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STUDIES ON THE TREATMENT OF CANCER AS VIEWED FROM CHANGES OF GLYCOLYTIC METABOLITES AND RELATED ENZYME ACTIVITY

Part 1. On Changes of Blood Values of Pyruvate, Lactate and α-Ketoglutarate and of Serum Lactic Dehydrogenase Activity

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The effectiveness of current cancer chemotherapy, although partly acceptable in certain malignancies such as leukemia, is generally limited. Hitherto it has been known that drugs with sufficiently potent carcinostatic effects are accompanied by toxicities to normal tissue cells. Thus the application of chemotherapy may result in an aggravation of the patient's condition. While side effects upon hemopoietic functions have been utilized as a parameter of the above adverse effects of the drugs, means for the objective estimation of the effectivenesses of the drugs selective to cancer tissues have been limited. Thus, attempts were made to study the effects of cancer chemotherapeutics through the metabolic aspects of cancer hosts. In the present study, results obtained by investigations in changes of the glycolytic metabolic pathway are reported.

Materials and Methods

1. Materials

For the observation of metabolites by types of malignant diseases, 82 carcinoma cases (of which 48 were stomach carcinoma and the remaining 34 were carcinomata of various other origins) and 6 cases of lymphosarcoma or reticulosarcoma, as well as 10 cases with acute or chronic myelogenic leukemia, were selected. For the observation of metabolites by stages of malignancy classified according to the TNM system, a total of 96 cases consisting of 14 incipient cases, 42 moderately advanced cases and 40 cases in the terminal stage of malignancy were selected.

2. Determinations of Blood Pyruvate, Lactate, α-Ketoglutarate and Serum Lactic Dehydrogenase Activity

The abovementioned cases were treated with cancer chemotherapeutics and the following values were determined throughout the clinical courses.

a) Determinations of pyruvate (Pyr) and α-ketoglutarate (α-K)

The dinitrophenyl hydrozine method according to Friedman (1) was used, in which the xylene layer was used for the determination of Pyr, and the water layer was used for the determination of α-K.

b) Determination of lactate (LA)

The p-hydroxyphenyl method according to Barker (2) was used.

c) Determination of serum lactic dehydrogenase (LDH) activity

The LDH activity was determined by the method of Cabaud (3) in

which Pyr was used as the substrate and dinitrophenyl hydrazine was used for the development of the color reaction.

3. Administrations of Anti-tumor Agents

In the present study, the majority of cases were treated with Mitomycin C (MMC) in a daily dose of 2 mg, or in a weekly dose of 8 mg. In a small number of cases, adrenal cortical steroid treatment was combined with the MMC treatment.

Results

1. Blood Pyr, LA and α-K Values, and Serum LDH Activities in Patients with Carcinoma and Other Malignancies

Normal values and values in patients with malignancies are shown in Table 1. In 26 out of the 48 cases (54%) of stomach carcinoma,

Table 1. Blood Pyruvate, Lactate and α-Ketoglutarate and Serum LDH Activity in Patients with Malignant Neoplastic Diseases

	Number of cases	Pyr(mg/dl)	LA(mg/dl)	α-K(mg/dl)	LDH(unit)
Normal Control	10	0.65±0.08 (0.51-0.76)	12.7±3.4 (7.4-17.8)	0.60±0.60 (0.27-0.90)	270±48 (190-335)
Carcinoma of the Stomach	48	0.82 (0.30-1.74)	20.2 (5.0-47.5)	0.78 (0.15-1.70)	361 (140-1050)
Other Carcinomata	34	0.83 (0.54-1.31)	20.5 (7.0-59.2)	0.91 (0.15-1.70)	495 (210-1380)
Lymphosarcoma and Hodgkin Sarcoma	6	0.99 (0.62-1.59)	34.7 (13.5-74.5)	0.86 (0.27-1.50)	672 (190-2000)
Myelogenic Leukemia	10	1.01 (0.52-2.45)	32.4 (11.5-74.5)	0.98 (0.34-2.05)	1089 (320-2000)

which was the predominant diseases in the present series, the blood Pyr values were abnormally high. Pyr values were also high in 23 of 34 cases (68%) of other carcinomata, including bronchogenic carcinoma, hepatoma and uterine carcinoma. Of the 6 cases with either lymphosarcoma or Hodgkin's sarcoma, 4 cases showed high Pyr values, and 6 of the 10 cases of leukemia also showed high Pyr values.

