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Studies on Methylglyoxal. II.[1] Changes of Methylglyoxal Level Accompanying the Changes of Glyoxalase I and II Activities in Mice Bearing L1210 Leukemia and Sarcoma 180

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Changes of methylglyoxal (MG) level accompanying the changes of glyoxalase I and II activities were examined in the liver and blood of mice inoculated with L1210 leukemia or Sarcoma 180 intraperitoneally. The amounts of MG in the liver and blood decreased for the first 3—5 d after the inoculation of L1210 leukemia or Sarcoma 180 and then increased significantly. Glyoxalase I and II activities, on the other hand, increased for the first 3—5 d and then decreased on day 8. The levels of dihydroxyacetone phosphate, glyceraldehyde-3-phosphate and fructose-1,6-diphosphate decreased gradually during the experimental period of 8 d in the liver and blood of mice with Sarcoma 180.

The present observations may suggest that the initial proliferation of the tumor cells is accompanied with a decrease of MG and further development of the tumor cells results in an inductive increase of MG level due to the acceleration of sugar catabolism and/or to the decrease of glyoxalase I and II activities.

Keywords——L1210 leukemia; Sarcoma 180; methylglyoxal; 2-oxopropanal; glyoxalase I; glyoxalase II; dihydroxyacetone phosphate; glyceraldehyde-3-phosphate; fructose-1,6-diphosphate

Methylglyoxal (MG, 2-oxopropanal) is known to be formed from dihydroxyacetone phosphate (DHAP) by MG synthase[2-4] or from glycine or threonine through aminoacetone by monoamine oxidase (MAO)[5] in mammalian tissues. MG is metabolized into *S*-lactoylglutathione by glyoxalase I (EC 4.4.1.5) and further converted into D-lactic acid by glyoxalase II (EC 3.1.2.6).[6,7]

Various studies have been made on the role of MG in mammals and it has been shown that MG is one of the regulator metabolites in cell division.[8-10] Apple and Greenberg have shown that MG inhibited the growth of Ehrlich ascites carcinoma, L1210, L4946 leukemia, TA_3 mammary carcinoma, 6C3HED lymphosarcoma and Sarcoma 180 in mice at daily dose levels of 80 mg/kg body weight, when injected intraperitoneally.[11] Winter *et al.* have reported that glyoxalase I and II activities in the liver of mice bearing Sarcoma 180 and L1210 leukemia in ascites form decreased progressively during the developing period of 8—9 d.[12]

In the present study, we determined MG periodically in the liver and blood of mice inoculated with L1210 leukemia and Sarcoma 180 by using our new fluorometric–high performance liquid chromatography (HPLC) method of analysis[1] and examined the relationship between MG and glyoxalase I and II activities. The amounts of DHAP, glyceraldehyde-3-phosphate (GAP) and fructose-1,6-diphosphate (FDP) were also determined in connection with the formation of MG in the liver and blood of mice bearing

Sarcoma 180.

Materials and Methods

Animals——Female DBA_2 and ICR mice (20 of each, 5 weeks old) were divided into controls (4 mice) and the experimental groups (16 mice). L1210 leukemia or Sarcoma 180 (each 1.0×10^6 cells/mouse) was inoculated into mice of the experimental group intraperitoneally. They were fed a standard diet (CE2, Clea Japan Inc.) and water *ad libitum*. On days 1, 3, 5 and 8 after implantation of the tumor cells, the mice (groups of 4) were sacrificed to obtain the blood and liver.

Preparation of the Test Solution——Mouse liver (0.2 g) was homogenized at 0 °C with a Potter-Elvehjem glass homogenizer in 8.875 mM phosphate buffer (I = 0.2, pH 7.0) containing 10 mM $MgCl_2$, in a total volume of 2 ml. The homogenate was centrifuged at 27000 × *g* for 20 min at 0 °C and the supernatant was used as the test solution.

