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Protective effects of dexpanthenol in an experimental model of necrotizing enterocolitis☆,☆☆

Ahmet Karadag [a], Ramazan Ozdemir [a,*], Ahmet Kurt [b], Hakan Parlakpinar [c], Alaadin Polat [d], Nigar Vardi [e], Elif Taslidere [e], Abdurrahman Karaman [f]

[a] Division of Neonatology, Department of Pediatrics, Inonu University School of Medicine, Malatya, Turkey
[b] Department of Pediatrics, Inonu University School of Medicine, Malatya, Turkey
[c] Department of Pharmacology, Inonu University School of Medicine, Malatya, Turkey
[d] Department of Physiology, Inonu University School of Medicine, Malatya, Turkey
[e] Department of Histology and Embryology, Inonu University School of Medicine, Malatya, Turkey
[f] Department of Pediatric Surgery, Inonu University School of Medicine, Malatya, Turkey

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ABSTRACT

Background/purpose: In pathogenesis of necrotizing enterocolitis (NEC), both oxidative stress and inflammation are considerable risk factors. The study was designed to evaluate whether administration of dexpanthenol (Dxp) is able to attenuate intestinal injury through the antioxidant and antiinflammatory mechanisms in a neonatal rat model of NEC.

Methods: Forty newborn pups divided into four groups were included in the study: control, control + Dxp, NEC, and NEC + Dxp. NEC was induced by hyperosmolar formula and additionally the pups were exposed to hypoxia/hyperoxia and cold stress. They were sacrificed on postnatal day four, and their intestinal tissues were analyzed biochemically and histopathologically.

Results: Dxp caused a significant decrease in intestinal damage as determined by the histological score, villus height and number of goblet cells in NEC groups ($p < 0.0001$). Tissue malondialdehyde, total oxidant status, and oxidative stress indexes levels were higher in the NEC group than in the control and control + Dxp groups ($p < 0.001$). These values were reduced in the pups treated with Dxp ($p \leq 0.004$). Superoxide dismutase, glutathione peroxidase, and reduced glutathione activities were significantly reduced in the NEC group compared to the others ($p < 0.005$). Treatment with Dxp significantly reduced elevations in tissue homogenate levels of tumor necrosis factor-α and interleukin-1β in the NEC + Dxp group ($p = 0.002$ and $p = 0.01$, respectively).

Conclusions: Dexpanthenol seems to have antiinflammatory and antioxidant properties. Prophylaxis with Dxp has a potential to reduce the severity of intestinal damage in NEC in the animals.

Necrotizing enterocolitis (NEC) remains a major cause of morbidity and mortality among premature infants born at less than 1500 g. Recent data suggest 7% of these infants develop NEC, and 20–30% of them do not survive [1]. Despite many advances in the management of the critically ill neonates, the exact etiology, attempts for the prevention and the best treatment strategy for NEC have been elusive [2].

The pathogenesis of NEC is multifactorial, and was poorly understood. The premium theory in demand is local intestinal inflammation initiated by perinatal stress. Intestinal bacteria and their products adhere to the epithelium, breach the immature and fragile intestinal mucosal barrier, and activate nuclear factor-κB in lamina propria immunocytes, causing them to secrete proinflammatory mediators and cytokines, chemokines, platelet-activating factor, and nitric oxide. Inflammatory mediators including tumor necrosis factor-α (TNF-α), and interleukins (IL-6, IL-8, IL-10, IL-12, and IL-18) were also proposed to have significant roles in the pathogenesis [3]. TNF-α, secreted predominantly by polymorphonuclear neutrophils, is a proinflammatory cytokine to induce apoptosis. The pivotal role of TNF-α in the pathogenesis of NEC has been well documented [4]. Interleukin-1β (IL-1β) is another well-recognized proinflammatory cytokine, and several studies have shown that it is associated with immune activation in NEC [5]. On the other hand, reactive oxygen species (ROS) have also been reported to play an important role in NEC pathogenesis [6,7]. Miller et al. demonstrated that ROS make a substantial contribution to intestinal injury in an experimental model of NEC, and that this injury was eliminated by the addition of superoxide dismutase (SOD) [8]. Recently, we also demonstrated that *all*-trans-retinoic acid, a derivate of vitamin A, and etanercept (a TNF-α inhibitor) therapy reduce the severity of NEC in

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* Corresponding author at: Division of Neonatology, Department of Pediatrics, Inonu University School of Medicine, Turgut Ozal Medical Center, 44280, Malatya, Turkey. Tel.: +90 422 3410660; fax: +90 422 3410736.
E-mail address: ramazanoz@yahoo.com.tr (R. Ozdemir).

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pups via their antiinflammatory and antioxidant properties [9,10]. The use of several antiinflammatory and antioxidants reduces the NEC severity in experimental models that were shown, as well [11,12]. Thus, we have assumed that preventing the generation of free radicals and/or inflammatory mediators, or neutralization of these factors might have a reducing effect on the severity of NEC.

Dexpanthenol (Dxp), an alcoholic analogue of pantothenic acid (PA), is oxidized to PA within tissues, which is known to protect against cell damage produced by ROS. PA that supports cellular antioxidant systems, including glutathione, glutathione peroxidase (GPx), catalase (CAT), SOD, and the other enzymatic reactions that prepare the host to encounter physiopathological conditions mediated by ROS, is well documented. PA and its derivatives increase the level of reduced glutathione, coenzyme-A and adenosine-5′-triphosphate synthesis within the cell [13–15]. All of them play a major role in cellular defense and in the repair systems against oxidative stress and the inflammatory response [16,17].

