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NECROTIZING ENTEROCOLITIS OF INFANTS – NEW TREATMENT POSSIBILITIES

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Review article

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SUMMARY. Necrotizing enterocolitis (NEC) of infants is a devastating disease that is both difficult to treat and to effectively predict its development. Its occurrence and progression are associated with a plethora of possible causes. That is why, in recent years, many studies were made with the intent of finding efficient new ways of treatment and prevention. Some of those were designed to study and isolate specific factors found in maternal milk and others were more focused on testing synthetic molecules or natural remedies. The etiology of NEC and its epidemiology, followed by a characterization of the pathology and pathogenesis, as well as symptomatology, clinical, radiological, and biochemical methods of detection.

New possible treatment methods and techniques are briefly described: insulin-like growth factor, human milk oligosaccharides, epidermal growth factor family, transforming growth factor β , an enzyme – alkaline phosphatase, vitamin A, trefoil factor family, branch chain fatty acids, erythropoietin, oxygen, and ozone. Particular emphasis is given to the role of maternal milk as a source of many of biochemical factors in prevention and even treatment of NEC. Further investigations are needed to decrease the incidence and severity as well as to improve the chances of NEC patients.

Introduction to NEC

Necrotizing enterocolitis is an acute inflammatory intestinal disease that afflicts infants and causes necrosis of the intestinal wall and may lead to perforation, sepsis, systemic organ failure. It is one of the leading causes of mortality and morbidity in premature infants.

Etiology is yet unclear with many factors being associated with NEC development. The most prominent ones are prematurity and low birth weight in relation to it, childbirth complications, and excessive enteral feeding. Among others, we can find respiratory illnesses, heart malformations, gut microbiome changes, caesarian section delivery, maternal diabetes, excessive antibiotic usage, chorioamnionitis, preeclampsia, prenatal exposure to indomethacin and the use of said medication for ductus arteriosus (Botalli) closure and many more.

As much as 90% of NEC patients are premature babies. The risk grows with shorter pregnancy and lower birthweight with 12% of very low birth weight (VLBW) infants becoming ill and roughly 30% of patients succumb to the disease. It is also a major driver of health-care costs with up to 19% infant care funds being used for its treatment.

Necrotizing enterocolitis primarily affects the large intestine, but it may also affect the small intestine or a combination of both. It mostly causes skip lesion rather than damaging the bowels in continuity. It can result in lesions of various thickness – ranging from epithelial damage to perforation. NEC is accompanied by intestinal edema, intraluminal bleeding, and interstitial pneumatosis – accumulation of gas bubbles in the intestinal wall. Many patients with full thickness lesions develop adhesion which can inhibit normal intestinal function and can lead to further complications.

Pathogenesis is complicated by many elements coming into play at the same time and exacerbating each other. The main factors are ischemia, which is suspected to be caused by an underdeveloped intestinal blood supply or an autonomous nervous system dysfunction, and pathological bacterial colonization. An increased susceptibility to bacterial damage might be the result of the underdeveloped intestinal selective barrier. In combination, these factors cause lesions that increase permeability and in term promote a more suitable environment for bacteria to thrive and that leads to worsening of ischemia.

The illness presents itself with a plethora of symptoms ranging from feeding intolerance, stagnation in weight gain or even in fact a weight loss, vomiting, fever, distended abdomen and bloody diarrhea. Later on as dehydration sets in the infant becomes hypotonic, tachycardic, exhibits abnormal breathing patterns as a result of acidosis, and finally loses consciousness.

It is diagnosed through a combination of clinical methods, i.e. paying special attention to prematurely born children and having an expectative attitude towards possible symptoms arising, radiological methods and blood tests. Significant X-ray signs of NEC are distended intestines and air-fluid levels that indicate ileus, and the presence of gas bubbles in the intestinal wall. Later perforation may lead to an accumulation of air under the diaphragm that is best visible in an upright stance. Another important part of diagnosing is complete and differential bloodwork, biochemical and microbiological tests. A decrease in red blood cells indicates bleeding. Increase in neutrophil levels in correlation with increased CRP and SE levels indicates the presence of inflammation and infection and a severe decrease in neutrophil numbers is also associated with a

worsened outcome. Thrombocyte levels in relation to coagulation parameters indicate susceptibility to bleeding. Acid-base status and ion concentrations are an important measure of hydration. Microbiological tests are necessary as NEC is often associated with bacteremia and sepsis. The severity of NEC is determined by Bell's criteria.