Blood hemolysate was prepared by adding 1.8 ml of H_2O to 0.2 ml of blood (10-fold dilution of blood). The resulting lysate was mixed and centrifuged at 27000 × *g* for 20 min under cooling and the supernatant was used as the test solution.

Determination of MG (and Related Substances), and Enzyme Activities——MG in the liver and blood of mice was determined by our HPLC–fluorometric method.[1] MG up to at least 50 pmol could be specifically determined by this technique. DHAP, GAP and FDP were determined by the method of Michal *et al.*[13] Glyoxalase I and II activities were determined by Racker's[14] method modified by Jerzykowski *et al.*[15]

Results

Changes of Body Weight of Mice Bearing L1210 Leukemia and Sarcoma 180

The body weight of mice in the control group showed a very small increase during the 8 d experiment. The group bearing L1210 leukemia gained weight to reach 110% and 115% of the control while that bearing Sarcoma 180 reached 110% and 133% of the control on days 5 and 8, respectively, due presumably to the growth of tumor cells (see the lower columns of Tables I and II).

Changes of MG and Glyoxalase I and II Activities in Mice Bearing L1210 Leukemia

The changes of MG and glyoxalase I and II activities in the liver and blood of the mice are shown in Table I. It was found that the amount of MG in the liver decreased gradually to 63%—43% during days 1—5 and then increased to 160% of the control on day 8. Glyoxalase I activities increased to 125%—128% during the first 5 d and decreased to 64% of the control on day 8. Glyoxalase II activities showed no significant change until day 8 when the activity decreased to 52%.

The amount of MG in the blood decreased to 43% on day 5. Glyoxalase II activity slightly increased on day 5, whereas glyoxalase I activity decreased to 83% on day 8, which may be responsible for the recovery of MG to the control level.

Changes of MG and Glyoxalase I and II Activities in Mice Bearing Sarcoma 180

Table II shows the changes of MG and glyoxalase I and II activities in the liver and blood of the mice. The amount of MG in the liver decreased to 72% of the control on day 3, and then increased to 129% on day 8. Glyoxalase I activity, on the other hand, increased to 129% on day 3 and then decreased to 81% on day 8. Glyoxalase II activity also increased to 133% of the control on day 3 and then decreased to 82% on day 8. The changes of MG are inversely related to those of glyoxalase I and II activities.

The amount of MG in the blood decreased to 67% and 69% on days 1 and 3, respectively, and then increased to the control levels on days 5 and 8. Glyoxalase I and II activities showed no significant change during 8 d.

Changes of DHAP, GAP and FDP in the Liver and Blood of Mice Bearing Sarcoma 180

The results are summarized in Table III. The amount of DHAP decreased to 62% on day 3 and then remained almost unchanged until day 8. GAP decreased progressively with the

Table I. Time Courses of Methylglyoxal and Glyoxalase I and II Activities in the Liver and Blood of Mice Inoculated with L1210 Leukemia, and Body Weight Change

	MG (μg/g or ml)	Glyoxalase I (units/g or ml)	Glyoxalase II (units/g or ml)
Liver			
1	2.18±1.47[a]	731.4±38.6[b]	54.5±6.2
3	2.67±0.19[a]	742.9±38.4[b]	48.6±4.7
5	1.83±0.63[b]	746.9±29.7[b]	48.1±8.0
8	6.91±1.68[a]	372.1±31.0[b]	24.9±3.3[b]
Control	4.22±0.95	582.6±41.3	47.3±6.5
Blood			
1	3.05±1.04	62.8±2.7	22.5±0.8
3	2.17±1.70	65.5±5.3	25.8±1.2
5	1.70±0.84[b]	64.0±3.3	27.4±1.3[b]
8	3.87±0.91	50.3±5.4[a]	23.1±2.2
Control	3.97±0.33	60.4±3.3	24.1±1.1

	Body weight (g)	
	Control	L1210
0	16.8±0.8	16.8±0.8
1	16.9±0.9	17.0±0.8
3	16.9±0.6	17.3±0.7
5	17.1±0.5	18.9±0.8
8	17.2±0.6	19.7±0.9

Female DBA_2 mice (16 mice, 5 weeks old) were inoculated intraperitoneally with L1210 leukemia (1.0×10^6 cells/mouse). Enzyme unit; enzyme activities are expressed in terms of 1 μmol of the substrate (methylglyoxal for glyoxalase I and *S*-lactoylglutathione for glyoxalase II respectively) consumed as the unit. Significantly different from the control (*a*) $p<0.05$, *b*) $p<0.01$).

