Research Square

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Caffeine citrate improves outcomes of apnea of prematurity based on gestational ages

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Abstract

Objective

This study aimed to evaluate the therapeutic effect of caffeine citrate on early premature infants with apnea of prematurity (AOP), and the impact on movement and neurobehavioral development in their early lives.

Study design

Amongst 397 premature infants whose gestational age (GA) were less than 32 weeks, 172 premature infants were eligible for this study from January 2014 to May 2017. 94 infants received caffeine citrate intervention, 20 infants were given aminophyline and the rest 58 infants didn't received any methylxanthines. According to the incidence of apnea, caffeine using was divided into two situations of therapeutic and preventive administration. The primary clinical outcomes were recorded which included length of stay (LOS), duration of mechanical ventilation, the incidence of complications and outcomes.

Results

62 cases (66%) were assigned to receive caffeine within 3 days after birth, and 69 cases (74%) received caffeine less than one month. Caffeine could reduce LOS, duration of MV and nasal continues positive airway pressure (nCPAP), the incidence of nosocomial infection and bronchial pulmonary dysplasia (BPD) ($p<0.05$). There was no significant difference in the incidence of abdominal distension, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) compared with aminophylline and conservative groups ($P >0.05$). Caffeine showed different effects on clinical outcomes in early premature infants based on their GAs, and premature infants with larger GA could benefit more from the intervention ($p<0.001$), and caffeine didn't affect their early lives in short-term prognosis by follow-up.

Conclusion

Caffeine can improve AOP clinical outcomes in those premature infants with larger GA, and didn't have side effects on the movement and neurobehavioral development in short-term prognosis.

Background

Apnea of prematurity (AOP) is one of the most common problems that occurs in neonatal intensive care unit (NICU), which is connected with gestational age (GA) and birth weight (BW). It occurs 7% to 14% in premature infants born at 32 to 35 weeks, 54% in premature born at 30 to 31 weeks and nearly 100% in those born <30 weeks or BW <1000g (1, 2). The occurrence of AOP is usually considered to be related to the immature control mechanism of respiratory center, that is, the insensitivity to the changes in carbon dioxide (CO_2) and hypoxia, as well as the wrong overreaction causes the closure of the larynx(3, 4). However, under certain situations, such as central nervous system injury, infection, environmental temperature fluctuations, congenital heart disease, metabolic disorders, anemia, upper airway structure abnormalities, necrotizing enterocolitis (NEC), gastroesophageal reflux, and drug products including opioids and general anesthetics can also trigger it (5).

Apnea is classified as central, obstructive or mixed based on the pathological mechanism. Central apnea is characterized by total cessation of inspiratory efforts with no evidence of obstruction (2), and obstructed apnea has obstruction in the upper airways. While mixed apnea is the most common type in preterm infants which has the both components of these two types (6). Methylxanthines, including caffeine, theophylline and aminophylline, are the most common admitted medicine to reduce the frequency and severity of apneic spells in preterm infants since 1970s(5, 7). Combined with the nasal continuous positive airway pressure ventilation (nCPAP), methylxanthines are able to reduce the incidence of apneic episodes and have become one of the common medicine used in neonatal intensive care unit (NICU). Methylxanthines can act by raising central sensitivity to CO_2 concentration and improving respiratory muscle function(8), which together lead to an increase in minute ventilation(9, 10). Recently, caffeine treatment has been reported to improve the rate of survival without neurodevelopmental disability in premature infants of very low birth weight(10), which displays different effects on premature infants by improving breath effort(11, 12).

Also, early caffeine therapy is suggested to reduce the incidence of bronchopulmonary dysplasia (BPD), and therefore decreases the burden of morbidities in preterm infants(13).

Despite the fact that caffeine has achieved widely benefits, there are few reports involved in its effect on early preterm infants of different GAs and their short-time follow-up. In the present study, we analyzed clinical outcomes of caffeine treatment and evaluated its therapeutic effect on AOP in premature infants of GA less than 32 weeks, and found that caffeine could improve premature infants clinical outcomes along with the GA's increase.

Methods

Patients and Methods

This is a retrospectively study conducted at the Neonatal Intensive Care Unit in Zhongnan Hospital of Wuhan University, China. Between January 2014 and December 2017, a total of 397 early premature birth infants were enrolled in this cohort. Premature infants with GA of 26^{+1}- 31^{+6} weeks and BW less than 2000g that were admitted at the NICU immediately after birth were included in this study. Cases were excluded if they had following situations: those had incomplete information, genetic abnormality or congenital disorders, need for two or more methylxanthine drugs or died during hospitalization. The consort flow diagram information is shown in figure 1. From these infants, 185 cases with incomplete information or irregularly methylxanthine drugs using, 37 deaths, and 3 premature infants who received both caffeine citrate and aminophylline were excluded. Finally, 172 premature infants were included for further analysis, from them, 120 cases were identified with apnea, and 52 cases without apnea. Premature infants were divided into five groups based on their apnea occurrence and different interfering protocol. Apnea infants were divided into caffeine, aminophylline and control groups, and infants without apnea were divided into caffeine intervention and controls.

