Caffeine and Bronchopulmonary dysplasia :Clinical Benefits and Its Mechanisms

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Abstract

Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease caused by a combination of prenatal and postnatal factors that leads to the disruption of lung development and abnormal repair, this is a condition that is commonly seen in premature infants. With the improvement of treatment technology, the survival rate of very early preterm infants has increased significantly compared with before, and the incidence of severe BPD has decreased, however, the prevalence of BPD has not decreased. The overall prevalence of BPD is 45%.The prevention of prematurity, the systematic use of non-aggressive ventilator measures, the avoidance of supra-physiological oxygen exposure, and the administration of diuretics, caffeine and vitamin A have all been shown to lead to a significant reduction in the risk of BPD development. A growing number of clinical studies have shown that caffeine not only prevents apnea, but also reduces the incidence of BPD. We review the clinical value of caffeine in the treatment of BPD and its potential mechanisms of action, include anti-inflammatory, antioxidant, anti-fibrotic, anti-apoptotic pathways, and the regulation of angiogenesis. Our aim was to provide a new theoretical basis for the clinical treatment of BPD.

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【Abstract 】:

Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease caused by a combination of prenatal and postnatal factors that leads to the disruption of lung development and abnormal repair, this is a condition that is commonly seen in premature infants. With the improvement of treatment technology, the survival rate of very early preterm infants has increased significantly compared with before, and the incidence of

severe BPD has decreased, however, the prevalence of BPD has not decreased. The overall prevalence of BPD is 45%.The prevention of prematurity, the systematic use of non-aggressive ventilator measures, the avoidance of supra-physiological oxygen exposure, and the administration of diuretics, caffeine and vitamin A have all been shown to lead to a significant reduction in the risk of BPD development. A growing number of clinical studies have shown that caffeine not only prevents apnea, but also reduces the incidence of BPD. We review the clinical value of caffeine in the treatment of BPD and its potential mechanisms of action, include anti-inflammatory, antioxidant, anti-fibrotic, anti-apoptotic pathways, and the regulation of angiogenesis. Our aim was to provide a new theoretical basis for the clinical treatment of BPD.

Key words :Caffeine, Bronchopulmonary dysplasia , Anti-inflammatory, Antioxidant , Anti-fibrotic, Anti-apoptotic

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease caused by a combination of prenatal and postnatal factors that leads to the disruption of lung development and abnormal repair, this is a condition that is commonly seen in premature infants[1] .With technological improvements in technology, the survival rate of very early preterm infants has increased significantly compared with previous years, and the incidence of severe BPD has decreased, However, the prevalence of BPD has not decreased[2] .The overall prevalence of BPD is 45%[3]. A previous study found that more than 70% of infants with a birth weight of less than 1000 grams went on to develop BPD[4].In a recent cohort study of the prevalence and risk factors for BPD in very low gestational age infants([?]28 weeks),the mean gestational age (GA) and birth weight (BW) of the cohort was 25. 3 $\pm$ 1. 4weeks and 724 $\pm$14g, respectively, with a moderate to severe BPD of 67%[5].The pathology of classical BPD is dominated by severe airway compromise, such as severe epithelial damage, smooth muscle hyperplasia in the airway, and fibrosis[6]. As medical treatments have advanced, the pathology of BPD has changed; new BPD is characterized by simplification of the alveolar structure, abnormal vascular development ,and impaired lymphatic function, as the main pathological features[7,8]. The prevention of prematurity, the systematic use of non-aggressive ventilator measures, the avoidance of supra-physiological oxygen exposure, and the administration of diuretics, caffeine and vitamin A have all been shown to lead to a significant reduction in the risk of BPD development[9],However, we have yet to develop a method that can completely prevent and treat BPD[10].A growing number of clinical studies have shown that caffeine not only prevents apnea, but also reduces the incidence of BPD[11-14].Therefore, caffeine is receiving increasing attention with regards to the prevention of BPD.

Caffeine, also known as 1, 3 and 7 methylxanthine, can act as an antagonist of adenosine receptors, an inhibitor of phosphodiesterase and an activator of ryanodine receptors. At physiological concentrations, caffeine acts primarily as an inhibitor of the adenosine receptor. However, with increasing plasma concentrations, caffeine may also inhibit other receptors, such as the gamma-aminobutyric acid receptor and cholinergic receptors [15]. Caffeine has been used for the treatment of apnea in preterm infants since the 1970s. The recommended dose for use in neonates is 20 mg/kg (loading)with a 5-10 mg/(kg-d) maintenance dose given after 24 h[16] . Furthermore, clinical studies have found that early caffeine treatment, and high-dose caffeine treatment, exerts a protective against BPD in the prevention and treatment of apnea in preterm infants[17,18].Early caffeine treatment is also known to reduce neurological sequelae, such as cerebral palsy and hearing impairment[19].

In this article, we review the clinical value of caffeine in the treatment of BPD and its potential mechanisms of action. Our aim was to provide a new theoretical basis for the clinical treatment of BPD.