Table 1. The severity of NEC is determined by Bell's criteria

Modified Bell's Stages	Clinical signs	Radiographical signs	Gastrointestinal signs
1	Apnea, bradycardia, and temperature variations	Normal gas pattern or mild signs of ileus	Mild abdominal distention, occult blood in the stool, gastric residuals
2a	Apnea, bradycardia, and temperature variations	Ileus with dilated bowel loops and focal pneumatosis	Moderate distention, hematochezia, absent bowel sounds
2b	Metabolic acidosis and thrombocytopenia	Widespread pneumatosis, gas in the portal vein, ascites	Abdominal tenderness and edema
3a	Mixed acidosis, coagulopathy, hypotension, oliguria	Severely dilated bowel loops, ascites, no free air	Abdominal wall edema, erythema, and induration
3b	Shock, deteriorating vital signs and laboratory values	Pneumo-peritoneum	Perforated bowels

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The treatment is proportional to disease severity and it includes non-surgical and surgical methods – in the most severe cases. Traditionally, conservative treatment is limited to supportive therapy which includes a cease in enteral food intake and intestinal decompression which is achieved through a nasogastric probe. Sufficient nutrient intake is accomplished by parenteral feeding in which a central venous line is inserted in the upper hollow vein through the subclavian vein. Dehydration and blood pH levels are corrected by administering saline and sodium bicarbonate solution. Application of various blood derivatives might be necessary to mitigate the loss of blood or specific coagulation factors, but they also might increase the likelihood of negative health outcomes. Antibiotics are administered to prevent the development of infections and to treat them when they do occur, but they also damage the natural, protective intestinal flora which in turn makes the intestines more vulnerable. Finally, in severe cases it might be unavoidable to perform a surgical procedure to remove the devitalized tissue. Needless to say, an operation is hard to bare for infants and it carries with it the risk of long lasting complications for digestion. This paper examines novel methods that would directly increase the fortitude of the intestinal wall and also increase its ability to recuperate so as to avoid the more aggressive forms of treatment that we have at our dis-

posal today. These new treatment options could also have a positive effect on weight gain and overall well-being.

New treatment methods for NEC of infants

Insulin-like growth factor 1 (IGF1)

Insulin-like growth factor 1 is a peptide hormone made up of four domains A, B, C, and D. It is mainly produced in the liver, but IGF 1 can be found in cells throughout the body including osteoblasts, skeletal muscle cells, renal cells, etc. The main stimulus for its production comes from human growth hormone, although insulin, nutritional status, and other factors influence its production. It is one of the main anabolic hormones in the body. It can also be found in human milk and a positive correlation between high concentrations of IGF 1 and faster velocity of infant growth has been observed (1). On the other hand, low serum levels of IGF 1 in premature infants play a role in a multitude of illnesses such as NEC, bronchopulmonary dysplasia, intraventricular hemorrhage, and they can stifle retinal and cerebral development (2). It was demonstrated in animal studies that intraperitoneal injections of IGF 1 can reduce intestinal apoptosis and necrosis as a result of hypoxia (3) and that enteral intake can reduce inflammation, incidence of NEC and its severity (4).

Human milk oligosaccharides (HMOs)

Human milk oligosaccharides are a group of roughly 200 oligosaccharides made up of two dozen different monosaccharides. The number of them in breastmilk varies from mother to mother, ranging from 23 to 130. Most of them are utilized as prebiotics for the intestinal flora as they are not digestible by infants. It was determined that a mixture of short chain galacto-oligosaccharides/long-chain fructo-oligosaccharides significantly reduces the incidence of NEC (5). In an attempt to single out specific oligosaccharides responsible additional *in vitro* studies were undertaken. They have shown that certain HMOs, namely 3'-sialyl-lactose and 3'-sialyl-3-fucosyl-lactose can reduce adhesion of leukocytes to endothelial cells which may provide anti-inflammatory properties (6). Furthermore, it was demonstrated that a specific oligosaccharide – disialyllacto-N-tetraose (DSNT) can reduce NEC incidence both in rats and humans (7), and that DSNT might also be used as a non-invasive marker for NEC prone infants (8). It was also shown, in an animal model, that 2'-fucosyllactose has a protective effect, although not as pronounced as DSNT, while galacto-oligosaccharides provide no apparent benefit (9).

Some studies show opposite effects, with one study demonstrating that a combination of short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides and acidic oligosaccharides does not reduce intestinal permeability (10). In addition to that, a very recent animal study has shown that even though HMOs reduce inflammation they do not change NEC incidence and

may, in fact, increase the likelihood of dehydration and diarrhea (11).

Epidermal growth factor family

EGF-family is a group of peptide growth factors consisting of epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor- α (TGF α), amphiregulin, betacellulin, epiregulin, epigen and the four neuregulins. They all share a similar structure and function and function as ligands for the epidermal growth factor receptor family. EGFR family consists of four members: ErbB1 (HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). From a perspective of pediatrics, the two most well studied and interesting factors in that group are EGF and HB-EGF. Both of them can be found in human milk, with EGF being present at 2 to 3 times higher concentrations. It was also determined that the concentration of EGF does not change with regard to the length of the pregnancy. On the other hand, it was shown that levels of salivary EGF vary with gestational age, being lower in prematurely born infants, and that lesser concentrations strongly correlate to the risk of NEC development (12). Furthermore, animal studies have demonstrated that enteral application of EGF reduces both the risk of NEC development and its severity (13). Those effects might be explained, in part, by the fact that EGF was demonstrated in an animal model to reduce the levels of proinflammatory cytokines, mainly IL 18, and increases anti-inflammatory ones like IL 10 (14). Other animal studies have pointed out its antiapoptotic effect that is achieved through reducing the activity of caspase-3 and Bax proteins and increasing Bcl-2 expression (15). In addition to that, it was also discovered, on a rat model, that EGF protects the integrity of the intestinal barrier through decreasing intestinal paracellular permeability, normalizing expression of two major tight junction proteins, occludin and claudin-3, and increasing goblet cell density and mucin production, in the ileum (16).