Therapeutic protocol for apnea

All premature infants were kept warmly in incubators and their respiratory tracts were kept unobstructed. Breath, heart beat rate, percutaneous oxygen saturation and blood pressure were monitored. For caffeine intervention group, premature were given caffeine in two conditions: treatment and prevention.

For apnea treatment, the caffeine citrate (Chiesi Farmaceutici SpA, Italy) was administrated immediately by intravenous injection when apnea happened and the initiate loading dose was 20mg/kg (equivalent to 10mg/kg of caffeine base) followed by a daily maintenance dose 10 mg/kg daily (equivalent to 5 mg/kg of caffeine base). If apneas persisted, the daily maintenance dose could be increased to a maximum of 20 mg/kg of caffeine citrate until apnea dispeared. The drug maintenance would be stopped when infants corrected gestational ages were approximately 35 weeks. The same protocol of initial loading dose and maintenance doses of caffeine were given in the first day after birth for prophylactic treatment in those premature infants without apnea. For aminophylline treatment, the aminophylline (Tianjin Jinyao Amino Acid Co. LTD, Tianjin, China) was intravenous injected with a first dose of 5mg/kg, and after 24 hours, the maintenance dose 2.5mg/kg once daily was adopted until the symptoms disappeared in three consecutive days. Infants were given aminophylline only when apnea occurred, and none was treated with aminophylline for apnea prevention.

The infants that received conservative treatment, in other words who didn't receive neither caffeine nor aminophylline, were assigned as controls. According to complications, other adjuvant therapies were adopted which included stimulating foot, adjusting the position, mask oxygen inhalation, and if premature infants appeared with the refratory apnea, the nasal continues positive airway pressure (nCPAP) would be taken for respiratory support. During the treatment in NICU, length of stay (LOS), duration of mechanical ventilation (MV), incidence of necrotizing enterocolitis (NEC), periventricular leukomalacia(PVL), intraventricular hemorrhage (IVH), bronchial pulmonary dysplasia (BPD) and nosocomial infection events were recorded.

Follow-up and development assessment

Follow-up was performed after premature left hospital with recovery. The development information was recorded every month in the first year, every two months in the second year, and six months in the third year, by using Children Development Monitor

System (CDMS, 0-6 Child Care Solution Co., Ltd., Beijing, China). The brain response of preterm infants was recorded with neonatal behavioral neurological assessment (NBNA) at corrected age of 40 weeks. Denver Development Screen Test II (DDST-II) was performed to evaluate children with developmental problems. From 125 items checked over the age range from birth to six-years, each item was scored as pass, fail, or refused, and final evaluations were recorded as normal, abnormal and suspicious.

Informed consent

This study was under the clinical study registration (ChiCTR-ORC-16008872), and approved by the Ethics Committee of Zhongnan Hospital, Wuhan University (protocol 2015019), where the study was performed and all guardians signed the inform consent to publish be included in this study.

Statistics analysis

Data are shown as mean ± SD. The comparison among three groups is analyzed by variance analysis, the comparison between two groups is performed by t-test, the linear regression is analyzed by Graphpad Prism 5.0, and the comparison between counting data is performed by chi-square test. $p < 0.05$ is considered statistically significant.

Results

Clinical information

Of 172 premature infants, 107 (62.2%) were males and 65 (37.8%) were females. The average GA was 29.8 weeks, the average BW was 1329g (500-2000g), of which 133 cases (77.3%) were very low birth weight (VLBW), 15 cases (8.7%) were extremely low birth weight (ELBW). 154 infants were suitable for gestational age (89.5%) and 16 infants were small gestational age (SGA) (9.3%). Of 120 premature infants with apnea, 67 infants were treated with caffeine, 20 infants received with aminophylline, and another 33 infants didn’t receive any methylxanthine drugs (Table 1). 52 premature infants didn’t present apnea, and 27 infants were given caffeine for preventive treatment, another 25 infants didn't receive any methylxanthine agents. BW, gender, GA, Apgar scores at 1 and 5 minute, mode of delivery, times of pulmonary surfactant (PS) use were recorded. There had no statistically significant difference of clinical features in AOP infants between caffeine, aminophylline using with conservative groups ($p>0.05$). The mean age of initial caffeine therapy was 3.8 days of life (DOL, 1 to 19 days), and 70% (84/120) premature infants were exposed to caffeine within 5 days after birth. The median caffeine using time were 23.5 days (2-56 days).