Multiple Benefits of Caffeine for the Treatment of BPD

1.1Improving the success rate of extubation and withdrawal and reducing the duration of mechanical ventilation

Mechanical ventilation is known to be one of the most important risk factors for BPD in preterm infants. The lung of preterm infants are poorly developed and require additional mechanical ventilation support, However,

invasive mechanical ventilation can exacerbate lung epithelial cell damage and lead to BPD.Caffeine may shorten the duration of mechanical ventilation and reduce the risk of BPD. Systematic reviews and meta-analyses have shown that methylxanthines reduce extubation failure, and strongly recommend the use of caffeine[20]. Previous studies in the apnea of prematurity (AOP) randomized controlled trial found that caffeine reduced the duration of positive pressure ventilation and attenuated the incidence of BPD in infants with very low birth weight[14]. With regards to the of timing of caffeine treatment, several retrospective cohort studies confirmed that the duration of mechanical ventilation was shorter in infants treated with caffeine in the early stages [21] and that the mortality rate and incidence of BPD were lower among infants treated early with caffeine than those who received caffeine later[22]. However, another study reported a lower incidence of BPD (23.1% versus 30.7%) but a higher mortality rate (4.5% versus 3.7%) in infants who received caffeine early[23].

In terms of caffeine dosing, two randomized controlled trials showed that high doses of caffeine led to a significant reduction in the failure rate of extubation and reduced the duration of mechanical ventilation in prematurely ventilated infants[24,25].In another study of the effect of maintenance doses of caffeine on extubation in preterm infants, higher maintenance doses of caffeine citrate reduced the incidence of extubation failure and apnea in preterm infants[26]. Interestingly, a systematic review and meta-analysis found that high-dose caffeine (>20 mg/kg.d) reduced cases of failed extubation and apnea and shortened the duration of mechanical ventilation, but had no effect on mortality or the incidence of BPD[27].Anis et al.conducted a further controlled study of the prophylactic use of high-dose caffeine for the prevention of apnea and found that lower doses of caffeine may be as effective as higher doses of caffeine in preventing apnea, but fewer adverse effects[28]. Unfortunately, the study only analyzed short-term efficacy and did not consider the effects of caffeine on long-term outcomes such as BPD. Therefore, the potential role of caffeine in reducing mechanical ventilation remains to be further investigated.

1.2 Improvement of lung function

A retrospective study of pulmonary function in preterm infants found that preterm infants born at less than 32 weeks may have significantly reduced airflow during childhood and adolescence and that impairment of pulmonary function was partially associated with the degree of BPD[29]. Young people with very low birth weight with combined BPD have significantly reduced lung ventilation, as evidenced by a low expiratory volume on exertion and reduced expiratory volume/exertional lung volume in one second on exertion[30].Caffeine improves lung function by increasing lung volume and airway compliance, decreases airway resistance, improves respiratory muscle strength, and helps wean premature infants from mechanical ventilation[31]. A clinical study on the effect of caffeine treatment on pulmonary function in preterm infants (birth weight <1500 g and gestational age <31 weeks) showed that early caffeine treatment significantly increased forced vital capacity(FVC) and forced expiratory volume(FEV) by five-fold in preterm infants without BPD, while this trend remained in preterm infants with BPD [32]. Doyle et al.demonstrated a significant effect of caffeine on expiratory flow ;a group of patients who weighed less than 1250 g at birth, and did not receive early caffeine treatment ,had a lower of expiratory forced expired volume in 1 second (FEV1), FVC, and the forced expired flow from 25% to 75% of the FVC (FEF25-75%) at 11 years of age[33]. Another study found that caffeine treatment resulted in a sustained rapid increase in diaphragmatic activity and tidal volume (VT) in preterm infants and that caffeine did not always affect end-expiratory lung volume (EELV), respiratory rate, or inspiratory and expiratory times[34].

Caffeine treatment has also been shown to improve lung function by increasing lung volume, total lung volume, and by improving lung elasticity and compliance in a rabbit model[35]. In a preliminary study, carried out in baboons, early caffeine treatment, combined with prophylactic surfactant treatment, was found to improve lung function over 24 hours by significantly reducing airway resistance and increasing lung compliance[36].

1.3 Other clinical benefits

Patent ductus arteriosus(PDA) is one of the postnatal risk factors for the development of BPD[37]. A prospec-

tive multicenter study of very preterm infants with respiratory distress syndrome confirmed that the early initiation of caffeine was associated with the reduced occurrence of PDA[38]. In a retrospective cohort of 140 newborns weighing [?] 1250 g at birth, early caffeine treatment was also found to reduce PDA requiring treatment in preterm infants[22]. In addition, active substances on the alveolar surface are closely associated with lung maturation. Fehrholz et al. demonstrated that caffeine enhanced the role of glucocorticoids in promoting the production, maturation and release of alveolar surface active substances both in vivo and in vitro in premature sheep with bronchoalveolar lavage solution[39].

2.The mechanism of action of caffeine in the lungs

It has been well established clinically that caffeine can reduce the incidence of BPD, although its protective mechanisms are still being investigated. The mechanisms that might be involved are shown in Figure 1 and include anti-inflammatory, antioxidant, anti-fibrotic, anti-apoptotic pathways, and the regulation of angiogenesis .