Table II. Time Courses of Methylglyoxal and Glyoxalase I and II Activities in the Liver and Blood of Mice Inoculated with Sarcoma 180, and Body Weight Change

	MG (μg/g or ml)	Glyoxalase I (units/g or ml)	Glyoxalase II (units/g or ml)
Liver			
1	3.57±0.28	1081.7± 35.1	96.1±27.3
3	2.80±0.64[a]	1310.4± 66.8[a]	115.5±20.5[a]
5	4.17±0.73	945.5±105.1	88.9±19.3
8	5.13±0.29[b]	829.4± 93.3	71.5± 4.2[a]
Control	3.88±0.37	1019.6±124.2	86.7± 9.3
Blood			
1	1.67±0.25[b]	44.1±11.4	11.9±3.0
3	1.71±0.54[a]	58.9±16.1	10.8±3.2
5	2.21±0.68	51.2± 7.4	13.1±4.3
8	2.20±0.91	52.8± 6.5	11.3±2.3
Control	2.49±0.30	57.0± 7.0	10.6±2.5

	Body weight (g)	
	Control	Sarcoma 180
0	21.0±0.5	21.5±0.6
1	21.7±0.6	21.8±0.9
3	22.4±0.4	21.9±0.8
5	22.4±0.5	24.8±1.1
8	22.8±0.4	30.5±2.0

Female ICR mice (16 mice, 5 weeks old) were inoculated intraperitoneally with Sarcoma 180 (1.0×10^6 cells/mouse). Enzyme units; enzyme activities are expressed in terms of 1 μmol of the substrate (methylglyoxal for glyoxalase I and *S*-lactoylglutathione for glyoxalase II) consumed as the unit. Significantly different from the control (*a*) $p<0.05$, *b*) $p<0.01$).

Table III. Time Courses of Dihydroxyacetone Phosphate, Glyceraldehyde-3-phosphate and Fructose-1,6-diphosphate in the Liver and Blood of Mice Inoculated with Sarcoma 180

	DHAP	GAP (nmol/g or ml)	FDP
Liver			
1	126.6± 9.5	14.9±2.5	96.1± 5.4
3	70.9±13.7[b]	10.8±2.2[b]	77.7±17.5
5	83.3±12.0[b]	10.5±1.9[b]	89.9± 8.0
8	70.4±13.0[b]	9.9±1.2[b]	102.7±14.8[a]
Control	115.0± 7.1	26.5±2.3	82.7±11.8
Blood			
1	69.8±11.1	9.0±3.5[b]	126.1±18.0[a]
3	50.9± 7.3[b]	9.0±3.5[b]	94.3± 4.1
5	58.2±11.2[a]	3.9±1.5[b]	80.5±18.5
8	65.4±19.7	12.1±3.7[b]	111.4±17.2
Control	72.8± 5.7	30.9±7.2	93.4±15.9

Female ICR mice (16 mice, 5 weeks) were inoculated intraperitoneally with Sarcoma 180 (1.0×10^6 cells/mouse). Significantly different from the control (*a*) $p<0.05$, *b*) $p<0.01$).

growth of tumor cells. The amount of FDP tended to decrease on day 3 and then increased to 124% of the control on day 8.

The amount of DHAP in the blood decreased to 70% on day 3 and then increased to near the control level on day 8. The change of GAP was most significant, and decreased gradually with the lowest level on day 5. FDP did not change significantly except on day 1.