Outcomes of premature infants

Clinical outcomes of premature infants were analyzed based on the effect of caffeine and aminophylline use compared with conservative group (Table 2). Caffeine group showed different effects on premature infants in length of stay (LOS) days (37.3±13.2) which is less than conservative and aminophylline group ($p<0.01$). There was no statistical significance between the aminophylline and conservative group ($p=0.483$). Caffeine reduced the duration the duration of MV(34.0±30.7 hours) compared to aminophylline ($p<0.05$), while reduced the duration of nCPAP (24.4±16.2 days) compared to conservation group, however, there was no significant difference compared with aminophylline group. The positive rates of sputum culture were 30.0% in aminophylline, 27.3% in conservative, and 10.4% in caffeine group ($p=0.033$). The incidence of BPD was 72.7% in conservative treatment group, 65% in minophylline group, and 34.3% in caffeine group ($p<0.001$). Blood culture was positive in only one case caffeine groups, and there was no statistical significance. Both caffeine and aminophylline increased the proportion of abdominal distension and the incidence of necrotizing enterocolitis (NEC), but the difference have no statistical significance ($p>0.05$). There were no significant differences in incidences of intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and patent ductus arteriosus (PDA) among three groups. Compared to aminophylline and controls, premature infants could benefit from caffeine treatment in reducing the length of stay, duration of MV and decreasing the incidence of BPD.

Prophylactic and early caffeine using

Prophylactic caffeine using had been recommended in premature infants earlier before apnea occurrence in recent years(14). Prophylactic treatment is different from early caffeine using, which is admitted in 2 days after birth whether apnea occurs or not.

For caffeine preventive intervention, infants were given drugs without the occurrence of apnea, especially in the situation of extubation. The median of caffeine initial times was 3 days DOL (2-7days). As shown in table 3, of 52 infants without apnea occurrence, 27 infants were given caffeine for apnea prevention, another 25 premature were assigned as controls. There were no significant statistically differences between the two groups in LOS, duration of MV, the incidence of PVL and IVH, as well as the positive rate of sputum or blood culture. However, prophylactic use of caffeine could decrease the risk of development of BPD (P=0.024).

67 premature infants with apnea were divided into two groups to evaluate the effect of caffeine on clinical outcomes according to their caffeine using occasion. Infants who were given caffeine within 48 hours after birth were defined as early caffeine using, and the others were defined as later using. The mean hospitalization days in the early group (36.82±13.01 days) were longer than those in the later group (34.25±10.92, days), but there was no significant difference (p=0.172), while the MV duration in the early group was shorter than that in the later medication group (24.30±16.77 hours, 40.58±36.08 hours) (p=0.02). In addition, there was no significant difference in other complications between the two groups (Table 4).

Effect of caffeine on infants of different GAs

The maturation of premature respiratory center development could affect their reaction to caffeine stimulation. Hence, we analyzed the therapeutic effect of caffeine on premature infants with different GA who were divided into less than 29 weeks, $30^{0\text{-}6\ wks}$ and $31^{0\text{-}6\ wks}$. It showed that caffeine significantly reduced the LOS, the duration of MV, NCPAP or nasal cannula oxygen regardless of GA, and the incidence of BPD decreased with the increase of GA (Table 5). In the GA less than 29 weeks, caffeine reduced LOS ($p<0.001$), and the positive rate of sputum culture ($p<0.05$), while in premature infants of $30^{0\text{-}6\ wks}$, caffeine reduced the duration of CPAP and cannula using and the incidence of BPD, in premature infants of GA with $31^{0\text{-}6\ wks}$, caffeine significantly shortened the duration of MV using. There was no difference in the incidence of NEC, abdominal distension, PDA and PVL/IVL among three GA groups ($p>0.05$).

Linear regression analysis was performed to evaluate the relative of caffeine treatment to clinical outcomes with GAs. As figure 2 shows, LOS is negative related to GA growth in caffeine, prevention and conservation group (Figure 2a), however, only caffeine showed statistic significance ($p<0.001$). The same results were observed in MV and CPAP, in which caffeine treatment reduced the duration of respiratory support along with the GA growth (Figure 2b, 2c). These results infer that premature infants could be benefit more by caffeine intervention in the clinical outcome along with GA growth ($p<0.001$).

