Effect of Triglyceride Chain Length on the Stability of Total Nutrient Admixtures

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Background : The triglyceride (TG) and long chain triglyceride (LCT) are the main components in TNA. Therefore, in this study, this research was conducted to compare the stability of TNA on the real and cold storage conditions with regard to the composition of LCT and MCT. Results : The TNA was prepared using LCT and MCT composition to compare the stability with TNA with one containing LCT only. As a result, no apparent changes were found in pH, osmolality, electrolytes, amino acids, glucose, triglyceride, total protein, sodium, vitamins, and minerals, etc.

Conclusion : The results of this study suggest that TNA with high osmolality can be used as a safe and effective alternative to IVH (intravenous hyperglycemia) or TNA with high osmolality. Therefore, TNA with high osmolality can be used as an alternative to IVH and TNA with LCT only.

Key words - LCT, MCT, TNA, Stability, pH
supply patients with more safe TNA formula. MCT is a triglyceride composed of medium chain fatty acids, and is an effective energy source in malnourished patients, hepatic failure patients, diabetic patients, new-born infants, postoperative sepsis patients and ICU patients since it oxidises fast and completely compared with LCT due to its smaller molecular weight and size. TNAs containing MCT are used in many cases in countries with active use of TNAs such as the United States. Since stability can differ according to pharmaceutical company, we used the products, IntraMCT and Intralipose and conducted the present study using TNAs made with IntraMCT containing MCT and LCT at 1:1 ratio and TNAs made with the LCT lipid emulsification, Intralipose, to determine and compare stability of these two TNAs.

MATERIALS AND METHODS

Study Subjects
According to the following formula table (Table 1), TNAs containing LCT (Intralipose) and LCT/MCT (IntraMCT) were prepared in two types, i.e., Peripheral and Central types; observations were made on appearance, particle distribution, pH, osmolarity, hyperoxide value, Zn content, and vitamin contents at room temperature and at refrigerated state for 3 days to compare the stability of the TNAs. TNA formulas were prepared at an amount applicable to 6 lots using 1 L TNA bags according to composition, and after placing glucose, amino acids, fats, and electrolytes in the order, the mixtures were shaken thoroughly. After preparation, TNAs were stored according to the storing condition, and a sample was taken from each TNA every day, and examinations were done on the applicable items. The study results were obtained by calculating an average from 3 measurements made per each lot.

Samples
IntraMCT (Fresenius Kabi Corp.), 20% Intralipose (Fresenius Kabi Corp.), Solgreen (Fresenius Kabi Corp.), 50% Dextrose (Choongwae Pharma Corporation), 20% Dextrose (Choongwae Pharma Corporation), Multivitamin (MVH, Whanin Pharm Co. Ltd.), Frutman (Choongwae Pharma Corporation), KH₂PO₄ (K-phos, Choongwae Pharma Corporation).

Appearance
The prepared TNA formulas were stored at room temperature and at a refrigerated state for 3 days, a sample was taken from each TNA formula to observe the presence of absence of coalescence under 1000 lux, visual observation on color change, picture taking, and picture taking under optical microscope at 0, 1, 2, and 3 days of storage.

Particle distribution
After diluting each sample in distilled water by 1000 folds, the samples were kept at room temperature out of refrigeration and the molecular distribution was measured with the dynamic light scattering (DLS) method using a laser particle analyzer (Otsuka electronics, Japan) for 3 days. The measurement was measured for 3 times per each sample and the average of the 3 measurements was calculated.

pH
After adjusting the temperature of each sample using cold water to 20°C, pH was measured using Horiba pH meter F-113. The measurements were made 3 times for each sample, and the average was calculated.