2.1 Anti-inflammatory effects

It is well known that inflammation is the most important risk factor for bronchopulmonary dysplasia and that inflammation leads to the increased secretion of pro-inflammatory cytokines. Over recent years, it has been found that caffeine can inhibit the inflammatory response by reducing the release of inflammatory factors and by regulating the expression of inflammatory pathway proteins and inflammatory receptors. Next, we describe how caffeine exerts its inhibitory effects on inflammation.

2.1.1Inflammatory factors

Numerous studies have confirmed the increased expression levels of pro-inflammatory factors in children with BPD, including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α[40-42].In vitro experiments showed that caffeine reduced IL-1β and IL-18 secretion in lipopolysaccharide (LPS)-induced THP-1 macrophages[43].In animal experiments involving hyperoxia-induced lung injury, it was confirmed that caffeine treatment not only reduced the expression of cyclooxygenase(COX-2),myeloperoxidase(MPO), TNF-α, and IL-1β[44],but also reduced leukocyte infiltration and decreased the release of pro-inflammatory factors and chemokines in the lungs, thus protecting against hyperoxia-induced alveolar dysplasia[45].In a young rat model of LPS-induced pro-inflammatory amnionitis, caffeine exerted anti-inflammatory effects and improved lung function by inhibiting the expression of IL-1β and CD68[46]. In a cohort study of preterm infants, Raul et al.found that BPD was associated with higher concentrations of IL-1 and IL-6, a greater imbalance between these cytokines and IL-10, and that alterations occurred in tracheal secretions and peripheral blood inflammatory factors before and after caffeine administration[47].At therapeutic levels of 10-20 mg/mL, caffeine prevented the sustained activation of the inflammatory cascade response, although serum caffeine levels >20 mg/mL resulted in elevated levels of IL-1b, IL-6,but TNF-a and decreased IL-10 concentrations. Thus, in vitro and in vivo experiments , together with clinical trials, have shown that caffeine reduces to lung injury by modulating changes in inflammatory factors.

2.1.2 Inflammasomes

Inflammasomes are complexes formed by the polymerization of multiple proteins that play an important regulatory role in the onset and progression of lung injury. The NLRP3 inflammasome is one of the most widely studied inflammatory vesicles, and mainly contains the recognition protein NLP, the bridging protein Asc, and the effector protein Caspase1[48]. Previous studies have found that activation of NLRP3 inflammatory vesicles is a critical step in the pathogenesis of BPD[49]. Zhao et al. found that caffeine-induced antagonism of adenosine 2A receptor (A2AR) reduced the production of reactive oxygen species(ROS) , decreased the levels of cleaved caspase 1 expression, and inhibited expression of the NLRP3 inflammasome expression in LPS-induced THP-1 macrophages[43]. Subsequently, Chen et al. used animal models to demonstrate that caffeine inhibits the production of inflammatory factors and protects against lung injury due to hyperoxia exposure by reducing NF-ϰB pathway activation and by inhibiting NLRP3 the formation of the inflammasome[44].

2.1.3 The ARA receptor

A2AR protein is considered to be an important mediator of inflammation and immune response. In monocytes, caffeine reduces the secretion of TNF-α by monocytes by blocking adenosine receptors[50]. Animal experiments found that mouse TNF-α, IL-1β, and phosphorylated-NF-ϰB proteins were positively correlated with A2AR proteins. Caffeine, as an A2A receptor antagonist, has been shown to reduce pulmonary inflammatory infiltration and apoptosis and promote the development of type II alveolar epithelial cells[44]. In LPS/ATP-induced THP-1 macrophages, caffeine was shown to inhibit the ROS-mediated activation of NLRP3 inflammatory vesicles and reduced the secretion of IL-1β and IL-18 in THP-1 macrophages by decreasing the gene transcription and protein expression of A2AR[43]. In addition,caffeine antagonism of A2AR was shown to modulate neutrophil production by inflammatory factors and reduce the inflammatory response to oxidative stress[51].

2.1.4 The TOLL receptor

Toll-like receptor (TLR) is a molecular pattern associated with the recognition of specific pathogens. Toll-like receptor-2 recognizes multiple microbial components of Gram-positive bacteria and TLR4 is required for the lipopolysaccharide response, an external component of Gram-negative bacterial membranes[52].TLR4 activation has been associated with neonatal lung inflammation[53,54]. Studies showed that caffeine had no significant effect on TLR2 and 4 in normal neonatal rat lung tissue, but increased TLR9 expression, limited TLR4 expansion, inhibited pro-inflammatory cytokine production, and reduced the severity of BPD[55]. In addition, Ren et al.found that caffeine may inhibit the TLR-mediated inflammatory cascade response in macrophages by suppressing calcium mobilization[56]. It was also demonstrated in human monocytes that high concentrations of caffeine (200μM) upregulated TLR4 to promote inflammation, while 50 and 100μM of caffeine downregulated TLR1 and TLR2 expression and was able to reduce TNF-α levels;this may be related to the reduced incidence of BPD by caffeine[57].