Follow-up of premature infants

77 infants were regularly followed-up by Children Development Monitor System successfully in 6 months to three years after their leaving hospital. Among them, 56 received caffeine treatment, and 21 cases didn't receive any methylxanthine (Table 6). One infant died of severe pneumonia at sixth month, another premature infant without caffeine using was diagnosed with cerebral palsy. All infants that were followed-up were evaluated by DDST-II regularly each month in the first year, every two months in the second year and six months in the third year. The test results were interpreted as normal, suspicious and abnormal (if untestable over three times). If infants had abnormal development in movement and language aspects, the test results were re-judgment after a period of rehabilitation intervention until the test results were normal. Finally, 46 (82.1%) infants were normal, 7 (12.5%) infants abnormal and 3 (5.4%) suspicious in caffeine group, there was no significant difference compared with the conservative controls (p=0.345).

Discussion

In this study, we analyzed the effect of caffeine citrate on early premature infants, and found that caffeine improved premature clinical outcomes in reducing the duration of MV, the length of hospitalization stay, and decreased the incidence of BPD. Prophylactic caffeine using reduced the incidences of BPD, while early caffeine using reduced the duration of MV. Premature infants with larger GA could benefit more in improving clinical outcomes by using caffeine, and shown no developmental side-effect during short-term follow-up.

AOP is a common and troublesome disorder which requires intervention to avoid potential morbidity in premature infants who need neonatal intensive care(15). The pathogenesis of apnea is due to immaturity of the respiratory control systems characterized by an abnormal ventilatory response to carbon dioxide and hypoxia combined with immature reflex responses(16). Caffeine, theophylline and aminophylline are commonly available forms of methylxanthine, which function in exciting the respiratory center, are widely used in the treatment of AOP(17, 18). In hemodynamic changes, caffeine demonstrates the similar effects on cardiac parameters as aminophylline, however, caffeine-treated small-for-gestation stratification gave rise to significant cardiac variations(19, 20)

Premature infants can benefit from caffeine intervention by improving the first successful extubation, reducing the frequency of apnea and the duration of MV(10, 17). Our results are consistent with previous reports that caffeine also reduced the positive rate of sputum culture compared with the aminophylline group. This could be contributed to decreased nosocomial of infection resulting of shortened intubation time. Most of these infants received high-dose intravenous immunoglobulin therapy for the prevention of infection and magnetic resonance imaging check for the evaluation of central nervous system injury(21, 22), there was no difference in the incidence of NEC and IVH. Caffeine therapy also reduces the incidence of BPD in infants with VLBW(23-26), it has been reported that caffeine is the blockade of the adenosine 2 receptor, which acts on inflammation and increases permeability and remodeling in the lung(27).

In recent years, it has been suggested that premature infants can be benefited from earlier using of caffeine, which was associated with improved hemodynamics(28) and reduced incidence and severity of acute kidney injury (29, 30). Early caffeine using was associated with improved blood pressure and systemic blood flow, and heart rate, left ventricular output, and stroke volume were not significantly affected. Cerebral oxygenation transiently decreased after caffeine administration (20, 30). However, in our study, there was no significant difference of earlier caffeine treatment compared with the controls in clinical outcomes, besides the MV duration. The reason could be the small samples, and should be accumulated more cases in future. Different from early using, prophylactic caffeine administration means the medicine is given before the occurrence of apnea, which has no initial time limitation and usually is preparation for extubation. By using this protocol, caffeine can prevent apnea in very premature infants almost four times decrease compared with the control group (14). In this study, we lost the data of relationship between prophylactic caffeine using with apnea occurrence, but we found that caffeine prevention only reduced the BPD occurrences, and there were no difference in other clinical features. These results are consistent with recent literatures and more evidence of higher quality is needed to guide optimal caffeine use (31, 32).

Another consideration is referred to the effect of caffeine using on different GAs, especially in those extremely early premature. We found that caffeine decreased the LOS and positive rates of sputum culture in GA less than 29, decreased the LOS, duration of CPAP and BPD in of GA 30^{0+6wks}, while LOS, duration of CPAP, MV and BPD in GA of 31^{0-6wks}. These results imply that the smaller the GA of premature infants was, the less they benefited. There is no reasonable explanation for that, one speculation may be owe to the immaturity of the early premature infants organs. Up to now, there are few studies involved in the effect of caffeine therapeutics on different GA(33), and some studies discussed caffeine effects on VLBW(14), evaluation is needed more clinical observation.