Hyperoxide value
Each sample was measured 3 times using a methrom

Table 1. Composition of four TNA formulations.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Formulation</th>
<th>P I</th>
<th>P II</th>
<th>C I</th>
<th>C II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose 50%</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose 20%</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Solgreen 12.5%</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>IntraMCT 20%</td>
<td>250</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamin</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Frutman</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>1000 IU</td>
<td>1000 IU</td>
<td>1000 IU</td>
<td>1000 IU</td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄ (13.6%)</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

IU (international units); P I (Peripheral I); P II (Peripheral II); C I (Central I); C II (Central II)
682 titroprocessor, and the average was calculated.

\[ \text{Oxidation value (mEq/L)} = \frac{(EP_1 - CO_1) \times \text{titration factor}}{CO_0} \]

EP1 = sample titrate amount (mL)
CO1 = blank titrate amount (mL)
CO0 = sample amount (mL)

**Osmolarity**

After diluting the sample by 2 folds using distilled water, the measurement was made using Fiske one-ten osmometer. The average of 3 measurements for each sample was calculated.

**Measurement of Zn content**

In order to observe changes in the Zn content in the Central I type, the TNA was stored in dark, samples were taken on 0, 1, 2, and 3 day, diluted by 5 folds with absolutely distilled water, and analyzed according to the atomic spectrophotometric method. As the standard solution, pure Frutman was diluted by 10,000 folds and prepared into the concentrations of 0.25, 0.5, and 0.75 ug/ml, and a calibration curve was prepared.

\[ \text{Zn (µmol/L)} = \frac{\text{measured value} \times \text{dilution folds}}{\text{Zn molecular weight} \times 100} \]

**Measurement of vitamin (nicotinamide, riboflavin) contents**

A sample was taken and diluted by 2 folds on 0, 1, 2, and 3 days after storing the TNA Central I type formula in dark, the samples were filtered with a 0.2 um filter and nicotinamide and riboflavin contents were measured using HPLC, and compared with the vitamin contents in multivitamin tablets.

\[ \text{nicotinamide content (µmol/L)} = \frac{\text{std. conc (mg/mL) \times area of sample peak} \times 2}{\text{area of std. peak}} \]

\[ \text{riboflavin content (µmol/L)} = \frac{\text{std. conc (mg/mL) \times area of sample peak} \times 2}{\text{area of std. peak}} \]

**Statistical analysis**

To evaluate the significance in the change of each measurement, statistical analysis was done using t-test (GraphPad Prism 4). P values less than 0.05 were considered to be statistically significant.

**RESULTS**

**Appearance**

Each lot was light yellow due to the effect of vitamins, and during the study period, no changes such as creaming, flocculation, breakage, and oiling out were observed.

**pH**

Although about 0.1 larger change was seen in TNAs containing LCT compared with TNAs containing MCT/LCT, no significance was present (P>0.05), and no changes in pH were present during the study period (Table 2).

**Osmolarity**

The Peripheral preparation at about 1000 mOsm, and Central preparation at about 1850 mOsm showed no changes, and though a minute change was present between LCT, Intralipose preparation and MCT:LCT=1:1, IntraMCT, no significance was present (P>0.05) (Table 3).

**Particle distribution**

No significant change was present by maintaining the ini-
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Table 3. Osmolarity change at room temperature (RT) and refrigerator (CT).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>CT</td>
<td>RT</td>
<td>CT</td>
<td>RT</td>
</tr>
<tr>
<td>P I</td>
<td>1071</td>
<td>1068</td>
<td>1070</td>
<td>1064</td>
</tr>
<tr>
<td>P II</td>
<td>1006</td>
<td>1013</td>
<td>1009</td>
<td>1007</td>
</tr>
<tr>
<td>C I</td>
<td>1892</td>
<td>1885</td>
<td>1878</td>
<td>1883</td>
</tr>
<tr>
<td>C II</td>
<td>1723</td>
<td>1727</td>
<td>1710</td>
<td>1720</td>
</tr>
</tbody>
</table>