2.2 Antioxidant effects

Increased oxidative stress due to exposure to hyperoxia in preterm infants is an important factor in the development of BPD.Caffeine has been shown to exert antioxidant effects in the nervous system[58]. Oxidative stress can lead to an increase in reactive oxygen species(ROS) and damage to cellular deoxyribonucleic acid (DNA) and lipid oxidation. In an in vitro model of pulmonary oxygen toxicity in human and mouse lung epithelial cells, a low concentration of caffeine (0.01 mM) reduced hydrogen peroxide (H_2O_2)levels but increased them at high concentrations of 1 mM[59]. Caffeine reduced hyperoxia-induced oxidative damage in mouse lung DNA and lipids, as well as increased superoxide dismutase, heme oxygenase-1 and Nrf2/Keap1 gene expression; it also blocked the expression levels of key proteins and superoxide dismutase (SOD) in oxidative stress, achieved antioxidant effects at different time points, and attenuated oxidative stress damage[60]. In addition, endoplasmic reticulum stress is one of the major manifestations of oxidative stress. Caffeine may have the pharmacological propertiesto improve leptin resistance by reducing endoplasmic reticulum stress[61].Teng et al.confirmed that caffeine treatment attenuated markers of endoplasmic reticulum stress and downstream effectors (C/EBP homologous protein (CHOP) or the splicing of X-box binding protein 1 (XBP-1))and attenuated the hyperoxia-induced oxidative stress-induced impairment of alveolar formation [62].

2.3 Anti-fibrotic effects

There is growing evidence that transforming growth factor-β(TGFβ), connective tissue growth factor(CTGF), and transgelin, play an important role in the pathogenesis of BPD in rodent models. Tatler et al. showed for the first time that caffeine exerted antifibrotic effects in a concentration-dependent manner in lung epithelial cells and fibroblasts[63]. Caffeine directly inhibited TGFβ activation in lung epithelial cells, whereas in lung fibroblasts, it suppressed the basal expression of α-smooth muscle actin genes and reduced the TGFβ-induced increase in pro-fibrotic genes *via* a pro-fibrotic response to TGFβ[63]. In a *vitro*precision-cut lung section model, caffeine was shown to reduce bleomycin-induced fibrosis[63].

CTGF is a downstream regulator of TGF-β that is responsible for abnormal extracellular matrix(ECM) deposition and tissue fibrosis. Transgelin is a cytoskeleton binding and stabilizing protein. Both of these

factors are regulated by TGF-β1 and play an important role in airway remodeling. The increased conditional expression of CTGF in alveolar type II epithelial cells was shown to disrupt alveolarization and vascular development, induce vascular remodeling, and led to pulmonary hypertension, a pathological feature of severe BPD[64]. Transgelin is an important target of TGFβ-regulated type II alveolar epithelial cell fibrosis. Increase dexpression of transgelinin ATII cells may lead to TGF-dependent alveolar type II (ATII) cell injury, along with repair and migration in pulmonary fibrosis[65].Caffeine antagonizes the TGF-β1-induced upregulation of CTGF and transgelin, and plays a role in airway remodeling in BPD[66]. Caffeine treatment has also been shown to attenuate glucocorticoid-induced CTGF expression and thus promote the restoration of intrapulmonary homeostasis[67]. Interestingly, recent studies have found that the stereological analysis of lung structure after caffeine administration found no effects of caffeine on alveolar simplification in lungs exposed to hyperoxia[68]. Caffeine was also found to enhance the ability of TGF-β to drive CTGF gene expression in type II alveolar epithelial cells and fibroblasts[68]. Therefore, the mechanism by which caffeine can exert regulatory effects on TGFβ, CTGF, and transgelin, still requires further research.

2.4 Anti-apoptotic effects

Previous studies have demonstrated the presence of apoptosis during BPD and found that caspase-3, caspase-9 and caspase-12 are important regulators of apoptosis. Li et al.evaluated 50 Wistar rats that received a dose of 25 mg/kg/day caffeine for the first 4 weeks of anexperiment and found that caffeine inhibited caspase 3 activation and reduced apoptosis incardiomyocytes[69]. Caffeine has also been shown to inhibit apoptosis in a mouse model of BPD, most notably in alveolar epithelial cells. In a hyperoxia-induced mouse model of lung. In terms of transcription of cell death-related mediators, caffeine inhibited the effect of hyperoxia on cell death-related mediators (caspase3, apoptosis-inducing factor(AIF) and glutamate cysteine ligase catalytic subunit (GCLC))[51]. Teng et al.also demonstrated that caffeine can inhibit endoplasmic reticulum stress-associated apoptosis by reducing the BCl2/Bax ratio and by inhibiting the expression of caspase12[62].Moreover, another *in vitro* study found that caffeine induced apoptosis in lung cancer epithelial cells in a concentration-dependent manner during a G2 phase block of the cell cycle; this was enhanced at a concentration of 5 mM and also induced a TP53 non-dependent G1 phase block[70]. In addition, high concentrations of caffeine abolished G2 phase block and increased the rates of cell death, whereas low and clinically relevant concentrations of caffeine had no effect on G2 phase block and exerted antioxidant effects[59]. Dayanim et al found that caffeine reduces the number of ATII cells and increases the levels of apoptosis by decreasing the expression of the A2AR in animal models of hyperoxia[71]. Therefore, the anti-apoptotic effects of caffeine still need to be investigated further.

