Correlation between plasma hydrogen sulfide and homocysteine in patients with acute promyelocytic leukemia

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\textbf{Objective:} To investigate the correlation between plasma hydrogen sulfide (H\(_2\)S), homocysteine (Hcy), folic acid and vitamin B\(_{12}\), in patients with acute promyelocytic leukemia (APL) before and after treatment. \textbf{Methods:} A total of 26 hospitalized patients with APL were randomly selected as case group and 26 healthy persons as control group. The concentration of H\(_2\)S, Hcy and folic acid in plasma of case group and control group were measured, respectively. The statistically significant difference was investigated by comparing the acute onset phase and clinical remission of case group with those of healthy control group. \textbf{Results:} The concentration of H\(_2\)S and Hcy of plasma in patients of case group during acute onset phase significantly increased, and concentration of folic acid significantly decreased, and there were both statistically significant differences as compared with those during clinical remission and those of control group (\(P<0.01\)). The concentration of H\(_2\)S and Hcy of plasma in patients of case group during clinical remission after treatment decreased, and concentration of folic acid increased. The result was close to that of healthy control group, and there was no statistically significant difference (\(P>0.05\)). The change in concentration of plasma H\(_2\)S was positively correlated with that of Hcy and negatively correlated with that of folic acid in patients of case group before and after treatment. \textbf{Conclusions:} The increase of plasma H\(_2\)S in patients with APL may be related to the changes in concentration of Hcy and folic acid.

\section*{1. Introduction}

In recent years, one of the gas signal molecule, namely, plasma hydrogen sulfide (H\(_2\)S) has already become a hotspot for many scholars to concern and study in the field of life sciences and medical science. Current studies have already found that H\(_2\)S plays an important role in regulating the physiology and pathophysiology in human, and it is greatly correlated with the occurrence, development and outcome of various diseases\cite{1-5}. We have found in our earlier study that H\(_2\)S also participates in the relative pathophysiological processe of acute promyelocytic leukemia (APL). H\(_2\)S is produced by the metabolism of amino acids containing sulphur in body such as homocysteine (Hcy), methionine and cysteine. In order to further confirm the mechanism that causes the change in concentration of H\(_2\)S in patients with APL, we designed the experiment. The experiment was conducted to measure the change in concentration of H\(_2\)S, Hcy, folic acid and vitamin B\(_{12}\) in patients with APL before and after treatment, investigate the correlation of change between them and study the mechanism of change in H\(_2\)S in patients with APL.

\section*{2. Materials and methods}

\subsection*{2.1. Study subjects}

A total of 26 hospitalized patients with APL in first affiliated
hospital of Hebei North University from January 1, 2014 to December 31, 2014 were selected as case group, including 12 males and 14 females, whose ages were 19–52 years old and middle age was 35 years old. Among patients who were taken treatment, 22 patients were in clinic remission including 9 males and 13 females, whose ages were 19–52 years old and middle age was 35 years old. Another 26 healthy persons who underwent a medical examination in our physical examination center were selected as control group, including 12 males and 14 females, whose ages were 22–50 years old and middle age was 36 years old. All the cases above did not include main organ diseases such as heart, cerebrum and lung. Diagnosis and therapeutic efficacy evaluation of the cases all accorded with the standard of diagnosis of hematopathy and therapeutic efficacy[6].

2.2. Methods

2.2.1. Collection of samples

A total of 3 mL venous bloods were drew from both case group and control group in fasting in the morning and kept in EDTA-K$_2$ anticoagulative tube. And after that they were centrifuged in 3 000 r/min for 10 min, the plasma was absorbed into EP tube and placed in low-temperature refrigerator at -75℃ for the measurement of concentration of H$_2$S. After treatment, 22 patients from case group were reviewed by drawing blood after complete remission. The collection of plasma and the way of processing from control group were same as those of case group.