HB-EGF was proven through numerous genetic studies to be an effective tool in fighting against NEC. One study with HB-EGF ((-/-)) knockout (KO) mice demonstrated a higher susceptibility to NEC development that was mitigated with enteral HB-EGF supplementation (17). Another study used an opposite approach in which transgenic mice with an overexpressed HB-EGF were exposed to an experimental NEC model. They showed a drastic decrease in NEC incidence and intestinal permeability (18). HB-EGF achieves its effect through several pathways. One of them seems to be that it significantly increases intestinal microvascular blood flow. Another way is its cytoprotective effect on intestinal cells exposed to hypoxia, and the ability to expedite proliferation and recovery. HB-EGF was also proven to reduce the expression of adhesion molecules such as platelet and endothelial selectins and of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1). It also, in animal models, reduces

polymorphonuclear cell infiltration (19). Furthermore, HB-EGF decreases reactive oxygen species (ROS) production in intestinal cells exposed to stress, and it also reduces apoptosis as a result of inflammation on *in vitro* models (20).

Transforming growth factor β (TGF- β)

Transforming growth factor β is divided into three different subgroups of signaling peptides: TGF- β sensu stricto, bone morphogenic proteins and activins. TGF- β has four structurally very similar sub variants that play a role in numerous cellular interactions. TGF- β 2 seems to be to most prominent member of the group when it comes to potential beneficial effects on intestinal health. It was shown in animal studies that lesser TGF- β 2 expression positively correlates with NEC development (21). In addition to that TGF- β 2 also has anti-inflammatory and anti-stress properties on intestinal cells as a result of changing the expression of up to 20 different proteins. It was also demonstrated on mice and in *in vitro* conditions that TGF- β 2 reduces the activity of intestinal macrophages and promotes their noninflammatory differentiation during gestation. That is one of the many reasons why premature infants have a higher risk of developing NEC due to their shorter development process (22). TGF- β is also present in human milk and it was shown that it had lower bioactivity in mothers who had shorter gestation. It was also determined that latent TGF- β can be activated through the use of enzymatic activation with neuraminidase (23). An additional study was undertaken to examine the effect of adding recombinated TGF- β 2 to human milk. It failed to increase its bioactivity as a result of TGF- β 2 being bound to chondroitin sulfate. Enzymatic treatment with chondroitinase increased the activity of both the endogenous and exogenous TGF- β 2 (24). Further human trials for TGF and its activation methods are necessary.

Alkaline phosphatase

Alkaline phosphatase is a homodimeric protein found in many organisms. It is an enzyme that catalyzes the reaction of dephosphorylation in alkaline environments. Humans have four iterations of this protein: intestinal (IAP), placental, tissue-nonspecific (liver-bone-kidney type) and germ cell. They are named after tissue in which they are most prevalent, but can also be found throughout the body. The most interesting one is the intestinal variant. Enteral supplementation with IAP was shown to reduce the expression of pro-inflammatory cytokines in the intestines and decrease nitrosative stress in animal models (25). It was also shown, on animal models, that enteral supplementation reduces the systemic levels of IL-1 β , IL-6, and TNF- α in a dose dependent manner (26). Additional studies demonstrated that IAP activity is lowered in the gut afflicted with NEC and that addition of IAP to the diet reduces intestinal injury severity and may improve the integrity of the intestinal barrier (27).

Vitamin A

Vitamin A is a generic term that encompasses a plethora of compounds. Retinol and retinyl esters are called preformed vitamin A, and β -Carotene and other carotenoids are called provitamin A and can be converted into retinol in the body. Retinol can then be turned into retinal, and it into retinoic acid. Vitamin A compounds are fat soluble and stored in the liver. They take part in numerous processes and regulate gene expression. It was demonstrated that very-low-birth-weight infants predominantly have a decreased concentration of vitamin A in the plasma and that it correlates to impaired development (28). It was also demonstrated in animal studies that intraperitoneal application of high doses of vitamin A can improve intestinal healing after injury, reduce colon wet weight, which is a sign of edema, and hasten weight gain (29). Further studies examined the specific molecular pathway through which vitamin A achieves its effect. It was demonstrated in vitro that all-trans-retinoic acid can increase the expression of TGF- β 2, whose role was already discussed in this text (30). Furthermore, as demonstrated on rats, another way might be by decreasing the expression of TNF- α and increasing the activity of antioxidative enzymes like superoxide dismutase and glutathione peroxidase (31).