2.5 The regulation of angiogenesis

In recent years, there is increasing evidence that pulmonary vascular injury has become a major manifestation of BPD. Jing et al.found that early caffeine treatment increased cAMP levels in a hyperoxicmodel of lung injury, improved endothelial nitric oxide synthase (eNOS) activity by inhibiting the degradation of GTP-cyclohydrolase-1, improved the bioavailability of tetrahydrobiopterin(BH4), and improved alveolar structure and vascular function[72]. In addition, Dumpa et al.used male miceto show that caffeine can improve pulmonary vascular remodeling in response to hyperoxia by increasing the vascular surface area of small pulmonary arteries and by inhibiting smooth muscle proliferation[73]. However, additional studies have found that caffeine inhibits embryonic angiogenesis[74]; therefore, furtherstudies are required to support the protective effects of caffeine against pulmonary vascular injury.

In summary, the clinical benefits of caffeine have been confirmed, although the molecular mechanisms responsible for its action remain controversial and are still being investigated.Currently, research is limited to five aspects: anti-inflammatory, antioxidant, anti-apoptotic, and anti-fibrotic effects, and the regulation of angiogenesis. Caffeine can act as a neurostimulant, thus leading to severe restrictions in the clinical research process in many countries. In recent years, caffeine has been shown to be protective against both Parkinson's disease[75]and colorectal cancer[76]by regulating flora and autophagy; however, there is still significant scope for investigating the effect of caffeine on BPD. This condition affects the long-term survival of newborns; consequently, we need to consider this disease carefully and find effective solutions for prevention and treatment

in order to promote human development.

References:

1. Bernard T, B., Goss KN, Laughon M, et al. Bronchopulmonary dysplasia.*Nat Rev Dis Primers.* 2019;5(1):78.

2. Smith VC, Zupancic JA, McCormick MC, et al. Trends in severe bronchopulmonary dysplasia rates between 1994 and 2002. *J Pediatr.* 2005;146(4):469-473.

3. Rutkowska M, Hożejowski R, Helwich E, Borszewska-Kornacka MK, Gadzinowski J. Severe bronchopulmonary dysplasia – incidence and predictive factors in a prospective, multicenter study in very preterm infants with respiratory distress syndrome. *The Journal of Maternal-Fetal & Neonatal Medicine.* 2018;32(12):1958-1964.

4. Klinger G, Sokolover N, Boyko V, et al. Perinatal risk factors for bronchopulmonary dysplasia in a national cohort of very-low-birthweight infants. *Am J Obstet Gynecol.* 2013;208(2):115 e111-119.

5. Geetha O, Rajadurai VS, Anand AJ, et al. New BPD-prevalence and risk factors for bronchopulmonary dysplasia/mortality in extremely low gestational age infants </=28 weeks. *J Perinatol.*2021;41(8):1943-1950.

6. Northway WH, Jr., Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med.* 1967;276(7):357-368.

7. Kalikkot Thekkeveedu R, Guaman MC, Shivanna B. Bronchopulmonary dysplasia: A review of pathogenesis and pathophysiology. *Respir Med.* 2017;132:170-177.

8. Wang SH, Tsao PN. Phenotypes of Bronchopulmonary Dysplasia. *Int J Mol Sci.* 2020;21(17).

9. Principi N, Di Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. *J Transl Med.* 2018;16(1):36.

10. Ogawa R, Mori R, Sako M, Kageyama M, Tamura M, Namba F. Drug treatment for bronchopulmonary dysplasia in Japan: questionnaire survey.*Pediatr Int.* 2015;57(1):189-192.

11. Moschino L, Zivanovic S, Hartley C, Trevisanuto D, Baraldi E, Roehr CC. Caffeine in preterm infants: where are we in 2020? *ERJ Open Res.* 2020;6(1).

12. Elmowafi M, Mohsen N, Nour I, Nasef N. Prophylactic versus therapeutic caffeine for apnea of prematurity: a randomized controlled trial. *J Matern Fetal Neonatal Med.* 2021:1-9.

13. Synnes A, Grunau RE. Neurodevelopmental outcomes after neonatal caffeine therapy. *Semin Fetal Neonatal Med.* 2020;25(6):101160.

14. Schmidt B, Roberts RS, Davis P, et al. Caffeine therapy for apnea of prematurity. *N Engl J Med.* 2006;354(20):2112-2121.

15. Aranda JV, Beharry KD. Pharmacokinetics, pharmacodynamics and metabolism of caffeine in newborns. *Semin Fetal Neonatal Med.*2020;25(6):101183.

16. Aranda JV, Cook CE, Gorman W, et al. Pharmacokinetic profile of caffeine in the premature newborn infant with apnea. *J Pediatr.*1979;94(4):663-668.

17. Kua KP, Lee SW. Systematic review and meta-analysis of clinical outcomes of early caffeine therapy in preterm neonates. *Br J Clin Pharmacol.* 2017;83(1):180-191.

18. Pakvasa MA, Saroha V, Patel RM. Optimizing Caffeine Use and Risk of Bronchopulmonary Dysplasia in Preterm Infants: A Systematic Review, Meta-analysis, and Application of Grading of Recommendations Assessment, Development, and Evaluation Methodology. *Clin Perinatol.*2018;45(2):273-291.