Despite caffeine benefits for preterm infants with AOP, the adverse effect of molecular and cellular on the infants developing brain must be vigilant, regardless of dose or duration of administration(34). Caffeine is an inhibitor of adenosine receptors, while adenosine was showed to play an important neuroprotective role in the initial stages of energy failure in anoxic and ischaemic mammalian brain and protecting the brain from cell death(35). Follow-up study shown that two dosing regimens of caffeine citrate for neonates born less than 30 weeks gestation did not result in adverse outcomes for development, temperament and behavior in 1 year later(36). Our data shown that using caffeine didn't increase the risk of neurological complications, among those follow-up infants, 82.1% infants were normal, 12.5% abnormal, and 5.4% suspicious in the duration of 6 months to 3 years, and there was no significant difference compared with those controls. Caffeine did not affect the development of the preterm infant's movement and language in their early life. However, there are limitations in this study that is a retrospectively observation, and some groups samples are not large enough.

In conclusion, caffeine treatment of AOP is beneficial for premature infants outcomes, due to the facts that it probably shortened hospitalization days, duration of MV and nCPAP, and reduced the incidence of BPD without increasing the risk of NEC. The lager

GA babies can benefit more than lower GA in early preterm infants. Further follow-up study, specifically well-designed randomized controlled trials, are needed to assess the safety and efficacy of caffeine especially on long-term neurodevelopmental outcomes.

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Declarations

Ethics statement

This study was under the clinical study registration (ChiCTR-ORC-16008872), and approved by the Ethics Committee of Zhongnan Hospital, Wuhan University (protocol 2015019), where the study was performed and all guardians signed the inform consent to publish be included in this study.

Consent to publish: All guardians signed the inform consent for publication.

Competing interests: The authors declare no competing interests.

Authors' contributions: LL collected the samples, performed the detection and wrote this paper. WX, YP and ZJ collected samples and analyzed the data. DZ designed this project and wrote this paper.

Availability of data and materials: All data and materials are availability.

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Abbreviations

nCPAP: nasal continuous positive airway pressure；LOS: length of stay；MV: mechanical ventilation；AP: ventilator-associated pneumonia；BPD: bronchial pulmonary dysplasia；IVH : intraventricular hemorrhage；NEC: necrotizing enterocolitis；PVL: periventricular leukomalacia； PDA : patent ductus arteriosus；AOP : Apnea of prematurity；NICU: neonatal intensive care unit；GA: gestational age；BW: birth weight；SGA: small gestational age；VLBW: very low birth weight；ELBW: extremely low birth weight；PS: pulmonary surfactant；MRI: magnetic resonance imaging；DOL: day of life

Tables

Table 1. Clinical features

Characteristics	Aminophylline	Caffeine	Conservative	F or X^2	P_1[a]	P_2[b]	P_3[c]	P[d]
	(n=20)	(n=67)	(n=33)					
GA, week, x ± s	29.36 ± 1.09	29.55 ± 1.30	29.95± 1.02	2.042	0.530	0.088	0.127	
BW,kg, x ± s[e]	1.29 ± 0.18	1.31 ± 0.22	1.37 ± 0.25	1.118	0.781	0.224	0.198	
PS,times, x ± s	1.2 ± 0.61	1.01 ± 0.36	1.15 ± 0.50	1.314	0.504	0.987	0.433	
Apgar, 1 min, x ± s	6.25 ± 1.2	6.21 ± 1.21	6.36 ± 1.22	0.180	0.895	0.742	0.550	
Apgar 5 min, x ± s	7.75 ± 0.96	7.61± 1.02	7.61 ± 1.05	0.159	0.599	0.622	0.979	
Delivery mode	11 (55%)	45 (67.2%)	23 (69.7%)	1.315				0.518
Cesarean,n[f] (%)								
SGA, n (%)	0	6 (9%)	3(9.1%)	2.743				0.602
Sex, male, n (%)	16 (80%)	42 (62.7%)	18 (54.5%)	3.502				0.174

Note: GA, gestation age; BW, birth weight; PS, pulmonary surfactant; SGA, small gestational age. a: P1 value: the compare between aminophylline group and Caffeine group. b: P2 value: the compare between aminophylline group and Conservative group. c: P3 value: the compare between Caffeine group and Conservative group. d: P value: For categorical data, using the Pearson chi-square test P values. Same as the other Tables. e: Mean ± standard deviation. f: Numbers. Same as the other tables.