Blood LA values were abnormally high in 32 cases (67%) of stomach carcinoma, 22 cases (65%) of other carcinomata, 4 cases (67%) of sarcomata and in 7 cases (70%) of leukemia.

The incidence of abnormally elevated blood α-K values was remarkably lower than the incidences of elevated Pyr and LA values. Abnormally high values were found only in 6 cases (13%) of stomach carcinoma, 9 cases (26%) of other carcinomata, 2 cases (33%) of sarcomata, and in 2 cases (20%) of leukemia.

Abnormally high serum LDH activities were found in 22 cases (46%) of stomach carcinoma, 26 cases (76%) of other types of carcinoma, 5 cases (83%) of sarcomata and in 10 cases (100%) of leukemia.

2. Blood Pyr, LA and α-K Values and Serum LDH Activities in Cancer Cases Classified According to Stages

As can be seen in Table 2, Pyr, α-K and LDH values were only slightly elevated to the upper normal limits or thereabouts in the incipient and moderately advanced stages, while LA values in the same stages were already strongly elevated beyond the upper normal limit, indicating that a retention of LA may occur in the relatively

early stages of malignancy.

A comparison of abnormally elevated values observed in the terminal stage indicated that the elevation of LDH activity was the most remarkable of the elevations of the three items excluding α-K which did not show a high incidence of abnormality.

3. Changes of Blood Values of Pyr, LA and α-K, as well as of Serum LDH Activity, Due to Carcinostatic Treatments of Acute and Chronic Myelogenic Leukemia

Leukemia was selected for a study of the effect of carcinostatic treatment upon the abovementioned items, since in this disease the anti-tumor agents manifested strongest effectiveness, and also since the course of the disease could be easily pursued with blood and bone marrow findings as well as with other clinical signs.

Table 2. Blood Pyruvate, Lactate and α-Ketoglutarate and Serum LDH Activity in Carcinoma Patients Classified According to Stages

	Number of cases	Pyr(mg/dl)	LA(mg/dl)	α-K(mg/dl)	LDH(unit)
Normal Control	10	0.65±0.08 (0.51-0.76)	12.7±3.4 (7.4-17.8)	0.60±0.60 (0.27-0.90)	270±48 (190-335)
Incipient Carcinoma	14	0.70 (0.41-1.00)	20.1 (11.0-35.0)	0.71 (0.27-1.70)	286 (60-590)
Moderately Advanced Carcinoma	42	0.79 (0.62-1.18)	21.4 (11.7-43.6)	0.75 (0.34-1.50)	398 (140-1710)
Terminal Carcinoma	40	0.85 (0.54-1.31)	26.2 (8.0-59.2)	0.93 (0.20-1.35)	696 (350-870)

The key substances of glycolytic pathway were determined in acute and chronic leukemia cases where the anti-tumor treatment resulted in ameliorations of clinical symptoms, and the values were compared with the pre-treatment values. As can be seen in Fig. 1, a decrease of LDH activity was observed in all instances, while values of Pyr,