Trefoil factor family (TFF)

Trefoil factor family is a group consisting of three small peptides expressed by mucus-producing cells in a variety of tissues. They are most prevalent in the intestinal tract. TFF 1 (also known as sP2), TFF 2 (also known as SP), and TFF 3 (also known as TFI) are abundantly present in the stomach, colon, and ductus of pancreas, whereas TFF 1 and 3 can also be found in other parts of the gastrointestinal tract. All of them are present in human milk, with TFF 3 being detectable in the highest concentrations. All of their levels drop significantly during the first few weeks after pregnancy. It was also determined that their enteral application drastically improves the colitis score, with TFF 3 dimer being the most effective. On the other hand, subcutaneous injections were shown to aggravate the inflammation in rats (32). TFF 3 was also demonstrated to be not only effective for treatment but also prevention. One of the reasons for their protective properties seems to be that they interact with mucins to create a more viscous, elastic mucus. TFF 2 was proven to be the most potent in doing that. Furthermore, TFF3 was shown to have strong anti-inflammatory properties with its effect on reducing IL 8, TNF- α , prostaglandin E2, thromboxane B2, nitric oxide and malondialdehyde expression in intestinal tissue.

Branch chain fatty acids (BCFAs)

Branch chain fatty acids are mostly saturated, methylated fatty acids. They are categorized as mono-, di-, or multi-methyl BCFA with regard to the number of methyl groups. They are rare in internal organs but can be found in the skin, and they are a core component of ver-

nix caseosa, a creamy white substance covering the newborns, making up 10–29% of its composition. Furthermore they can also be found in human milk. BCFAs have been shown to reduce inflammation in intestinal cells in vitro and more recent studies demonstrated that oral supplementation can increase the production of IL 10 – a pleiotropic immunoregulating peptide with a multitude of effects that include the downregulation of pro-inflammatory cytokines and co-stimulatory molecules, and a reduced expression of HLA2 antigens. In addition to that, it was demonstrated on rats, that they promote the development of a healthier gut flora and reduce NEC incidence by 50% (33).

Erythropoietin (EPO)

Erythropoietin, sometimes also called hematopoietin, is a glycopeptide hormone produced mostly by peritubular fibroblasts in the renal cortex. He is also produced in the brain, liver, spleen, lung and testis, but in minute quantities. Prenatal EPO is mainly produced by hepatocytes. The main stimulus for its production is hypoxia. In turn, he acts as an anti-apoptotic agent for the erythrocytic lineage, mainly the colony-forming units-erythroid (CFU-Es) in the bone marrow and other hematopoietic organs. Although elevated blood levels of EPO in preterm infants were shown to correlate with NEC development (34), possibly because high levels are a sign of severe anemia, recent studies have shown the beneficial effect of EPO on enterocytes. EPO is a component of human breastmilk and was proven to reduce cell necrosis in vitro, possibly through increasing glutathione levels (35). It also reduces autophagy and apoptosis both in vitro and in vivo, on rats, in intestinal cells via the different signaling pathways (36).

Oxygen and ozone

Hyperbaric oxygen (HO) has been used in a variety of conditions ranging from necrotizing fasciitis to decompression sickness. It is proven to promote expression of antioxidative enzymes and enhance angiogenesis. A study has demonstrated that an application of HO can reduce inflammation markers and injury scores in a NEC model. Furthermore, a human pilot study has demonstrated that a brief application of HO has no adverse effects and might be used for NEC treatment (37). Ozone (O₃) is a triatomic allotrope of oxygen. He is often associated with detrimental effects on human health. When inhaled, it was shown to irritate the respiratory tract mucosa, exacerbate asthma and bronchitis, increase the likelihood of heart attacks, and may even lead to premature death. For all of those reasons it is understandable that there is skepticism towards ozone and its application in medicine. Nevertheless, recent studies have demonstrated that rectal and intraperitoneal application of ozone has the ability to reduce the levels of inflammation markers in the intestines. In addition to that, intraperitoneal administration can also reduce oxidative stress and mortality, and ameliorate weight loss in rats (38). It was also demonstrated, on

animal models, that when compared, ozone has a better overall effect on NEC than HO (39).

Proper enteral feeding

Given the fact that a significant number of compounds mentioned in this article are contained in maternal milk and that for the foreseeable future maternal milk will stay as the primary source of these molecules, at least until they are verified in their effectiveness and until we are able to produce them in larger quantities, it is appropriate to give a review of various combinations of enteral feeding methods. They include maternal milk, fortified maternal milk, formula, and combinations of the aforementioned. It is also important to note that when it comes to prematurely born infants there are great variations in gestational age and birth weight and as a result of that – great variations in gastrointestinal tract development. That naturally leads to different enteral nutrient intake capacity and risk of feeding intolerance and other complications, such as NEC. It is very hard to draw the line between different age and weight classes of infants and assign a specific feeding regimen to them. That is why the common wisdom about the diets of VLBW and extremely low birth weight (ELBW) infants changes throughout the years and is a topic of fierce debate. There is a general consensus of scientific evidence that shows that maternal milk diet is highly preferable to formula feeding. Not only that, it was also shown that exclusive human milk diet outperforms maternal milk with bovine fortifier, and a combination of fortified milk and formula. The reduction in NEC incidence is significant with other benefits such as: reduced feeding intolerance, days to full enteral feeding, and shorter hospital stay. In a situation when maternal milk is not available we are left with the choice of donated milk or formula. Studies have shown that formula based nutrition, in the short run, leads to faster weight gain, and an increased head circumference and body length. In the long run those infants did not retain their growth advantage nor did they have superior neurodevelopmental outcomes. On the other hand, infants that were fed with donated milk had a lower incidence of NEC (40). Given the fact that the majority of children, in a case that they do not have access to maternal milk, will also not have access to donated milk, doctors must apply formula in a proper manner. Studies have demonstrated that while maintaining a sufficient calorie intake of 110–130 kcal/kg/day, and a protein intake of 3–4 g/kg/day for VLBW infants and 4–4.5 G/kg/day for ELBW infants (41) is necessary, it might be worth considering lowering the concentration of the formula so as to reduce the risk of feeding intolerance. Further studies are necessary.