Table 4. Changes in particle distribution at room temperature (RT) and refrigerator (CT).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>CT</td>
<td>RT</td>
<td>CT</td>
<td>RT</td>
</tr>
<tr>
<td>P I</td>
<td>180</td>
<td>178</td>
<td>171</td>
<td>170</td>
</tr>
<tr>
<td>P II</td>
<td>224</td>
<td>219</td>
<td>218</td>
<td>218</td>
</tr>
<tr>
<td>C I</td>
<td>166</td>
<td>152</td>
<td>149</td>
<td>140</td>
</tr>
<tr>
<td>C II</td>
<td>227</td>
<td>237</td>
<td>229</td>
<td>228</td>
</tr>
</tbody>
</table>

Table 5. Changes in hyperoxide value at room temperature (RT) and refrigerator (CT).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>CT</td>
<td>RT</td>
<td>CT</td>
<td>RT</td>
</tr>
<tr>
<td>P I</td>
<td>0.07</td>
<td>0.16</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>P II</td>
<td>0.14</td>
<td>0.16</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>C I</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>C II</td>
<td>0.03</td>
<td>0.06</td>
<td>0.07</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Vitamin contents

During the study period, Nicotinamide showed almost no change but riboflavine showed a slight decrease of ±0.44 ug/ml without significance (P>0.05) (Fig. 2, 3).

DISCUSSION

As a preparation that supplies patients with sugars, amino acids, lipids, electrolytes, and required nutrients in one container, TNA is a preparation that requires caution at the time of mixing since the stability of the preparation changes according to the mixing order. Especially, since a physicochemically unstable lipid state can be induced when lipids are mixed with high concentrations of glucose solution, many studies were conducted on stability of TNAs. TNAs using LCT have been used in 8 hospitals in Korea, and studies on stability were also conducted.
The results of the study conducted by Suh et al on stability of TNA prepared using LCT showed that TNAs containing LCT should be used within 1 day and 3 days when kept refrigerated to prevent the phenomena such as creaming, flocculation, coalescence, and breakage. In the present study, coalescence was shown in all samples left at room temperature within 24 h, and increases in pH, particle size, and hyperoxide value were seen. We developed a TNA formula using MCT/LCT (1:1, IntraMCT) that could overcome the problem of TNAs prepared with LCT, and compared appearance, pH, osmolarity, particle size, and hyperoxide value with TNAs prepared with LCT. The results of the present study showed that when the TNAs were kept at room temperature and in refrigeration for 3 days each, no significant changes were present in appearance, pH, osmolarity, hyperoxide value, Zn content, and nicotinamide content (P>0.05), but riboflavin showed a decrease of about 0.4 ug/ml (P>0.05), and the particle size of TNAs containing MCT/LCT was maintained about 40 um smaller compared with TNAs containing only LCT. Although we concluded that the two type of TNAs all maintained stability during the 3 day study period according to the study results, we think that TNAs containing MCT/LCT would be more physicochemically stable compared with TNAs containing LCT. These results agree with the study results of Jiang, J. M., M.D. and fast hydrolysis occurs with small particle size with MCT rather than LCT, suggesting a higher utilization as an energy source.\textsuperscript{15)} Conclusively, the stability of TNAs prepared with MCT/LCT (1:1, Intra MCT) was maintained for 3 days at room under 25\textdegree C temperature and refrigeration, and we think that it can be more effective energy source than TNAs prepared with LCT.

In conclusion, when we examined the stability of TNAs prepared with Intra MCT with MCT: LCT= 1:1, the results showed that the appearance of the formula was light yellow due to the vitamins present but no change in color was observed during the study period. During the study period, pH was decreased about 0.1, which was higher than the TNAs prepared with LCT, but at no significance. No significant changes were observed in osmolarity and hyperoxide value during the study period, and smaller particle distribution was shown compared with TNAs prepared with LCT, confirming that our TNAs can be more effective physiologic energy source compared with TNAs prepared with LCT.
TNAs prepared with Intra MCT at MCT:LCT= 1:1 can maintain stability for 3 days at room temperature lower than 25°C and refrigeration as more effective energy source than the existing TNAs prepared with LCT.

REFERENCES