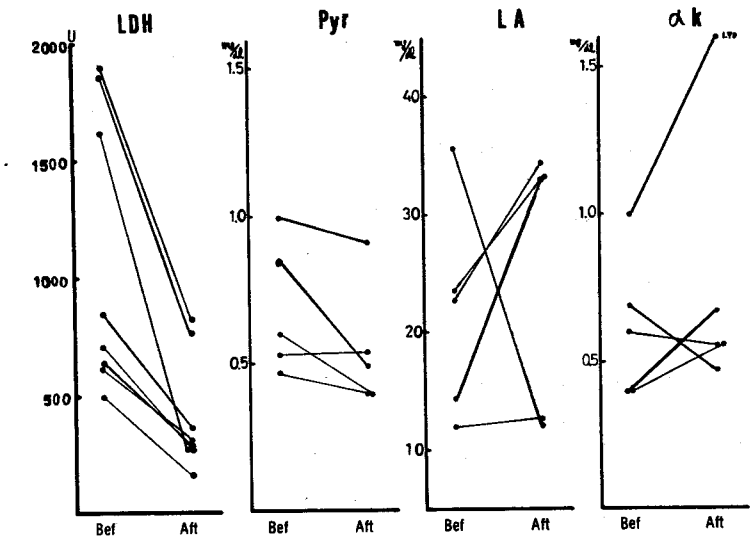

Fig. 1. Changes of serum LDH activity and blood levels of Pyr, LA and α -K in leukemia patients in whom chemotherapy resulted in clinical ameliorations.

LA and α -K were relatively unstable. On the other hand, cases in which clinical aggravation was observed in spite of the treatment were characterized in each case with an increase of serum LDH

activity and with variable Pyr, LA and α-K values (Fig. 2).

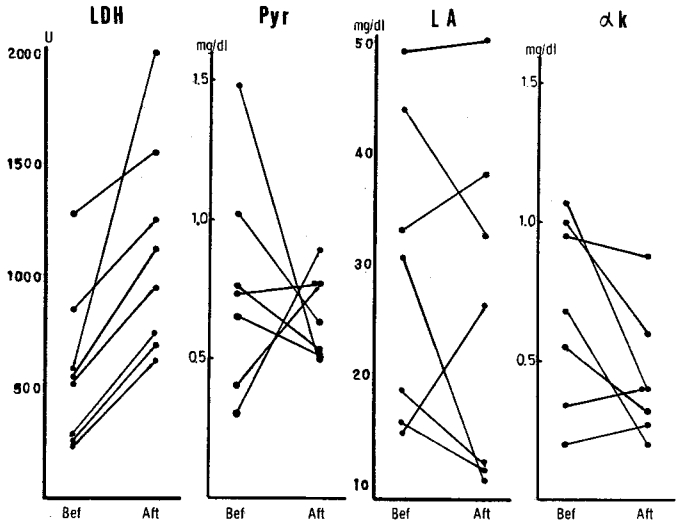

Fig. 2. Changes of serum LDH activity and blood levels of Pyr, LA and α-K in leukemia patients in whom chemotherapy resulted in clinical aggravations.

4. Comparison of Changes of Blood Pyr, LA and α-K Values Between Cancer Cases in Which Normalization of LDH Activity Took Place and Those in Which Aggravation of the Activity was Observed

Normalization and re-elevation of serum LDH activity after treatment were tentatively termed as amelioration and aggravation, respectively. Changes of Pyr, LA and α-K values due to amelioration and aggravation were studied. As can be seen in Fig. 3, there were

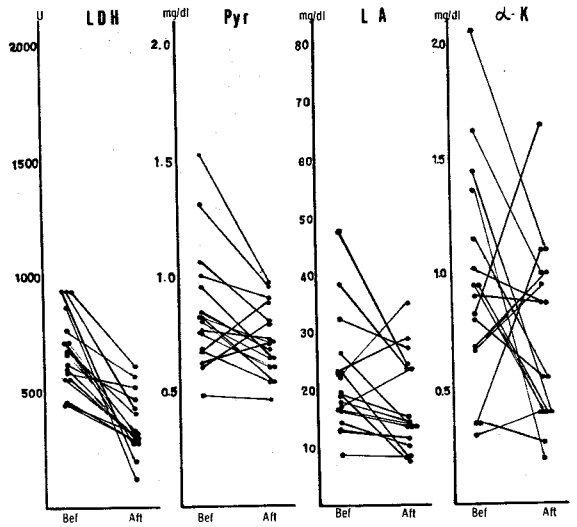

Fig. 3. Changes of blood levels of Pyr, LA and α -K in carcinoma patients in whom chemotherapy resulted in ameliorations of serum LDH activity.