Two other important aspects of enteral nutrition that must be considered are the proper time after birth to introduce enteral nutrition, and a proper volume of food that ought to be applied. It is a general attitude that par-enteral nutrition should be reduced given the fact that it carries a risk of malnutrition and serious metabolic dis-

balances. Majority of the studies agree that minimal enteral nutrition should be started within the first four days for VLBW infants with the intent of stimulating mucosal function, intestinal motility and preventing atrophy. Beyond that point there is a divergence in findings and attitudes towards the increase in volume of intake. Certain studies propose that for the first ten days the volume should not be greater than 24 mL/kg/day and that this regimen has a positive effect on the reduction of feeding intolerance, and the risk of NEC and its severity (42). On the other hand there are studies that propose a more aggressive approach which includes increasing the feeding volume by 20–30 mL/kg/day for VLBW and 15–25 mL/kg/day for ELBW infants. Their reasoning is that it will reduce the chance of insufficient calorie intake, increase growth, reduce the time necessary to achieve full enteral feeding, and that it does not increase the risk of NEC (41). Finally, there is evidence that for ELBW infants under 750g it might be beneficial, in terms of NEC avoidance, to have a regimen that completely excludes enteral nutrition for up to two weeks and then slowly introduces it through a period of several weeks (43).

Discussion

NEC is still one of the main problems of infant health care and there haven't been many drastic changes in patient health outcome in the last decade. It has a relatively high mortality and morbidity and it is a problem that is not going to go away anytime soon due to the fact that the capacity of modern medicine to sustain life in extremely premature infants is ever expanding. That is why this article focuses on new methods of treating NEC that may provide a much-needed breakthrough. As far as treatment is concerned there is a multitude of compounds that have a possibility of being used one day on their own, or in combination, for prevention, treatment, and reduction of NEC severity and mitigating its influence on the body. They can be molecules found in human milk like insulin-like growth factor 1, human milk oligosaccharides, epidermal growth factor family, transforming growth factor β , erythropoietin. Others are a part of human milk and are also produced by the infant, such as branch chain fatty acids and trefoil factor family. There is also alkaline phosphatase, vitamin A, ozone and oxygen – a gas already present in the treatment of respiratory illnesses that afflict newborns.

Finally, the best NEC prevention tool is maternal milk. The most difficult thing is to resolve the puzzle of its administration in terms of volume and the proper time frame in which it ought to be applied. A certain level of consensus exists but further studies are required to fully optimize the clinical use.

This paper focused to highlight relevant human and animal studies, as to bring them to the attention of the medical community. It must be emphasized that all of these clinical tools are still in their experimental and developmental stage and that they require much further

study, but that they also provide a wide arrange of opportunity that ought to be explored.

In conclusion, it must be noted that the best treatment is prevention and early detection. Additional studies must also focus on a wide range of molecules, currently being investigated, that have a possibility of one day giving us an insight in the early stages of NEC development so as to facilitate a quick and appropriate response.

