gradually from the first day to the third day, then tended to be stable (Figure 1).

PCNA expression in colonic, ileal, lung, liver and kidney

PCNA is expressed in intestinal epithelial cells, lung epithelium, hepatocytes, interstitial lung, renal cortex and tubular nucleus of the renal corpuscle. In the NEC group, the expression of PCNA on the 1st day after modeling was lower than that of control group. Expression gradually increased to the 4th and 5th day. Quantitative analysis showed that the expression of PCNA in lung, liver and kidney in the observation group was higher than that in the control group ($P<0.05$), except for the lower expression of PCNA in liver

on Day 1. The expression of PCNA in the lungs of the observation group was higher than that of the normal control group on the 1st day after modeling, and gradually increased to the 3rd day and then decreased to the 7th day. The expression in liver gradually increased up to Day 3 and remained stable. The expression increased gradually in the kidney (Figure 2).

PAF expression in colonic, ileal, lung, liver and kidney

The expression of PAF was the same as that of Bax. In the NEC group, expression of PAF was higher than that of control group on the 1st day, then increased gradually up to the 4th day, after which expression began to decrease and approached normal levels by the 7th day. In the observation group, the expression of PAF gradually increased to the maximum at the 4th day after lung modeling, and then gradually decreased. In the liver, it increased gradually from the 2nd day to the 4th day, and then decreased gradually. However, 1-7 days after termination of NEC induction, its expression in the kidney was stable. Quantitative analysis showed that the expression of PAF in the lung and liver of the observation group was higher than that of the control group ($P< 0.05$), but there was no significant difference in the expression of PAF in the kidney ($P> 0.05$) (Figure 3).

Discussion

NEC model establishment and evaluation

Studies have confirmed that the cause of NEC is multifactorial. In recent years, researchers began to try to establish animal models of NEC by multifactorial combination [6]. In this study, 3-day-old rat pups were used as subjects to establish NEC through artificial feeding, hypoxia and cold stimulation. The results showed that NEC of neonatal SD rats causes obvious pathological changes that are in agreement with the diagnostic criteria of NEC. The NEC rats are consistent with the pathological changes and clinical manifestations of human neonatal NEC [7]. It can therefore be used as a model for NEC.

Time-series pathological changes of multiple organ injury induced by NEC

SIRS refers to a kind of uncontrolled systemic inflammatory response caused by various severe infections and non-infectious factors. Further development of SIRS can cause MODS [8]. The experimental results showed that the organs of the newborn rats in the observation group are congested and swollen to varying degrees. On the 1st day after termination of the 3-day NEC induction, inflammatory changes were observed in the intestine, lung, liver and kidney. Unger et al. showed that intestinal flora imbalance can lead to NEC, which leads to SIRS [9]. Therefore, this experiment further confirmed that NEC may lead to an increase in the incidence of MODS.

Significance of apoptosis, proliferation and inflammatory factors in multi-organ in NEC

After induction of NEC, the pathological changes in the intestinal tract, lung, liver and kidney, inflammatory response and repair after injury are accompanied by changes of cell proliferation, apoptosis and inflammatory factors [10]. This may be due to extensive necrosis of typical intestinal

tissue at the end of NEC [11]. Apoptosis may be the main mode of death of intestinal epithelial cells in NEC [12]. In the liver and kidney, apoptotic cells induced by secondary injury, caused by inflammatory mediators after NEC, showed different trends as their different reactions. In the lung, the duration of apoptosis may be longer because the lung itself is greatly affected by cold stimulation.

At present, PCNA has been widely used in the study of cell proliferation kinetics [13]. In this study, we found that lung, liver and kidney cells proliferated to repair their corresponding organs after injury, but the duration of proliferation and degree of each organ were also different due to their differing abilities to self-repair and mechanism of each organ. In addition, in this study, the intestinal injury was in the positive repair stage only after the 4th day after the model has been established.

In recent years, studies have confirmed that PAF plays a key role in the inflammatory chain reaction of NEC [14]. In this study, the expression of PAF in lung and liver of the observation group was significantly higher than that of the control group, but there was no change of PAF in the kidney. This may be related to the production of PAF in the kidney and the metabolic level of endogenous PAF.Human beings, like SD rats, produce these cytokines when they are exposed to NEC.Because the above three cytokines are produced in both human and SD rats when NEC occurs, the animal research may be transferred to other species including human.

Conclusion

In summary, NEC can cause secondary injuries to the colon, ileum, lung, liver and kidney, and the degree and time of injury repair differ. In general, organ repair plays a leading role on the 4th day after modeling.

Abbreviations

Bax: Bcl2-related X gene; PCNA: Proliferating cell nuclear antigen; PAF: Platelet-activating factor; NEC: Necrotizing enterocolitis; SD:Sprague-dawley; SIRS: Systemic inflammatory response syndrome; MODS: Multiple organ dysfunction syndrome.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Medical Animal Care and Welfare Committee of Shantou University Medical College (Approval number SUMC2019-363).We obtained written informed consent to use the animals in our study from the Shantou Univercity Medical College Lavoratory Animal Center.