17 cases in which serum LDH values were abnormally high before treatment and which decreased to normal values after treatment. Of these, 12 cases had abnormally high pre-treatment Pyr values which in all cases were lowered after the treatment. On the other hand, in two cases the treatment was accompanied by aggravations of Pyr values. Also 12 of the 17 amelioration cases showed elevated pre-treatment LA values, of which 9 cases were characterized with normalization of the post-treatment LA values. On the other hand, 3 cases showed even higher post-treatment values, while none of the cases which had normal pre-treatment values showed abnormally elevated post-treatment LA values.

Of the 17 cases, 4 cases had high pre-treatment α-K values, and the post-treatment values in these cases were uniformly lower than the pre-treatment values. On the other hand, there was one case in which the normal pre-treatment α-K value showed an abnormal elevation after the treatment.

There were 21 cases in which the post-treatment serum LDH activity showed higher values than before the treatment. As shown in Fig. 4, the post-treatment Pyr values of these cases showed

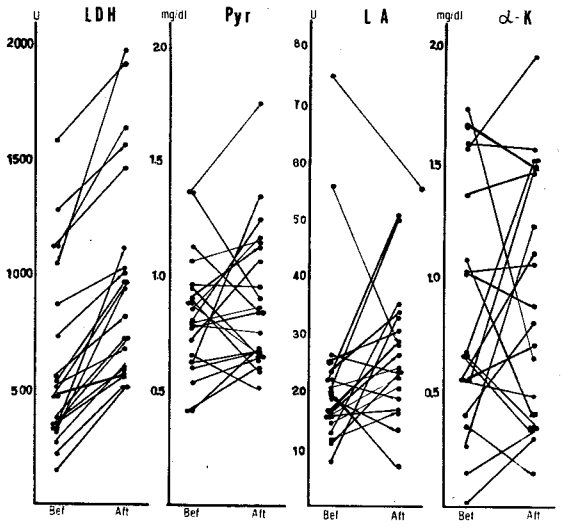

Fig. 4. Changes of blood levels of Pyr, LA and α-K in carcinoma patients in whom chemotherapy resulted in aggravations of serum LDH activity.

amelioration in 6 cases, aggravation in 7 cases, and constancy in 6 cases, when compared with the pre-treatment values. The incidences of amelioration, aggravation and constancy for LA after the treatments were 6, 12 and 2, respectively, and the corresponding incidences for α-K were 3, 5 and 12, respectively.

Discussion

Acceleration of glycolysis in cancer hosts had been reported by a number of investigators (4-8). The results of the present study also showed apparently abnormal increases of blood Pyr and LA, as well as of serum LDH activity. The present results also showed that the increases were generally proportional to the stages of the malignancies and that while the increase of LA was observed in a relatively early stage, abnormally high levels of LDH activity in the terminal stage were remarkable. Nieper (9) reported that one of the metabolic characteristics of cancer hosts was the decrease of Pyr/LA ratio, since the retention of LA was more remarkable than that of Pyr. The present results appeared to support this view.

This was further studied by observing the effect of anti-tumor agents upon leukemia cases which were chosen for this purpose since in this disease the objective observation of amelioration and aggravation was possible. Changes of Pyr, LA and α-K in leukemia cases were to some extent proportional to the grade of morbidity,

although there were also not a few exceptional cases.

On the other hand, it was noted that serum LDH activity was more closely correlated to the patient's condition, all cases treated with the anti-tumor agents showing decreases of activity against normal values, while cases which showed re-aggravations of clinical conditions manifesting abnormal re-elevations of the activity.