Discussion

By applying our new and sensitive analytical method,[1] the changes of MG during the growth of syngeneic and allogeneic tumor cells in mice were quantitatively studied. The amount of MG in DBA_2 mouse liver and blood decreased during the first 5 d and then increased on day 8 after the inoculation of L1210 leukemia. In ICR mouse liver and blood, MG similarly decreased for the first 3 d and then increased in the later period of cell growth after the inoculation of Sarcoma 180. The activities of glyoxalase I and II, which metabolize MG, increased on days 5 and 3 in DBA_2 and ICR mice, respectively, and then decreased on day 8.

It is thus demonstrated that the amount of MG in the liver and blood first decreased and then increased after the inoculation of tumor cells regardless of their type. The present study also demonstrates that the changes of MG are mainly controlled by the activities of its metabolizing enzymes, glyoxalase I and II, the activities of which were first enhanced and then decreased in the later stage of tumor growth. The above results are not completely consistent with those of Winter *et al.*, who observed that glyoxalase I and II activities both decreased progressively in the liver of mice bearing L1210 leukemia or Sarcoma 180, reaching 60%—40% of the control in 8—9 d. The discrepancy between these results may be due to the differences of mouse strain (BDF_1 and DBA_2 mice were used by Winter *et al.*) and of the number of cells inoculated (0.1 ml of ascites fluid containing about 6×10^6 cells was used by Winter *et al.* for the experiment with Sarcoma).

In our other experiments with mice after partial hepatectomy, the amount of MG decreased to 55% at 24 h after surgery and increased to the control level after 72 h, accompanied with liver regeneration. It was also demonstrated in our preliminary experiment that the growth of Sarcoma 180 ascites was promoted in mice fed a vitamin E-deficient diet for 14 weeks; the MG level in the liver was significantly lower than the control, and the activities of glyoxalase I and II were about 31% and 102% higher, respectively.

It may thus be possible to speculate that the proliferation of cells is related to the initial and transient decrease of MG, and this may indirectly support the idea of Szent-Györgyi that the cell growth is dexterously regulated by MG. It can be seen from Table III that the pattern of decreasing amounts of both DHAP and GAP during the first 3 d is similar to that of MG in the liver of mice bearing Sarcoma 180. The decreased MG level at the initial stage of the development of tumor cells, therefore, might be due to the decrease of synthesis from DHAP or GAP and/or to the increase of the enzymatic conversion to *S*-lactoylglutathione or D-lactic acid.

It may be suggested on the basis of all the above results that the decrease of MG in the liver is likely to accelerate the proliferation of tumor cells, and the maintenance of MG at a certain level might be important to avoid the proliferation.

As for the increase of MG level in the liver of mice at 5 and 8 d after the inoculation of Sarcoma 180 or L1210 leukemia, it might be aimed at limiting tumor cell proliferation or it may simply be due to the promotion of sugar catabolism. The amounts of MG and FDP increased to 132% and 124% respectively, of the control on day 8 in the liver of mice with Sarcoma 180. It should be noted, however, that the levels of DHAP and GAP remained almost unchanged after 5 d and their levels were 61% and 37% of the original values,

respectively, on day 8. The activities of glyoxalase I and II decreased on day 8. The increase of MG in the later stage of tumor development seems thus to be mainly due to the decrease of the enzymatic transformation to D-lactic acid, though the activity of MAO is known to increase as tumor cells develop.[16)]

The level of MG in the blood of mice inoculated with the tumor cells showed a similar decreasing pattern to that in the liver for the first 3 or 5 d and recovered to the original level on day 8, though the activities of glyoxalase I and II were not significantly changed during the whole 8-d experiment in mice with Sarcoma 180. Among DHAP, GAP and FDP, GAP showed the most remarkable change in the blood of mice with Sarcoma 180; it dropped to 13% on day 5 and recovered to 39% of the original level on day 8.

Further investigation is necessary to clarify the mechanism of MG regulation in the proliferation process of Sarcoma 180 and L1210 leukemia ascites cells.

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