Consent for publication

Not Applicable.

Availability of data and materials

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GHW provide the research idea and organize the implementation. FSW and MLY write and revise the manuscript. WZL count and analyze experimental data, KH and CBX built models and experiment.All authors have read and approved the manuscript

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References

1. Caplan MS, Fanaroff Necrotizing: A historical perspective. SeminPerinatol. 2016;41:2-6.
2. Tanner SM, Berryhill TF, Ellenburg JL, Jilling T, Cleveland DS, Lorenz RG, et al. Pathogenesis of necrotizing enterocolitis: modeling the innate immune response. Am J Pathol. 2015;185: 4-16.
3. Castilho A, Vershinin A, Heslopharrison JS. Role of Asphyxia and Feeding in a Neonatal Rat Model of Necrotizing Enterocolitis. PediatrPathol Mol M. 1994;14:1017-28.
4. Gomes RO, Neto AR, Neto GJ, de Freitas J, Nunes AP, Montero EFDS, et al. Neonatal necrotizing enterocolitis rat model attenuated by a remote ischemic preconditioning in the pregnant. Acta Cir Bras. 2017;32:236.
5. Sodhi C, Richardson W, Gribar S, Hackam DJ. The development of animal models for the study of necrotizing enterocolitis. Dis Mod Mech. 2008;1:94-8.
6. Samuels N, Van dGRA, De Jonge RCJ, Reiss IK, Vermeulen MJ. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. BMC Pediatr. 2017;17:105.

7. Zani A, Cordischi L, Cananzi M, De Copp P, Smith VV, Eaton S, et al. Assessment of a neonatal rat model of necrotizing enterocolitis. Eur J Pediatr Surg. 2008;18:423-6.
8. Taniguchi LU, Pires E, Vieira Jr JM, de Azevedo LCP. Systemic inflammatory response syndrome criteria and the prediction of hospital mortality in critically ill patients: a retrospective cohort study. Rev Bras Ter Intensiva. 2017;29:317-24.
9. Unger S, Stintzi A, Shah P, Mack D, O'Connor DL. Gut Microbiota of the Very Low Birth Weight Infant. Pediatr Res. 2015;77:205.
10. Ballance WA, Dahms BB, Shenker N, Kliegman RM. Pathology of neonatal necrotizing enterocolitis: a ten-year experience. J Pediatr. 1990; 117:S6-S13.
11. Lu P, Sodhi CP, Jia H, Shaffiey S, Good M, Branca MF, et al. Animal models of gastrointestinal and liver diseases. Animal models of necrotizing enterocolitis: pathophysiology, translational relevance, and challenges. Am J Physiol-Gastr L. 2014;306:917-28.
12. Hackam D, Caplan M. Necrotizing enterocolitis: pathophysiology from a historical context. SeminPediatr Surg. 2018;27: 11-8.
13. Trasviña-Arenas CH, Cardona-Felix CS, Azuara-Liceaga E, Díaz-Quezada C, Brieba LG. Proliferating cell nuclear antigen restores the enzymatic activity of a DNA ligase I deficient in DNA binding. FEBS Open Bio. 2017;7: 659-74.
14. Ewer AK. Role of platelet-activating factor in the pathophysiology of necrotizing enterocolitis. Acta Paediatr. 2002;91: 2-5.

Figures

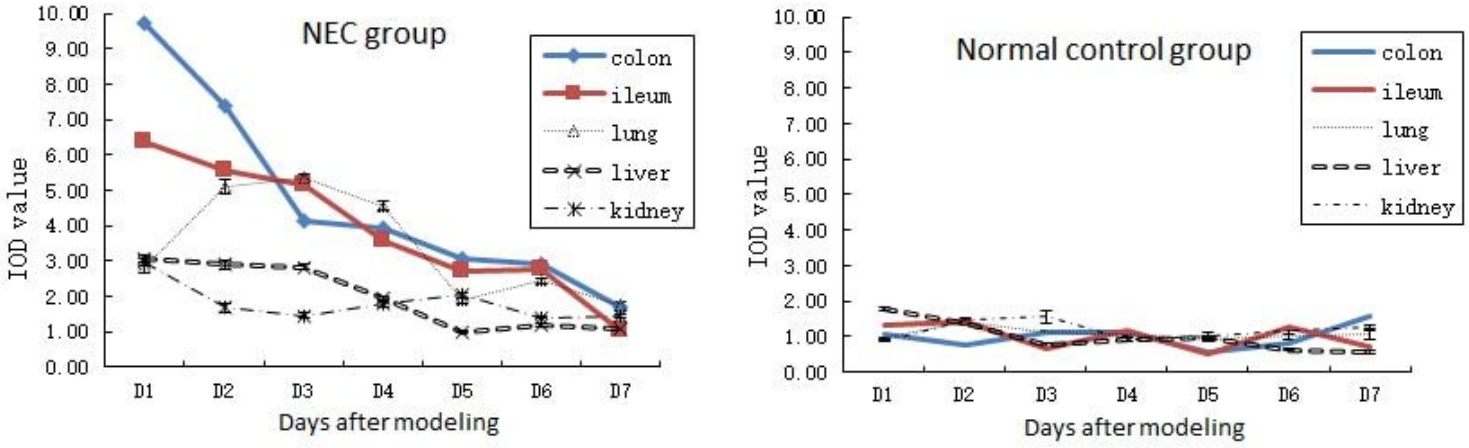

Figure 1

Expression of BCL2-related X gene in the necrotizing enterocolitis and normal control groups.