In carcinoma cases where the effects of the drugs were more difficult to determine clinically, blood values of Pyr, LA and α-K were compared between two groups classified according to the post-treatment LDH activities. In most cases of a group of patients in which the post-treatment LDH activities showed a tendency to be normalized, blood values of Pyr, LA and α-K also showed a tendency to be normalized, while in most cases in a group of patients in which the post-treatment LDH activities were still elevated, the blood values of the glycolytic metabolities also remained high. It is therefore concluded that the determination of serum LDH activities may serve as an indicator of the effects of the carcinostatic treatments.

Undoubtedly, although changes of the blood values of Pyr, La and α-K in cancer patients may to some extent reflect the clinical conditions of the patients, changes of the values caused by treatment with anti-tumor agents may not necessarily be proportional to the effect of the treatment. This may partly be explained by differences between the mechanism of the drug action upon the glycolytic metabolic pathway and that upon the tumor itself, and also partly by case-to-

case differences of the pathological physiological conditions underlying the process of the elevation of blood levels of the glycolytic metabolites. Therefore, a complicated combination of factors related to these processes must be considered. The fact that serum LDH activity more closely reflects the stages of the morbidity of cancer patients, as well as the effectiveness of the anti-tumor agents, than the blood LA level, may be explained by the fact that the latter substance is lible to be effected by a wider variety of metabolic environments, especially such as the patient's duration of rest before withdrawal of the test blood sample, as compared to the relative in vivo stability of LDH as far as the sample blood is carefully treated until subjected to the determination of the enzyme activity, avoiding possible loss of activity due to low temperature (10) and apparent increase of activity due to hemolysis (11).

Conclusion

1. Patients with malignant neoplastic disease such as carcinoma, sarcoma and leukemia were characterized by increased blood values of glycolytic metabolic factors such as pyruvate, lactate and LDH activity. Close correlations were found between the clinical stages of malignancies and the blood levels of the abovementioned metabolic factors.

2. Patients with leukemia were treated with anti-tumor agents, and the changes of the blood levels of the metabolic factors were

assayed throughout the course of the treatment. Cases in which clinical improvement was observed were accompanied with normalizations of serum LDH activity, which were more clearly demonstrated than the decreases of other metabolites determined. On the countrary, cases in which clinical aggravation was observed were accompanied by abnormal elevations of LDH activity.

3. A majority of carcinoma cases in which normalization of serum LDH activity due to carcinostatic treatments were encountered were also characterized with normalizations of blood levels of pyruvate, lactate and α-ketoglutarate. On the countrary, carcinoma cases in which re-elevation of serum LDH activity followed the carcinostatic treatment were characterized with elevations of blood levels of the above glycolytic metabolites. These results seemed to suggest that the changes of serum LDH activity in cancer patients also reflected to a certain extent the improvement and aggravation of metabolic abnormality of the patients.

It is concluded, therefore, that the determination of serum LDH activity in relation to the changes of glycolytic metabolic factors has a practical significance as an indicator of the effectiveness of treatment with carcinostatic agents.

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References

1. Friedman, T. E., & Hanger, G. E.: J. Biol. Chem., 144, 67 (1942); 147, 415 (1943).

2. Barker, S. B., & Summerson, W. H.: J. Biol. Chem., 138, 535 (1941).

3. Cabaud, P. G., & Wróblewski, F.: Amer. J. Clin. Pathol. 30, 234 (1958).

4. Lasch, F.: Dtsch. med. Wschr., 78, 975 (1953).

5. Kipping, H.: Dtsch. Arch. klin. Med., 199, 162 (1957).

6. Norman, T. D., & Smith, A. B.: Cancer Res., 16, 1027 (1956).

7. Tsirimbas, A., & Stich, W.: Klin. Wschr., 38, 1196 (1960).

8. Hill, B. R., & Levi, C.: Cancer Res., 14, 513 (1954).

9. Nieper, H. A.: Ärztl. Forsch., 15, 510 (1961).

10. Kreutzer, H. H., & Fennis, W. H. S.: Clin. Chim. Acta, 9, 64 (1964).

11. Kingsley, D. P. E., Cook, J., & Vartan, A. E.: Clin. Chim. Acta, 12, 489 (1965).