References

- Kon IY, Shilina NM, Gmshinskaya MV, Ivanushkina TA. The study of breast milk IGF-1, leptin, ghrelin and adiponectin levels as possible reasons of high weight gain in breast-fed infants. *Ann Nutr Metab.* 2014;65(4):317–23. doi: 10.1159/000367998.
- Hellström A, Ley D, Hansen-Pupp I, Hallberg B, Ramenghi LA, Löfqvist C, Smith LE, Hård AL. Role of Insulinlike Growth Factor 1 in Fetal Development and in the Early Postnatal Life of Premature Infants. *Am J Perinatol.* 2016 Sep;33(11):1067–71. doi: 10.1055/s-0036-1586109.
- Ozen S.a, Akisu M.a, Baka M.b, Yalaz M.a, Sozmen E.Y.c, Berdeli A.a, Kultursay N. Insulin-Like Growth Factor Attenuates Apoptosis and Mucosal Damage in Hypoxia/Reoxygenation-Induced Intestinal Injury. *Biol Neonate* 2005;87:91–6. <https://doi.org/10.1159/000081897>
- Tian F, Liu GR, Li N, Yuan G. Insulin-like growth factor I reduces the occurrence of necrotizing enterocolitis by reducing inflammatory response and protecting intestinal mucosal barrier in neonatal rats model. *Eur Rev Med Pharmacol Sci.* 2017 Oct; 21(20):4711–4719.
- Armanian AM, Sadeghnia A, Hoseinzadeh M, Mirlohi M, Feizi A, Salehimehr N, Saeed N, Nazari J. The Effect of Neutral Oligosaccharides on Reducing the Incidence of Necrotizing Enterocolitis in Preterm infants: A Randomized Clinical Trial. *Int J Prev Med.* 2014 Nov;5(11):1387–95.
- Bode L, Kunz C, Muhly-Reinholz M, Mayer K, Seeger W, Rudloff S. Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. *Thromb Haemost.* 2004 Dec;92(6):1402–10.
- Yu H, Yan X, Autran CA, Li Y, Etzold S, Latasiewicz J, Robertson BM, Li J, Bode L, Chen X. Enzymatic and Chemoenzymatic Syntheses of Disialyl Glycans and Their Necrotizing Enterocolitis Preventing Effects. *J. Org. Chem.*, 2017, 82 (24), pp 13152–13160. DOI: 10.1021/acs.joc.7b02167
- Autran CA, Kellman BP, Kim JH, Asztalos E, Blood AB, Spence EC, Patel AL, Hou J, Lewis NE, Bode L. Human milk oligosaccharide composition predicts risk of necrotising enterocolitis in preterm infants. *Gut.* 2017 Apr 5. pii: gutjnl-2016-312819. doi: 10.1136/gutjnl-2016-312819.
- Autran CA, Schoterman MH, Jantscher-Krenn E, Kamerling JP, Bode L. Sialylated galacto-oligosaccharides and 2'-fucosyllactose reduce necrotising enterocolitis in neonatal rats. *Br J Nutr.* 2016 Jul;116(2):294–9. doi: 10.1017/S0007114516002038.
- Westerbeek EA, van den Berg A, Lafeber HN, Fetter WP, van Elburg RM. The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants. *Br J Nutr.* 2011 Jan;105(2):268–74. doi: 10.1017/S0007114510003405.
- Rasmussen SO, Martin L, Østergaard MV, Rudloff S, Roggenbuck M, Nguyen DN, Sangild PT, Bering SB. Human milk oligosaccharide effects on intestinal function and inflammation after preterm birth in pigs. *J Nutr Biochem.* 2017 Feb; 40:141–154. doi: 10.1016/j.jnutbio.2016.10.011.
- Warner BB, Ryan AL, Seeger K, Leonard AC, Erwin CR, Warner BW. Ontogeny of salivary epidermal growth factor and necrotizing enterocolitis. *J Pediatr.* 2007 Apr;150(4):358–63.
- Dvorak B, Halpern MD, Holubec H, Williams CS, McWilliam DL, Dominguez JA, Stepankova R, Payne CM, McCuskey RS. Epidermal growth factor reduces the development of necrotizing enterocolitis in a neonatal rat model. *Am J Physiol Gastrointest Liver Physiol.* 2002 Jan;282(1):G156–64.
- Halpern MD, Dominguez JA, Dvorakova K, Holubec H, Williams CS, Meza YG, Ruth MC, Dvorak B. Ileal cytokine dysregulation in experimental necrotizing enterocolitis is reduced by epidermal growth factor. *J Pediatr Gastroenterol Nutr.* 2003 Jan;36(1):126–33.
- Clark JA, Lane RH, MacLennan NK, Holubec H, Dvorakova K, Halpern MD, Williams CS, Payne CM, Dvorak B. Epidermal growth factor reduces intestinal apoptosis in an experimental model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol.* 2005 Apr;288(4):G755–62.
- Clark JA, Doelle SM, Halpern MD, Saunders TA, Holubec H, Dvorak K, Boitano SA, Dvorak B. Intestinal barrier failure during experimental necrotizing enterocolitis: protective effect of EGF treatment. *Am J Physiol Gastrointest Liver Physiol.* 2006 Nov;291(5):G938–49.
- Radulescu A, Yu X, Orvets ND, Chen Y, Zhang HY, Besner GE. Deletion of the heparin-binding epidermal growth factor-like growth factor gene increases susceptibility to necrotizing enterocolitis. *J Pediatr Surg.* 2010 Apr;45(4):729–34. doi: 10.1016/j.jpedsurg.2009.06.035.
- Radulescu A, Zhang HY, Yu X, Olson JK, Darbyshire AK, Chen Y, Besner GE. Heparin-binding epidermal growth factor-like growth factor overexpression in transgenic mice increases resistance to necrotizing enterocolitis. *J Pediatr Surg.* 2010 Oct;45(10):1933–9. doi: 10.1016/j.jpedsurg.2010.05.002.
- Xia G, Martin AE, Besner GE. Heparin-binding EGF-like growth factor downregulates expression of adhesion molecules and infiltration of inflammatory cells after intestinal ischemia/reperfusion injury. *J Pediatr Surg.* 2003 Mar;38(3):434–9.
- Michalsky MP, Kuhn A, Mehta V, Besner GE. Heparin-binding EGF-like growth factor decreases apoptosis in intestinal epithelial cells in vitro. *J Pediatr Surg.* 2001 Aug;36(8):1130–5.
- Namachivayam K, Blanco CL, MohanKumar K, Jagadeeswaran R, Vasquez M, McGill-Vargas L, Garzon SA, Jain SK, Gill RK, Freitag NE, Weitkamp JH, Seidner SR, Maheshwari A. Smad7 inhibits autocrine expression of TGF-β2 in intestinal epithelial cells in baboon necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol.* 2013 Jan 15;304(2):G167–80. doi: 10.1152/ajpgi.00141.2012.
- Maheshwari A, Kelly DR, Nicola T, Ambalavanan N, Jain SK, Murphy-Ullrich J, Athar M, Shimamura M, Bhandari V, Aprahamian C, Dimmitt RA, Serra R, Ohls RK. TGF-β2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. *Gastroenterology.* 2011 Jan;140(1):242–53. doi: 10.1053/j.gastro.2010.09.043.
- Namachivayam K, Blanco CL, Frost BL, Reeves AA, Jagadeeswaran R, MohanKumar K, Safarulla A, Mandal P, Garzon SA, Raj JU, Maheshwari A. Preterm human milk contains a large pool of latent TGF-β, which can be activated by exogenous neuraminidase. *Am J Physiol Gastrointest Liver Physiol.* 2013 Jun 15;304(12):G1055–65. doi: 10.1152/ajpgi.00039.2013.

