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Stability of Aminophylline, Ceftriaxone Sodium and Ampicillin/Sulbactam in Total Parenteral Nutrient Solution

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고영양수액제 중 아미노필린, 세프트리악손 및 암피실린/설박탐의 안정성에 관한 연구

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Aminophylline, ceftriaxone 및 ampicillin/sulbactam (Unasyn)을 미숙아용 고영양수액제에 직접 첨가하거나 Y-site로 투여하는 경우의 안정성에 관해 조사하였다. Aminophylline 주사액 (25 mg/mL) 300 μl와 ceftriaxone sodium (37.5 mg/mL) 2 mL를 각각 고영양수액제에 직접 첨가하였다. 또한, Y-site에서의 안정성 조사를 위해 ceftriaxone sodium (37.5 mg/mL)을 고영양수액제에 각각 1:1 및 1:2의 부피비가 되도록 혼합하였고 Unasyn (25 mg/mL)은 고영양수액제와 1:1의 부피비로 혼합하였다. 이상과 같이 조제한 혼합액을 25°C와 4°C에 보관하여 aminophylline은 48시간 동안 그리고 항생제들은 24시간 동안의 경시변화를 HPLC를 이용하여 분석하였다. Aminophylline은 위 보존조건에서 48시간동안 안정하였다 (변화율 < 10%). Ceftriaxone sodium을 고영양수액제에 직접 첨가한 경우 ceftriaxone의 잔존률은 25°C에서 4시간째에 90.5±1.8%이었고 4°C에는 95.1±1.4%로 측정되었다. Y-site에서의 안정성과 관련하여 ceftriaxone sodium을 고영양수액제와 1:1로 혼합한 경우 양 보존조건에서 ceftriaxone은 24시간 동안 안정하였으나 1:2로 혼합한 경우에는 4°C 보관 시에만 안정하였고 25°C에서는 24시간째에 약 14% 정도 분해되는 것으로 나타났다. 한편, Unasyn의 경우 ampicillin은 24시간째에 4°C에서는 안정하였으나 25°C에서는 30%까지 감소되는 것으로 분석되었고 sulbactam은 24시간째에 온도와 관계없이 안정한 것으로 나타났다. Ceftriaxone sodium을 TPN과 Y-site 혼합 후 1-2시간 이내에 침전이 형성되었고 Unasyn의 경우에는 12시간째에 침전이 형성되었다. 혼합액의 pH나 색상은 연구기간 동안 일정하였다. 이러한 연구결과에 기초할 때, aminophylline은 고영양수액제와 혼합가능하고 ceftriaxone과 Unasyn은 고영양수액제와 혼합시 최소 1시간 동안은 안정한 것으로 평가되었다.

□ Key words - Stability, Aminophylline, Ceftriaxone, Unasyn, TPN

Aminophylline is frequently administered to premature infants because recurrent apnea is a common complication of prematurity. Repeated episodes of apnea lasting longer than 20 seconds are known to cause irreversible neurologic damage secondary to hypoxia and acidosis, and eventually result in death if untreated[1]. Aminophylline decreases the frequency of apnea by increasing respiratory minute volume and the ventilatory response to carbon dioxide, and decreasing the frequency of hyperoxic and hypoxic episode[2-3].

To prevent central venous catheter-related bacterial infection in premature neonates who receive the total parenteral nutrient (TPN) therapy, some antibiotics are used. In our institution, ceftriaxone sodium or ampicillin/sulbactam is frequently being prescribed, which is reported to be resistant to many of the bacterial β-lactamases and has good activity against many gram-positive and gram-negative aerobic bacteria[4].

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Due to limited number of intravenous sites, it is valuable to reduce a second administration site by co-infusion. However, only limited data are available to support the combination of medications and TPN solution. In this study, we evaluated the stability of aminophylline, ceftriaxone sodium and ampicillin/sulbactam when they are added to TPN solution directly and/or under simulated Y-site administration.

Methods

Preparation of Mixtures. All TPN solutions were compounded in ethylene vinyl acetate sterile bags[a] in a clean room under a horizontal-laminar-airflow hood (Table 1). Aminophylline injection[b], ceftriaxone sodium[c] and ampicillin/sulbactam[d] were used for the stability studies. Before the experiments, ceftriaxone sodium and ampicillin/sulbactam were constituted with distilled water for injection to produce the concentrations of 37.5 and 25 mg/mL, respectively.

For the stability of aminophylline in TPN solution, 300 μl of aminophylline injection (25 mg/mL) was added to TPN (538.5 mL); three sets were stored at 4 °C in a refrigerator and another three sets at 25°C in a temperature-controlled water bath[e]. Ten milliliter of samples was taken immediately after mixing and at 1, 12, 24 and 48 hr, and assayed by HPLC. The pH of the mixtures was measured using a pH meter[f] on each of the sampling times. The appearance and color of the mixtures were assessed by observing them against black and white background.