19. Lodha A, Entz R, Synnes A, et al. Early Caffeine Administration and Neurodevelopmental Outcomes in Preterm Infants. *Pediatrics.*2019;143(1).

20. Ferguson KN, Roberts CT, Manley BJ, Davis PG. Interventions to Improve Rates of Successful Extubation in Preterm Infants: A Systematic Review and Meta-analysis. *JAMA Pediatr.* 2017;171(2):165-174.

21. Davis PG, Schmidt B, Roberts RS, et al. Caffeine for Apnea of Prematurity trial: benefits may vary in subgroups. *J Pediatr.*2010;156(3):382-387.

22. Patel RM, Leong T, Carlton DP, Vyas-Read S. Early caffeine therapy and clinical outcomes in extremely preterm infants. *J Perinatol.*2013;33(2):134-140.

23. Dobson NR, Patel RM, Smith PB, et al. Trends in caffeine use and association between clinical outcomes and timing of therapy in very low birth weight infants. *J Pediatr.* 2014;164(5):992-998 e993.

24. Mohammed S, Nour I, Shabaan AE, Shouman B, Abdel-Hady H, Nasef N. High versus low-dose caffeine for apnea of prematurity: a randomized controlled trial. *Eur J Pediatr.* 2015;174(7):949-956.

25. Steer P, Flenady V, Shearman A, et al. High dose caffeine citrate for extubation of preterm infants: a randomised controlled trial.*Arch Dis Child Fetal Neonatal Ed.* 2004;89(6):F499-503.

26. Wan L, Huang L, Chen P. Caffeine citrate maintenance doses effect on extubation and apnea postventilation in preterm infants. *Pediatr Pulmonol.* 2020;55(10):2635-2640.

27. Brattstrom P, Russo C, Ley D, Bruschettini M. High-versus low-dose caffeine in preterm infants: a systematic review and meta-analysis.*Acta Paediatr.* 2019;108(3):401-410.

28. Mohd Kori AM, Van Rostenberghe H, Ibrahim NR, Yaacob NM, Nasir A. A Randomized Controlled Trial Comparing Two Doses of Caffeine for Apnoea in Prematurity. *Int J Environ Res Public Health.* 2021;18(9).

29. Segerer FJ, Speer CP. Lung Function in Childhood and Adolescence: Influence of Prematurity and Bronchopulmonary Dysplasia]. *Z Geburtshilfe Neonatol.* 2016;220(4):147-154.

30. Saarenpaa HK, Tikanmaki M, Sipola-Leppanen M, et al. Lung Function in Very Low Birth Weight Adults. *Pediatrics.* 2015;136(4):642-650.

31. Kassim Z, Greenough A, Rafferty GF. Effect of caffeine on respiratory muscle strength and lung function in prematurely born, ventilated infants. *Eur J Pediatr.* 2009;168(12):1491-1495.

32. Sanchez-Solis M, Garcia-Marcos PW, Aguera-Arenas J, Mondejar-Lopez P, Garcia-Marcos L. Impact of early caffeine therapy in preterm newborns on infant lung function. *Pediatr Pulmonol.* 2020;55(1):102-107.

33. Doyle LW, Ranganathan S, Cheong JLY. Neonatal Caffeine Treatment and Respiratory Function at 11 Years in Children under 1,251 g at Birth.*Am J Respir Crit Care Med.* 2017;196(10):1318-1324.

34. Kraaijenga JV, Hutten GJ, de Jongh FH, van Kaam AH. The Effect of Caffeine on Diaphragmatic Activity and Tidal Volume in Preterm Infants.*J Pediatr.* 2015;167(1):70-75.

35. Nagatomo T, Jimenez J, Richter J, et al. Caffeine Prevents Hyperoxia-Induced Functional and Structural Lung Damage in Preterm Rabbits. *Neonatology.* 2016;109(4):274-281.

36. Yoder B, Thomson M, Coalson J. Lung function in immature baboons with respiratory distress syndrome receiving early caffeine therapy: A pilot study. *Acta Paediatrica.* 2005;94(1):92-98.

37. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia.*Birth Defects Res A Clin Mol Teratol.* 2014;100(3):145-157.

38. Borszewska-Kornacka MK, Hozejowski R, Rutkowska M, Lauterbach R. Shifting the boundaries for early caffeine initiation in neonatal practice: Results of a prospective, multicenter study on very preterm infants with respiratory distress syndrome. *PLoS One.*2017;12(12):e0189152.

39. Fehrholz M, Hutten M, Kramer BW, Speer CP, Kunzmann S. Amplification of steroid-mediated SP-B expression by physiological levels of caffeine.*Am J Physiol Lung Cell Mol Physiol.* 2014;306(1):L101-109.

40. Rocha G, Proenca E, Guedes A, et al. Cord blood levels of IL-6, IL-8 and IL-10 may be early predictors of bronchopulmonary dysplasia in preterm newborns small for gestational age. *Dis Markers.*2012;33(1):51-60.