24. Namachivayam K, Coffing HP, Sankaranarayanan NV, Jin Y, MohanKumar K, Frost BL, Blanco CL, Patel AL, Meier PP, Garzon SA, Desai UR, Maheshwari A. Transforming growth factor- β 2 is sequestered in preterm human milk by chondroitin sulfate proteoglycans. *Am J Physiol Gastrointest Liver Physiol*. 2015 Aug 1;309(3):G171–80. doi: 10.1152/ajpgi.00126.2015.
25. Rentea RM, Liedel JL, Fredrich K, Pritchard K Jr, Oldham KT, Simpson PM, Gourlay DM. Enteral intestinal alkaline phosphatase administration in newborns decreases iNOS expression in a neonatal necrotizing enterocolitis rat model. *J Pediatr Surg*. 2013 Jan;48(1):124–8. doi: 10.1016/j.jpedsurg.2012.10.026.
26. Rentea RM, Liedel JL, Fredrich K, Welak SR, Pritchard KA Jr, Oldham KT, Simpson PM, Gourlay DM. Intestinal alkaline phosphatase administration in newborns decreases systemic inflammatory cytokine expression in a neonatal necrotizing enterocolitis rat model. *J Surg Res*. 2012 Oct;177(2):228–34. doi: 10.1016/j.jss.2012.05.039.
27. Biesterveld BE, Koehler SM, Heinzerling NP, Rentea RM, Fredrich K, Welak SR, Gourlay DM. Intestinal alkaline phosphatase to treat necrotizing enterocolitis. *J Surg Res*. 2015 Jun 15;196(2):235–40. doi: 10.1016/j.jss.2015.02.030.
28. Spears K, Cheney C, Zerzan J. Low plasma retinol concentrations increase the risk of developing bronchopulmonary dysplasia and long-term respiratory disability in very-low-birth-weight infants. *Am J Clin Nutr*. 2004 Dec;80(6):1589–94.
29. Nafday SM, Green RS, Chauvin SN, Holzman IR, Magid MS, Lin J. Vitamin A supplementation ameliorates butyric acid-induced intestinal mucosal injury in newborn rats. *J Perinat Med*. 2002;30(2):121–7.
30. Namachivayam K, MohanKumar K, Arbach D, Jagadeeswaran R, Jain SK, Natarajan V, Mehta D, Jankov RP, Maheshwari A. All-Trans Retinoic Acid Induces TGF- β 2 in Intestinal Epithelial Cells via RhoA- and p38 α MAPK-Mediated Activation of the Transcription Factor ATF2. *PLoS One*. 2015 Jul 30;10(7):e0134003. doi: 10.1371/journal.pone.0134003.
31. Ozdemir R, Yurttutan S, Sari FN, Oncel MY, Erdeve O, Unverdi HG, Uysal B, Dilmen U. All-trans-retinoic acid attenuates intestinal injury in a neonatal rat model of necrotizing enterocolitis. *Neonatology*. 2013;104(1):22–7. doi: 10.1159/000350510.
32. Poulsen SS, Kissow H, Hare K, Hartmann B, Thim L. Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat. *Regul Pept*. 2005 Mar 30;126(3):163–71.
33. Ran-Ressler RR, Khailova L, Arganbright KM, Adkins-Rieck CK, Jouni ZE, Koren O, Ley RE, Brenna JT, Dvorak B. Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PLoS One*. 2011;6(12):e29032. doi: 10.1371/journal.pone.0029032.
34. Holm M, Skranes J, Dammann O, Fichorova RN, Allred EN, Leviton A. Systemic endogenous erythropoietin and associated disorders in extremely preterm newborns. *Arch Dis Child Fetal Neonatal Ed*. 2016 Sep;101(5):F458–63. doi: 10.1136/archdischild-2015-309127.
35. Martin C, Patel M, Melendez-Ferro M, Sims B. Erythropoietin-induced cytoprotection in intestinal epithelial cells is linked to system Xc. *Exp Cell Res*. 2017 Mar 15;352(2):202–206. doi: 10.1016/j.yexcr.2017.02.002.
36. Yu Y, Shiou SR, Guo Y, Lu L, Westerhoff M, Sun J, Petrof EO, Claud EC. Erythropoietin protects epithelial cells from excessive autophagy and apoptosis in experimental neonatal necrotizing enterocolitis. *PLoS One*. 2013 Jul 25;8(7):e69620. doi: 10.1371/journal.pone.0069620.
37. Sánchez EC. Use of hyperbaric oxygenation in neonatal patients: a pilot study of 8 patients. *Crit Care Nurs Q*. 2013 Jul-Sep;36(3):280–9. doi: 10.1097/CNQ.0b013e318294e95b.
38. Guven A, Gundogdu G, Vurucu S, Uysal B, Oztas E, Ozturk H, Korkmaz A. Medical ozone therapy reduces oxidative stress and intestinal damage in an experimental model of necrotizing enterocolitis in neonatal rats. *J Pediatr Surg*. 2009 Sep;44(9):1730–5. doi: 10.1016/j.jpedsurg.2009.01.007.
39. Altinel O, Demirbas S, Cakir E, Yaman H, Ozerhan IH, Duran E, Cayci T, Akgul EO, Ersoz N, Uysal B, Kurt B, Yasar M, Oter S, Peker Y. Comparison of hyperbaric oxygen and medical ozone therapies in a rat model of experimental distal colitis. *Scand J Clin Lab Invest*. 2011 May;71(3):185–92. doi: 10.3109/00365513.2010.548875. Epub 2011 Jan 4.
40. Quigley M, McGuire W. Formula versus donor breastmilk for feeding preterm low birth weight infants. *Cochrane Database of Systematic Reviews* 2014, Issue 4. Art. No.: CD002971. DOI: 10.1002/14651858.CD002971.pub3.
41. Senterre T. Practice of enteral nutrition in very low birth weight and extremely low birth weight infants. *World Rev Nutr Diet*. 2014;110:201–14. doi: 10.1159/000358468.
42. Qiu-fang Li, Hua Wang, Dan Liu, Yi Tang, Xin-fen Xu. Application of prolonging small feeding volumes early in life to prevent of necrotizing enterocolitis in very low birth weight preterm infants. *International Journal of Nursing Sciences* Volume 3, Issue 1, March 2016, Pages 45–49. <https://doi.org/10.1016/j.ijnss.2016.02.015>
43. Viswanathan S, McNelis K, Super D, Einstadter D, Groh-Wargo S, Collin M. Standardized Slow Enteral Feeding Protocol and the Incidence of Necrotizing Enterocolitis in Extremely Low Birth Weight Infants. *JPEN J Parenter Enteral Nutr*. 2015 Aug;39(6):644–54. doi: 10.1177/0148607114552848.