Table 1. TPN formula for premature infants

Components	Volume (mL)
Dextrose 20%	300
Amino acid 8.5%	70
Aqua	130
Heparin (2500 Unit)	0.1
NaCl (Na-40 mEq)	4
KCl (K-40 mEq)	1
$MgSO_4$	4
KH_2PO_4	4.5
$ZnSO_4$	1.5
Ca-gluconate	20
$CuSO_4$	1
Multivitamin	2.5
Total Volume	538.5
Calorie	227.8 kcal

The stability of ceftriaxone sodium and ampicillin/sulbactam was examined under conditions simulating administration via a Y-site injection into TPN solution by the method reported in the literature[5]. The constituted antibiotics were added to TPN solution to achieve 1:1 (ampicillin/sulbactam), and 1:1 and 1:2 (ceftriaxone sodium) ratios of drug solutions to TPN solution. Samples of these admixtures were stored at 4 and 25°C and assayed by HPLC immediately after mixing and at 1, 2, 4, 8, 12 and 24 hr. The constituted ceftriaxone sodium (37.5 mg/mL) 2 mL was also directly added to TPN solution (538.5 mL); the mixture was stored at the same temperatures as the above and assayed by HPLC immediately after mixing and at 1, 2 and 4 hr. The changes of pH, appearance and color were also tested. All of the samples were prepared in triplicate.

High-performance liquid chromatography (HPLC). Slightly modified HPLC methods of aminophylline[6], ceftriaxone sodium[7] and ampicillin/sulbactam[8] were employed. The HPLC system consisted of a pump[g] with a UV detector[h], and an automatic injector[i]. An ODS column[j] was used for all samples studied. The mobile phases used for the HPLC analysis of aminophylline, ceftriaxone sodium and ampicillin/sulbactam were 9% acetonitrile in phosphate buffer (0.25 N, pH 2.5), phosphate buffer (0.2 N, pH 7.4) methanol acetonitrile (100:20:10) and tetrabutylammonium hydroxide (0.05 M, pH 5.0) · acetonitrile (165:35), respectively. For the assay of aminophylline, ceftriaxone sodium and ampicillin/sulbactam, the mobile phase prepared was delivered at a flow rate of 1.0, 0.7 and 1.5 mL/min and the UV wavelength was 275, 270 and 230 nm, respectively.

To determine that the drugs' breakdown products did not interfere with the parent compounds, acid degradation method was employed, which 5 mL of stock solution in its mobile phase (1000 μg/mL) and 5 mL of 6N HCl were combined and placed at 60°C in a temperature-controlled water bath[e]. And then, they were neutralized to pH 4-5 with 6N NaOH. The resulting solution was analyzed by our chromatography methods.

Data analysis. The stability of drugs was assessed by evaluating the percentage of the initial concentration remaining at each time interval. Less than 90% recovery was considered to indicate instability.

Results

Chromatographic analysis of the solution obtained after deliberate degradation of the drug revealed that no breakdown products interfere with the chromatographic peak of its parent compound. The retention times of ceftriaxone, ampicillin and sulbactam were 5.4, 3.9 and 12.6 min while the retention times of their breakdown products were 4.4, 9.2 and 25.2 min, respectively. It was found that aminophylline had two degraded products, and the retention times of the parent product and the degraded products were 7.4, 10.6 and 14.9, respectively. Likewise, no interference of the drug peak with other nutrients was detected.

Aminophylline was stable in TPN solution during the study period. The percent initial concentration remaining was 100.8±2.4, 101.2±1.1, 100.6±10.3 and 98.7± 9.3 at 1, 12, 24 and 48 hr at 25°C respectively. Similar results were obtained in a refrigerator; the percent initial concentration remaining at 48 hr was 102.1±4.0. All mixtures remained clear with no visual indication of physical instability. The pH variations of all test solutions were minimal, which ranges from 5.6 to 6.2.

As listed in Table 2, ceftriaxone sodium was stable for 24 hr when it was mixed with TPN solution in the 1:1 ratio. In the 1:2 ratio, it had significant (≥10%) degradation at 25°C at 24 hr even though it was stable for 24 hr in a refrigerator. Trace microparticulates were formed 1-2 hr after mixing regardless of the temperature or ratio. When ceftriaxone sodium was directly added to TPN solution, its recovery at 4 hr at 25°C and under refrigeration was 90.5±1.8 and 95.1±1.4%, respectively; the degradation was more rapid than that in the Y-site simulation. However, any precipitate was not observed in this mixture.