41. Floros J, Londono D, Gordon D, et al. IL-18R1 and IL-18RAP SNPs may be associated with bronchopulmonary dysplasia in African-American infants. *Pediatr Res.* 2012;71(1):107-114.

42. Hogmalm A, Bry M, Strandvik B, Bry K. IL-1beta expression in the distal lung epithelium disrupts lung morphogenesis and epithelial cell differentiation in fetal mice. *Am J Physiol Lung Cell Mol Physiol.* 2014;306(1):L23-34.

43. Zhao W, Ma L, Cai C, Gong X. Caffeine Inhibits NLRP3 Inflammasome Activation by Suppressing MAPK/NF-kappaB and A2aR Signaling in LPS-Induced THP-1 Macrophages. *Int J Biol Sci.*2019;15(8):1571-1581.

44. Chen S, Wu Q, Zhong D, Li C, Du L. Caffeine prevents hyperoxia-induced lung injury in neonatal mice through NLRP3 inflammasome and NF-kappaB pathway. *Respir Res.* 2020;21(1):140.

45. Weichelt U, Cay R, Schmitz T, et al. Prevention of hyperoxia-mediated pulmonary inflammation in neonatal rats by caffeine.*Eur Respir J.* 2013;41(4):966-973.

46. Koroglu OA, MacFarlane PM, Balan KV, et al. Anti-inflammatory effect of caffeine is associated with improved lung function after lipopolysaccharide-induced amnionitis. *Neonatology.*2014;106(3):235-240.

47. Chavez Valdez R, Ahlawat R, Wills-Karp M, Nathan A, Ezell T, Gauda EB. Correlation between serum caffeine levels and changes in cytokine profile in a cohort of preterm infants. *J Pediatr.*2011;158(1):57-64, 64 e51.

48. Groslambert M, Py BF. Spotlight on the NLRP3 inflammasome pathway.*J Inflamm Res.* 2018;11:359-374.

49. Liao J, Kapadia VS, Brown LS, et al. The NLRP3 inflammasome is critically involved in the development of bronchopulmonary dysplasia.*Nat Commun.* 2015;6:8977.

50. Chavez-Valdez R, Wills-Karp M, Ahlawat R, Cristofalo EA, Nathan A, Gauda EB. Caffeine modulates TNF-alpha production by cord blood monocytes: the role of adenosine receptors. *Pediatr Res.*2009;65(2):203-208.

51. Endesfelder S, Strauss E, Bendix I, Schmitz T, Buhrer C. Prevention of Oxygen-Induced Inflammatory Lung Injury by Caffeine in Neonatal Rats.*Oxid Med Cell Longev.* 2020;2020:3840124.

52. Reis e Sousa C. Toll-like receptors and dendritic cells: for whom the bug tolls. *Semin Immunol.* 2004;16(1):27-34.

53. Wedgwood S, Gerard K, Halloran K, et al. Intestinal Dysbiosis and the Developing Lung: The Role of Toll-Like Receptor 4 in the Gut-Lung Axis. *Front Immunol.* 2020;11:357.

54. Malash AH, Ali AA, Samy RM, Shamma RA. Association of TLR polymorphisms with bronchopulmonary dysplasia. *Gene.*2016;592(1):23-28.

55. Tunc T, Aydemir G, Karaoglu A, et al. Toll-like receptor levels and caffeine responsiveness in rat pups during perinatal period. *Regul Pept.* 2013;182:41-44.

56. Ren H, Teng Y, Tan B, et al. Toll-like receptor-triggered calcium mobilization protects mice against bacterial infection through extracellular ATP release. *Infect Immun.* 2014;82(12):5076-5085.

57. Chavez-Valdez R, Ahlawat R, Wills-Karp M, Gauda EB. Mechanisms of modulation of cytokine release by human cord blood monocytes exposed to high concentrations of caffeine. *Pediatr Res.* 2016;80(1):101-109.

58. Endesfelder S, Zaak I, Weichelt U, Buhrer C, Schmitz T. Caffeine protects neuronal cells against injury caused by hyperoxia in the immature brain. *Free Radic Biol Med.* 2014;67:221-234.

59. Tiwari KK, Chu C, Couroucli X, Moorthy B, Lingappan K. Differential concentration-specific effects of caffeine on cell viability, oxidative stress, and cell cycle in pulmonary oxygen toxicity in vitro.*Biochem Biophys Res Commun.* 2014;450(4):1345-1350.

60. Endesfelder S, Strauss E, Scheuer T, Schmitz T, Buhrer C. Antioxidative effects of caffeine in a hyperoxia-based rat model of bronchopulmonary dysplasia. *Respir Res.* 2019;20(1):88.

61. Hosoi T, Toyoda K, Nakatsu K, Ozawa K. Caffeine attenuated ER stress-induced leptin resistance in neurons. *Neurosci Lett.*2014;569:23-26.

62. Teng RJ, Jing X, Michalkiewicz T, Afolayan AJ, Wu TJ, Konduri GG. Attenuation of endoplasmic reticulum stress by caffeine ameliorates hyperoxia-induced lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2017;312(5):L586-L598.