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NEKROTIZIRAJUĆI ENTEROKOLITIS DOJENČADI – NOVE MOGUĆNOSTI LIJEČENJA

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Stručni članak

Ključne riječi: nekrotizirajući enterokolitis, dojenčad, nove mogućnosti liječenja

SAŽETAK. Nekrotizni enterokolitis (NEC) dojenčadi razorna je bolest koja zahtijeva intenzivno liječenje te čiji je razvoj teško predvidjeti. Njegova pojava i progresija povezani su s mnoštvom mogućih uzroka. Zato su posljednjih godina mnoge studije napravljene s namjerom pronalaženja učinkovitih novih načina liječenja i prevencije. Neki od njih su dizajnirani za proučavanje i izoliranje specifičnih čimbenika pronađenih u majčinom mlijeku, a drugi su bili više usredotočeni na ispitivanje sintetičkih molekula ili prirodnih lijekova. Etiologija NEC-a i njezine epidemiologije, nakon čega slijedi karakterizacija patologije i patogeneze, kao i simptomatologije, kliničke, radiološke i biokemijske metode detekcije.

Kratko su opisane nove metode i tehnike liječenja: faktor rasta sličan inzulinu, oligosaharidi ljudskog mlijeka, obitelj epidermalnog faktora rasta, transformirajući faktor rasta, enzim-alkalna fosfataza, vitamin A, obitelj trefoil faktora, masne kiseline lanca grana, eritropoetin, kisika i ozona. Poseban naglasak stavljen je na ulogu majčinskog mlijeka kao izvora mnogih biokemijskih čimbenika u sprječavanju i čak liječenju NEC-a. Potrebne su daljnje istrage kako bi se smanjio učestalost i ozbiljnost, kao i poboljšali šanse za pacijente s NEC-om.