2.2.2. Measurement method

The measurement method of plasma H$_2$S was as follow. Before the measurement, plasma samples were thawed at room temperature and centrifuged in 3 000 r/min for 10 min, and then the supernatant was collected for detection. Then the method of deproteinization was taken[6]. First, the standard curve was drew by using different concentration of standard liquid NaHS, and its linear regression equation was $y=1.5131x + 2.362$, $r^2 = 0.998$. The reaction was done in 5 mL glass tube and added with following reagents in sequence. First, 0.5 mL zinc acetate (10 g/L) was added into glass tube, and then 0.1 mL plasma sample was added and slightly shaken. Then 0.5 mL phenylene diamine hydrochloride (20 mmol/L), 0.5 mL ferric trichloride (30 mmol/L) were added in sequence and then incubated at room temperature for 20 min till they fully reacted. And 10% trichloroaetic acid of 1 mL was added to precipitate the protein. Last, 2.5 mL distilled water was added to expand the volume into 5 mL. The samples were fully mixed again and centrifuged in 6 000 r/min for 5 min. Then the supernatant was collected whose absorbancy was measured at wave length of 670 nm by using 722 spectrophotometer. The contents (μmol/L) of H$_2$S in the supernatant were calculated according to the finished H$_2$S standard curve. The level of Hcy of serum was measured by enzymic colorimetric, and the levels of folic acid and vitamin B$_{12}$ were measured by chemiluminescence.

2.3. Statistical analysis

Software SPSS version 17.0 was used for statistically analysis. Mean±SD was used to express the measurement data. Software SPSS version 16.0 was used to carry out the test of homogeneity of variances. Data of homogeneity of variance ($P>0.10$) in multiple groups were compared using One-way ANOVA, and student’s $t$-test was used for the comparison of two groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Concentration of plasma H$_2$S, Hcy, folic acid and vitamin B$_{12}$ (Figure 1 and Table 1)

The concentration of H$_2$S and Hcy of plasma in patients of case group during acute onset phase significantly increased, and concentration of folic acid significantly decreased, and there were both statistically significant differences as compared with those during clinical remission and those of control group ($P<0.01$). The concentration of H$_2$S and Hcy of plasma in patients of case group during clinical remission after treatment decreased, and concentration of folic acid increased. The result was close to that of healthy control group, and there was no statistically significant difference ($P>0.05$) (Table 1).

3.2. Correlation of each index

The level of H$_2$S had a positive linear correlation with that of Hcy.
in patients of case group before and after treatment ($r = 0.611$, $r = 0.588$, $P < 0.01$). The level of H$_2$S had a negative linear correlation with that of folic acid in patients of case group before and after treatment ($r = -0.607$, $r = -0.668$, $P < 0.01$). The level of Hcy had a negative linear correlation with that of folic acid in patients of case group before and after treatment ($r = -0.536$, $r = -0.579$, $P < 0.01$).

4. Discussion

H$_2$S is the third gas signal molecule found by human. It is produced by the metabolism of amino acids containing sulphur in body such as Hcy, methionine and cysteine which were catalyzed by pyriodoxal-5-phosphate (including CBS and CSE) and cysteine transferase[6]. Study results showed that endogenous H$_2$S participates in the pathophysiological process of many clinical diseases such as nephrosis, left ventricular hypertrophy, cerebral infarction, tumor and pancreatitis[1-5].

We have found in our study that during the acute onset phase of patients with APL, the concentration of plasma H$_2$S in patients significantly increased and then it decreased to a level close to that of control group with the remission after treatment. For further investigate the mechanism of the increasing H$_2$S, we determined the concentration of Hcy of plasma, and the results showed that the variation trend in concentration of Hcy was the same as that of H$_2$S, and there was a positive linear correlation between them. Therefore, we confirmed that one of the obvious mechanism for the increasing concentration of plasma H$_2$S in patients was that the materials that can produce H$_2$S for some reasons (Hcy, methioninem and aminothiopropionic acid) were increased.

Hcy can also be referred as homocysteine, namely, 2-amino-4-sulphur butyric acid, which is formed after the demethylation of methionine in cells. It is the intermediate products decomposed by methionine. Folic acid and vitamin B$_{12}$ are the necessary cofactors during the metabolic process of Hcy. The lack of folic acid especially can increase the Hcy of plasma. By carrying out the study, we found that the level of folic acid in patients obviously decreased during the acute onset phase of patients with APL. And the reasons of decrease could be the rapid proliferation of leukemia cell leading to the malignant consumption and decreased level of folic acid in body, which further resulted in the metabolic block and accumulation of Hcy in body[7]. Therefore, the change in concentration of H$_2$S in patients with APL could relate to the increase of Hcy, and its specific mechanism needs further study.

References