63. Tatler AL, Barnes J, Habgood A, Goodwin A, McAnulty RJ, Jenkins G. Caffeine inhibits TGFβ activation in epithelial cells, interrupts fibroblast responses to TGFβ, and reduces established fibrosis in ex vivo precision-cut lung slices. *Thorax.* 2016;71(6):565-567.

64. Wang X, Cui H, Wu S. CTGF: A potential therapeutic target for Bronchopulmonary dysplasia. *Eur J Pharmacol.* 2019;860:172588.

65. Yu H, Konigshoff M, Jayachandran A, et al. Transgelin is a direct target of TGF-beta/Smad3-dependent epithelial cell migration in lung fibrosis. *FASEB J.* 2008;22(6):1778-1789.

66. Fehrholz M, Speer CP, Kunzmann S. Caffeine and rolipram affect Smad signalling and TGF-beta1 stimulated CTGF and transgelin expression in lung epithelial cells. *PLoS One.* 2014;9(5):e97357.

67. Fehrholz M, Glaser K, Speer CP, Seidenspinner S, Ottensmeier B, Kunzmann S. Caffeine modulates glucocorticoid-induced expression of CTGF in lung epithelial cells and fibroblasts. *Respir Res.*2017;18(1):51.

68. Rath P, Nardiello C, Surate Solaligue DE, et al. Caffeine administration modulates TGF-beta signaling but does not attenuate blunted alveolarization in a hyperoxia-based mouse model of bronchopulmonary dysplasia. *Pediatr Res.* 2017;81(5):795-805.

69. Li XY, Xu L, Lin GS, et al. Protective effect of caffeine administration on myocardial ischemia/reperfusion injury in rats.*Shock.* 2011;36(3):289-294.

70. Qi W, Qiao D, Martinez JD. Caffeine Induces TP53-Independent G1-Phase Arrest and Apoptosis in Human Lung Tumor Cells in a Dose-Dependent Manner. *Radiation Research.* 2002;157(2):166-174.

71. Dayanim S, Lopez B, Maisonet TM, Grewal S, Londhe VA. Caffeine induces alveolar apoptosis in the hyperoxia-exposed developing mouse lung. *Pediatr Res.* 2014;75(3):395-402.

72. Jing X, Huang YW, Jarzembowski J, Shi Y, Konduri GG, Teng RJ. Caffeine ameliorates hyperoxia-induced lung injury by protecting GCH1 function in neonatal rat pups. *Pediatr Res.* 2017;82(3):483-489.

73. Dumpa V, Nielsen L, Wang H, Kumar VHS. Caffeine is associated with improved alveolarization and angiogenesis in male mice following hyperoxia induced lung injury. *BMC Pulm Med.* 2019;19(1):138.

74. Yeh CH, Liao YF, Chang CY, et al. Caffeine treatment disturbs the angiogenesis of zebrafish embryos. *Drug Chem Toxicol.*2012;35(4):361-365.

75. Ren X, Chen JF. Caffeine and Parkinson's Disease: Multiple Benefits and Emerging Mechanisms. *Front Neurosci.* 2020;14:602697.

76. Cui WQ, Wang ST, Pan D, Chang B, Sang LX. Caffeine and its main targets of colorectal cancer. *World J Gastrointest Oncol.*2020;12(2):149-172.

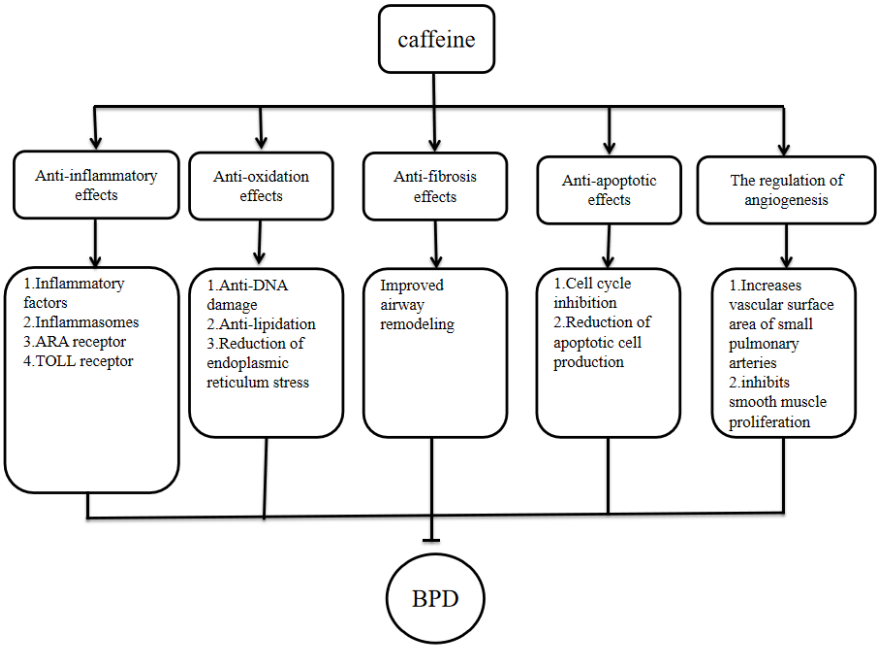

Figure 1:Mechanism of action of caffeine in bronchopulmonary dysplasia