Given these characteristics of Dxp, using Dxp that might prevent or reduce inflammatory responses is suggested. Therefore, in this histopathological and biochemical study it was aimed to evaluate whether Dxp administration could attenuate intestinal injury, and prevent NEC in a neonatal rat model.

1. Materials and methods

1.1. Animals and experimental design

After the ethics committee approval, the present study was performed at the Animal Laboratory of Inonu University, School of Medicine, according to the Guidelines for the Care and Use of Laboratory Animals of the US National Institutes of Health (Washington, DC).

The pregnant rats were kept in identical cages, and were left to feed with regular laboratory chow and water. Forty newborn pups from Wistar-albino pregnant time-mated rats were divided randomly into four groups in the first day of life. Group 1 (n = 10) was assigned as control which were only nursed by their mothers; Group 2 (n = 10) was also nursed by their mothers, but received Dxp. Group 3 (n = 10) was subjected to NEC procedure, and treated with intraperitoneal physiological saline. Group 4 (n = 10) was also assigned to NEC procedure and treated with Dxp. To prevent from protective effect of mother's milk, Groups 3 and 4 were immediately separated from the mothers, and kept at 37 °C in a humidified incubator. These animals were fed orally using a plastic sheath of 24-gauge polyflon Venocath with 0.2 mL of special rodent formula prepared with 15 g Similac 60/40 (Ross Pediatrics, Columbus, Ohio, USA) and 75 mL of puppy-canine milk replacement (Beaphar BV, Raalte, Netherlands).

1.2. NEC procedure and Dxp application

Hypoxia was accomplished by placing the pups in an airtight plexiglass chamber that was perfused with 100% CO_2 for 10 min. At the end of this period, the animals became cyanotic and began gasping. After the hypoxia procedure, the animals were exposed to +4 °C cold for 5 min, and 97% O_2 for 5 min twice a day to induce NEC. The procedure was applied to the pups for three days [9,11]. The pups were weighed daily.

NEC-induced pups in Group 3 were given physiologic saline (0.2 mL) by intraperitoneal (IP) injection. Those in Groups 2 and 4 were treated with Dxp (Bepanthene ampul®, 500 mg, Bayer Corp., Istanbul, Turkey) and were administered once a day by IP injection at a dose of 500 mg/kg of body weight, starting from birth until postnatal day four. The dosage of Dxp was chosen based on the previous studies which have been reported to cause marked antioxidative effects in rats [18,19].

1.3. Tissue preparation

All rats were sacrificed on the 4th day of experiment. For each rat, the abdomen was opened, and the intestines were inspected for macroscopic evidence of NEC such as intestinal discoloration, edema, fragility, weakness of tissue integrity, ileal distension, intestinal hemorrhage, pneumatosis intestinalis, perforation, and necrosis. Three centimeters of the terminal ileum, including the caecum, was harvested for biochemical and histological evaluations. Tissue specimens were flushed with cold saline solution, and half of the distal intestine was fixed in 10% buffered formalin for histological evaluation. The other half was frozen in liquid nitrogen, and then stored at −80 °C for biochemical examination.

1.4. Biochemical analysis

On the day of analysis, phosphate buffer (pH 7.4) was added to the frozen tissues, which shortly after were homogenized on an ice cube using a homogenizer in order the mechanical process and the heat not to contribute to the oxidation of the tissue. The supernatant was used for the entire assay. The protein content of tissue homogenates was determined as described by Lowry et al. [20] by means of the standard bovine serum albumin. The malondialdehyde (MDA, one of the specific markers and end product of lipid peroxidation [LPO]) concentrations of the homogenates were determined spectrophotometrically [21] by determination of the presence of thiobarbituric acid reactive substances (TBARS). The amount of lipid peroxides was calculated as TBARS of LPO. Another oxidation marker, SOD activity, was assayed using the nitroblue tetrazolium method of Sun et al. [22]. GPx activity was assayed using the method described by Paglia and Valentine [23]. The GSH content in kidney tissue as nonprotein sulfhydryls was analyzed following a previously described method [24]. Tissue total antioxidant status (TAS), total oxidant status (TOS), and oxidative stability index (OSI) markers were assayed as described previously [25] using commercial assay kits (Rel Assay Diagnostics, Gaziantep, Turkey). Intestinal tissue levels of TNF-α and IL-1β were analyzed in duplicate with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China) according to the manufacturer's instructions.

1.5. Histological evaluation

Intestinal tissues were fixed in 10% formalin, and were embedded in paraffin. Sections of tissue were cut at 5 μm, mounted on slides, stained with hematoxylin-eosin (HE) and periodic acid Schiff (PAS). The sections were examined by a Leica DFC 280 light microscope. Intestinal injury was classified as follows: Grade 0, no injury; Grade 1, for swelling of the surface epithelial cell less than 25% of total villous; Grade 2, for swelling of the surface epithelial cell 25–75% of total villous; Grade 3, for swelling of the surface epithelial cell more than 75% and Grade 4, for loss of villi. The median height of the intestinal villi was measured from 100 villi per each of the groups. Goblet cells were counted under 40× objective magnification using Leica Q Win Image Analysis System (Leica Micros Imaging Solution Ltd. Cambridge, UK).

1.6. Statistical analysis

Statistical analysis was carried out using SPSS® for Microsoft Windows. Data are expressed as medians (minimum to maximum). We analyzed differences among groups using the Kruskal-Wallis test. Posthoc comparisons among groups with significant values were evaluated with the Bonferroni-corrected Mann-Whitney U tests. Statistical significance was defined as $p < 0.05$.

2. Results

One rat per NEC and NEC + Dxp groups died throughout the study.