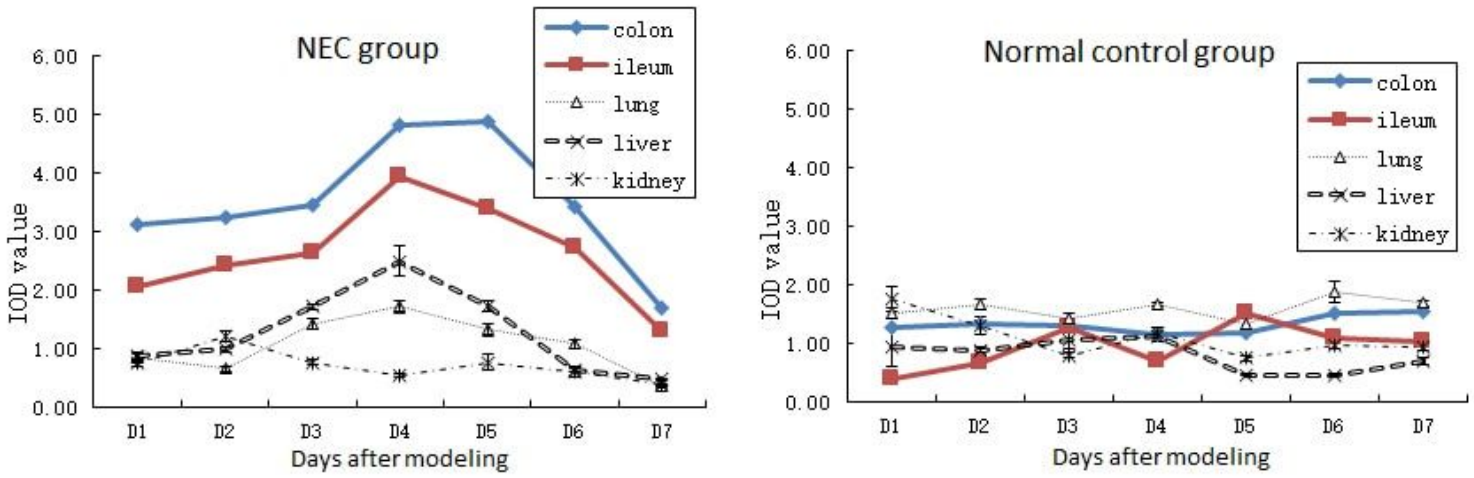

Figure 2

Expression of proliferating cell nuclear antigen in the necrotizing enterocolitis and normal control groups.

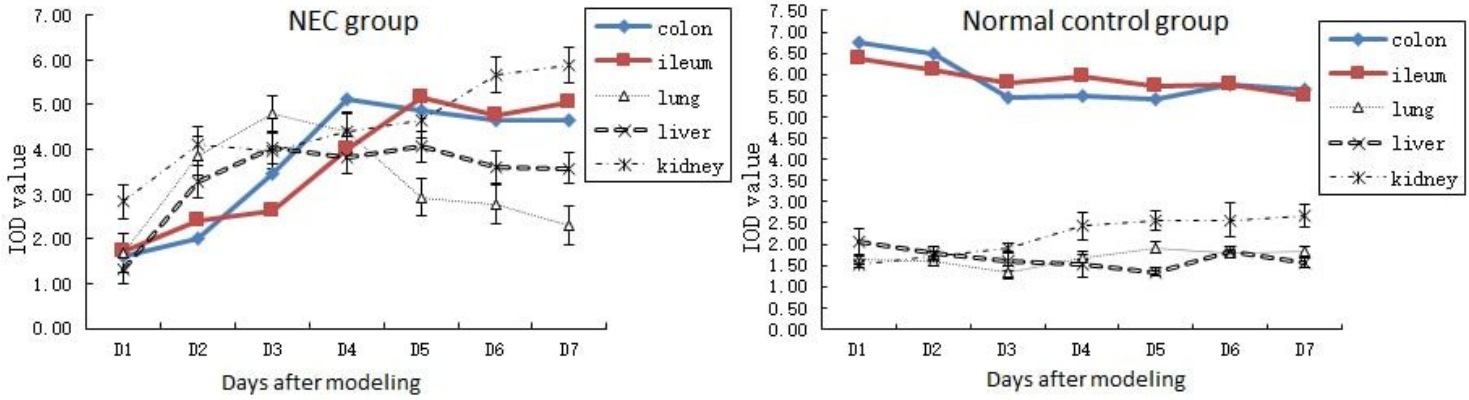

Figure 3

Expression of platelet-activating factor in the necrotizing enterocolitis and normal control groups.