Table 2. Different protocols on the premature clinical outcomes

	Aminophylline	Caffeine	Conservative	F or X^2	P_1	P_2	P_3	P
Clinical outcomes								
	(n=20)	(n=67)	(n=33)	value				
LOS, days, x ± s	47.50 ± 14.64	37.30±13.17	50.06 ±10.88	12.31	0.002	0.483	<0.001	
MV, hours, x ± s	70.10 ± 40.31	34.01±30.73	56.55±44.77	7.723	0.003	0.592	0.036	
CPAP, days, x ± s	30.05 ± 16.06	24.42±16.18	33.76 ± 9.95	5.060	0.440	0.731	0.002	
Blood culture, positive, n (%)	0	1 (1.5%)	0	NA				NA
Sputum culture, positive, n (%)	6 (30%)	7 (10.4%)	9 (27.3%)	10.517				0.033
Abdominal distension, n (%)	16 (80%)	41 (61.2%)	18 (54.5%)	3.553				0.169
NEC, n (%)	3 (15%)	9 (13.4%)	3 (9.1%)	0.518				0.772
PDA, n (%)	2 (10%)	4 (6%)	2 (6.1%)	4.026				0.403
BPD, n (%)	13 (65%)	23 (34.3%)	24 (72.7%)	15.200				0.001
PVL or IVH, n (%)	5 (25%)	19 (28.4%)	12 (36.4%)	3.573				0.467

Note: LOS, length of stay; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchial pulmonary dysplasia; PVL, periventricular leukomalacia; IVH, intraventricular hemorrhage. a: P1 value: compared between caffeine and

aminophylline group. b: P2 value: compared between aminophylline and conservative group. c: P3 value: compare between caffeine and conservative group. d: P value: Pearson chi-square test.

Table 3. The effect of prophylactic caffeine on the clinical outcomes

	Prophylactic	Control	X value	P value
Clinical outcomes				
	(n=27)	(n=25)		
LOS, days, x ± s	32.85±11.63	35.24±8.61	0.836	0.407
MV, hours, x ± s	33.26±16.46	30.64±28.75	0.407	0.686
CPAP or nasal cannula, days, x±s	22.30±15.66	24.24±12.68	0.489	0.627
Blood culture, positive, n (%)	3 (11.1%)	2 (8%)	0.145	0.704
Sputum culture, positive, n (%)	4 (14.8%)	5 (20%)	5.298	0.071
Abdominal distension, n (%)	17 (63%)	12 (48%)	1.178	0.278
NEC, n (%)	1(3.7%)	2 (8%)	0.441	0.507
PDA, n (%)	1(3.7%)	0	NA	NA
BPD, n (%)	5(18.5%)	12 (48%)	5.127	0.024
PVL or IVH, n (%)	5(18.5%)	9 (36%)	2.755	0.252

Note: LOS, length of stay; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchial pulmonary dysplasia; PVL, periventricular leukomalacia; IVH, intraventricular hemorrhage.

Table 4. Clinical outcome of early and late caffeine using

Clinical outcomes	Early using	Late using	X^2 or T	p value
	(n=27)	(n=40)		
LOS，days	36.82±13.02	34.25±10.92	2.386	0.172
Duration of MV，hours	24.31±16.82	40.58±36.08	2.484	0.016
Duration of CPAP or nasal cannula, days	28.70±18.01	21.55±14.36	1.795	0.077
Blood culture, positive, n (%)	1(3.7%)	0	NA	NA
Sputum culture, positive, n (%)	4(14.8%)	3(7.5%)	2.529	0.282
Abdominal distension, n (%)	18(66.7%)	23(57.5%)	0.570	0.450
NEC, n (%)	4(14.8%)	5(12.5%)	0.074	0.785
PDA, n (%)	2(7.4%)	2（5%）	0.639	0.727
BPD, n (%)	10(37%)	13(32.5%)	0.147	0.701
PVL or IVH, n (%)	8(29.6%)	11(27.5%)	0.036	0.982

Note: LOS, length of stay; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchial pulmonary dysplasia; PVL, periventricular leukomalacia; IVH, intraventricular hemorrhage.

Table 5. The effect of caffeine on premature infants of different GAs

Clinical outcomes	≤29 week			30 week			31 week		
	Conservative (17)	Caffeine (40)	p value	Conservative (8)	Caffeine (11)	p value	Conservative (8)	Caffeine (16)	p value
LOS, days, x ± s	54.5±10.3	41.5±13.5	0.001	48.5±8.9	34.9±8.9	0.005	42.4±9.9	25.6±10.4	0.005
MV, hours, x ± s	51.2±37.2	41.5±30.3	0.304	47.5±50.0	31±42.4	0.448	76.9±53.4	17.4±10.9	0.016
CPAP or cannula oxygen, days, x±s	33.1±11.5	31.2±15.1	0.644	33.9±8.0	19±13.9	0.015	35.1±9.2	11.3±10.1	0.000
Abdominalbdominal distension, n (%)	9（52.9%）	22（55%）	0.886	6（75%）	9（81.8%）	0.719	2（25%）	10（62.5%）	0.083
NEC, n (%)	2（11.8%）	3（7.5%）	0.603	0	3（27.3%）	NA	1（12.5%）	3（18.8%）	0.699
Blood culture, positive, n (%)	0	0	NA	0	0	NA	0	1（6.3%）	NA
Sputum culture, positive, n (%)	5（29.4%）	3（7.5%）	0.023	2（25%）	1（9.1%）	0.473	2（25%）	3（18.8%）	0.309
PDA, n (%)	2（11.8%）	2（5%）	0.587	0	NA	0.292	0	1（6.3%）	NA
BPD, n (%)	11（64.7%）	20（50%）	0.308	6（75%）	2（18.2%）	0.013	7（87.5%）	1（6.3%）	0.000
PVL or IVH, n (%)	6（35.3%）	8（20%）	0.471	3（37.5%）	6（54.5%）	0.463	3（37.5%）	5（31.3%）	0.626