Another antibiotics studied, ampicillin and sulbactam were physically compatible with TPN solution up to 12 hr. The mixture appeared clear. Participate was formed around 24 hr after mixing. As listed in Table 3, ampicillin was stable at 4 hr after mixing with TPN solution and degraded significantly from 8 hr; its concentration decreased by almost 30% at 24 hr at 25°C. When the mixture was stored in a refrigerator, ampicillin concentration was not changed. On the contrary, sulbactam

Table 2. Stability of ceftriaxone in 1:1 (18.75 mg/mL) and 1:2 (12.5 mg/mL) mixture of ceftriaxone sodium and TPN

Time after mixing	% Initial Concentration Remaining			
	1 : 1		1 : 2	
	25°C	4°C	25°C	4°C
1 hr	98.5 ± 6.7	101.3 ± 7.6	101.2 ± 1.7	96.6 ± 4.2
2 hr	97.7 ± 10.6	99.8 ± 6.5	97.3 ± 6.2	96.9 ± 4.1
4 hr	98.2 ± 5.7	103.9 ± 4.0	94.7 ± 8.1	101.1 ± 3.3
8 hr	103.1 ± 3.3	96.7 ± 7.2	95.2 ± 5.2	102.2 ± 2.7
12 hr	100.4 ± 7.5	100.1 ± 6.2	94.8 ± 5.2	99.3 ± 8.3
24 hr	102.2 ± 5.6	105.7 ± 4.6	86.6 ± 4.7	94.5 ± 3.2

Data represent the mean±S.D. (n=3).

Table 3. Stability of ampicillin and sulbactam in 1:1 Mixture (12.25 mg/mL) of Unasyn and TPN

Time after mixing	% Initial Concentration Remaining			
	Ampicillin		Sulbactam	
	25°C	4°C	25°C	4°C
1 hr	96.4 ± 1.9	104.5 ± 5.2	100.2 ± 3.4	98.4 ± 1.3
2 hr	100.7 ± 3.0	101.7 ± 4.3	99.0 ± 1.0	105.8 ± 6.1
4 hr	93.3 ± 1.0	100.3 ± 3.5	99.9 ± 6.2	103.6 ± 2.4
8 hr	87.8 ± 3.7	101.5 ± 2.8	99.1 ± 1.9	104.1 ± 7.3
12 hr	82.1 ± 9.9	104.2 ± 5.9	98.7 ± 1.2	104.3 ± 3.6
24 hr	72.2 ± 4.7	101.7 ± 0.1	98.7 ± 1.5	103.8 ± 0.3

Data represent the mean±S.D. (n=3).

was stable regardless of the temperature.

There were no appreciable pH or color changes of all solutions studied during the study period.

Discussion

Based on the results, it was thought that aminophylline could be mixed with TPN solution for premature infants. Ceftriaxone sodium in TPN solution was more stable as the portion of ceftriaxone sodium increased in the room temperature. However, in that case, the mixture was more physically incompatible. Thus it was thought that the microparticulate formation in the mixture of 1:1 or 1:2 ratio was attributed to the precipitation of some components in TPN solution with addition of relatively large amount of ceftriaxone sodium. Even though ceftriaxone sodium was stable and compatible up to 12 hr when it was directly added to TPN solution, direct admixture of ceftriaxone sodium in a TPN bottle is not practical. In the Y-site administration, ceftriaxone sodium with TPN solution at the 1:1 or 1:2 ratio was compatible and stable for at least 1 hr. Ampicillin and sulbactam were physically compatible with TPN at the 1:1 ratio for at least 12 hr at 25°C or under refrigeration. Also, the drugs were stable for 2 hr. Even though actual contact time between antibiotics and TPN solution during administration via a Y-site would be less than 1 hr in clinical practice, considering that the stability of TPN was not evaluated in this study, health care practitioners should be cautious when these antibiotics infuse during Y-site infusion of TPN.

[a]Ethylene vinyl acetate sterile bags, Baxter Co., Seoul, Korea

[b]Aminophylline injection 250 mg/10 mL, Daewon Pharmaceutical Co., Ltd., Seoul, Korea, lot D010

[c]Triaxone 500mg, Hanmi Pharmaceutical Co., Ltd., Seoul, Korea, lot H20004

[d]Unasyn 750mg, Pfizer Pharmaceuticals Korea Ltd., Seoul, Korea, lot 12512108

[e]Water bath, BS-06, Jeio Tech, Seoul, Korea

[f]pH meter, WPA CD500, Linton, Cambridge, United Kingdom

[g]HPLC pump, LC-10AD, Shimadzu, Tokyo, Japan

[h]HPLC UV detector, SPD-10A, Shimadzu, Tokyo, Japan

[i]HPLC automatic injector, SIL-10A, Shimadzu, Tokyo, Japan

[j]HPLC ODS column, μ Bondapak C18, Waters Co., Milford, MA, USA

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