Note: LOS, length of stay; MV, mechanical ventilation; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchial pulmonary dysplasia; PVL, periventricular leukomalacia; IVH, intraventricular hemorrhage.

Table 6. Follow-up of premature infants

Outcome	Caffeine (56)	Conservative (21)	X^2 value	p value
Normal, n(%)	46（82.1%）	14（66.7%）		
Abnormal, n(%)	7（12.5%）	5（23.8%）		
Suspicious, n(%)	3（5.4%）	2（9.5%）	2.131	0.345

Figures

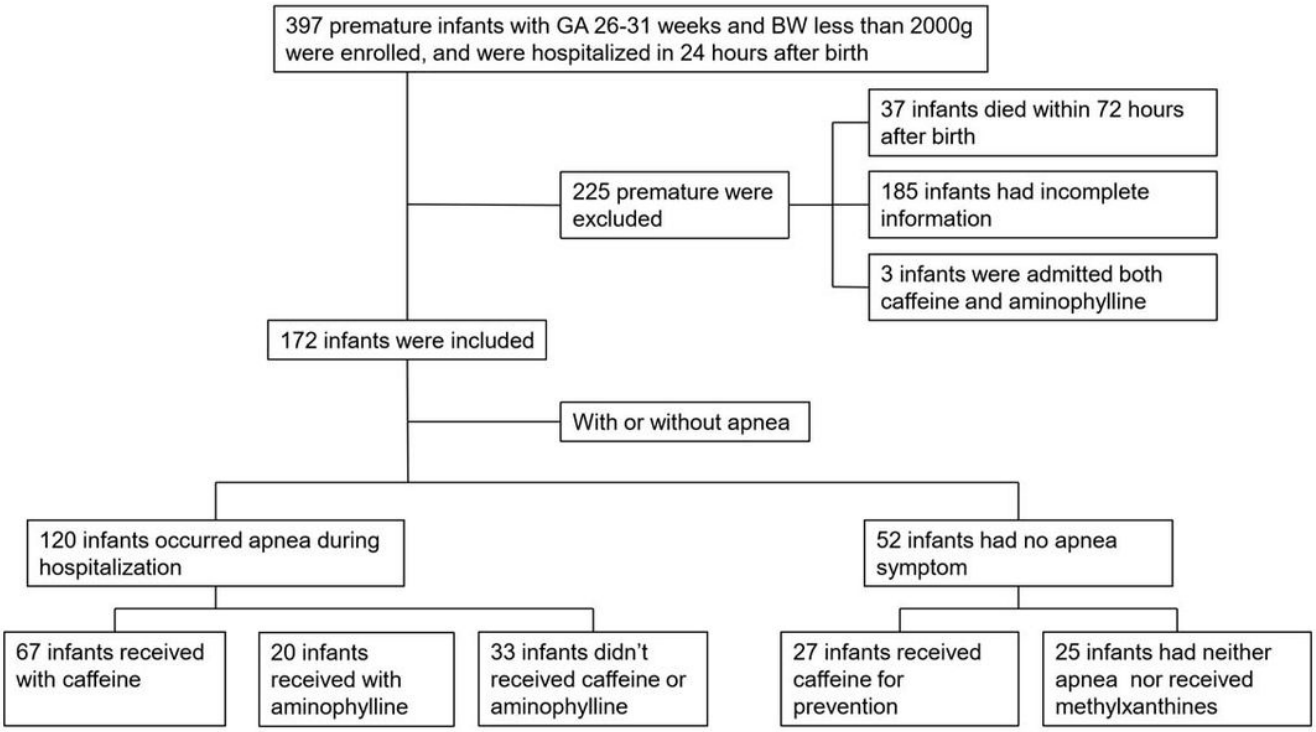

Figure 1

The information of CONSORT flow diagram

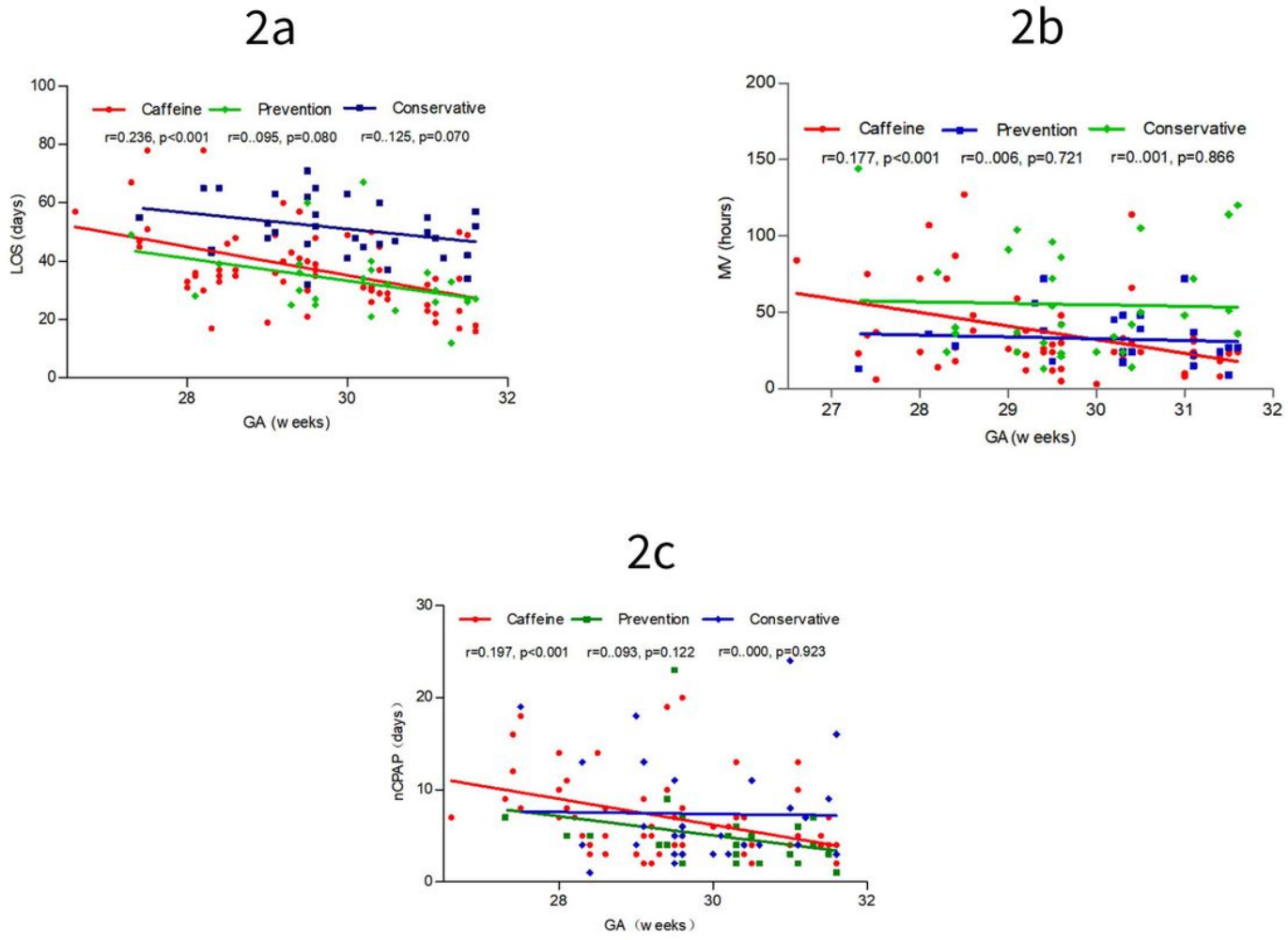

Figure 2

Caffeine treatment is correlated to premature infants clinical outcomes with GA. a, linear regression analysis of length of stay related to gestation age (95% CI of slope, caffeine -7.067 to -2.712; prevention, -5.869 to 0.3545; conservative -7.983 to 0.3406). b, linear regression analysis of length of stay related to the duration of mechanical ventilation (95% CI of slope, caffeine -13.72 to -4.111; prevention, -7.952 to 5.593; conservative -12.83 to 10.86). c, linear regression analysis of the duration to continuous positive airway pressure(95% CI of slope, caffeine -2.087 to -0.7302; prevention, -2.364 to 0.2965; conservative -2.219 to 2.016).