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Antitumor Activities of Iodoacetate and Dimethylsulphoxide Against Solid Ehrlich Carcinoma Growth in Mice

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ABSTRACT

Treatment of tumor-bearing mice with $LD_{12.5}$ values of iodoacetate; IAA (1.84 mg/100g b.w.) and/or dimethylsulphoxide; DMSO (350 mg/ 100g b.w.) significantly increased the cumulative mean survival time and percentage of survivors and reduced the mean tumor weight, compared to tumor-bearing controls, however, a more pronounced effect is recorded in the combined treatment. Also, an increase in the life span (ILS%) and tumor growth inhibition ratio (T/C%) are reported and amounted to 145.78 and 43.80%, 195.54 and 61.30% and 220.77 and 78.40% in IAA, DMSO and combined-treated groups, respectively. Results obtained from biochemical studies reveal that a single IAA treatment of tumor-bearing mice significantly increased the levels of plasma lactate dehydrogenase (LDH) activity, while it also significantly decreased the levels of plasma glucose and liver total protein, RNA and DNA, compared to normal controls. On the other hand, a single DMSO treatment significantly elevated the activities of blood antioxidant enzymes, i.e. glutathione peroxidase (GP_x) and glucose-6-phosphate dehydrogenase (G6PDH) and decreased the liver RNA and DNA levels. Combined treatment increased significantly the levels of plasma LDH and erythrocytes G6PDH activities, as well as liver glycogen, and in contrast it decreased the levels of liver total protein, RNA and DNA, compared to normal controls.

Key terms: antioxidant enzymes, carbohydrate metabolism dimethylsulphoxide, iodoacetate, solid Ehrlich carcinoma

INTRODUCTION

In cancer cells, glycolysis is the main process involved in the production of ATP caused by hypoxia, which results from defective or insufficient tissue vasculature (Kairento *et al.*, 1985). The pentose phosphate pathway (PPP) is also important in the tumor proliferation process because of its role in supplying tumor cells with pentoses, reduced $NADP^+$ and carbons for the intracellular anabolic processes. However, the direct involvement of PPP in tumor DNA and RNA synthesis is not considered to be significant, as it is in lipid and protein syntheses (Boros *et al.*, 1998).

Iodoacetic acid (IAA) is reported as a classical inhibitor of anaerobic glycolysis, acting primarily on the enzyme glyceraldehyde-3-phosphate dehydrogenase (G3PDH), which catalyzes the conversion of glyceraldehyde-3-phosphate into the high-energy phosphate compound, 1,3 bisphosphoglycerate with the reduction of NAD^+ to NADH (H^+) (Bickis and Quastel, 1965). IAA reacts with the sulfhydryl (-SH) group of the cysteine residue at the active site of the enzyme to prevent the formation of thiohemiacetal (Harris, 1992). IAA is also reported to be an inhibitor of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase, the key enzymes of the oxidative segment of PPP (Badwey and Karnovsky,1980). It is reported that the addition of IAA (1×10^{-4} M) to ascites tumor cells suspended in

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glucose in air increases the intracellular concentration of fructose diphosphate, which accounts for more than 50% of the consumed glucose (Wu and Racker, 1958).

Dimethylsulphoxide (DMSO) is a dipolar aprotic solvent that has the ability to cross cellular and vascular membranes with great ease and has been used for administration of compounds that are difficult to solubilize, and thus enhances their effectiveness (Jeffrey and Haschek, 1988). DMSO is a non-enzymatic free oxygen radical detoxifying agent with a high affinity for the hydroxyl radicals, and thus modulates the antioxidant enzyme system of the host (Akyurek *et al.*, 2000). It is known that an increase in the antioxidant enzyme activity could be a mechanism to protect the cells against the hyperproduction of reactive oxygen species by the tumor. Higgins (1986) reported that DMSO, which is a differentiation-inducing agent, has the ability to suppress hepatic tumor cell proliferation by inducing their entrance into the low RNA non-replicating phase (Quiescent phase).

The present study was undertaken to investigate the anti-tumor activities of the single and combined treatments of IAA and DMSO at sub-lethal doses against an experimental non-metastasizing murine tumor model, solid Ehrlich carcinoma, through inhibition of glucose metabolism and provoking the antioxidant defense system in tumor-bearing mice.

MATERIALS AND METHODS

Tumor

The initial inoculum of Ehrlich ascites carcinoma (EAC) cells was kindly provided by the National Cancer Institute (Cairo University). EAC cells were thereafter propagated in our laboratory by weekly intra-peritoneal injection of 0.3 ml of 1:5 saline solution of freshly drawn ascites fluid from a donor mouse bearing a 6-8 day old ascites tumor. Solid Ehrlich carcinoma was induced by s.c. inoculation of $40x10^6$ cells/ 0.25 ml saline in the back between the thighs of each animal (Fahim *et al.*, 1997).

Animals

Adult female Swiss albino mice weighing 27±3 g obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. The animals were maintained on a commercial standard pellet diet supplemented with lettuce and carrots and tap water *ad libitum*.

Chemical compounds

Iodoacetic acid "sodium salt", (M.W. 209.7, Sigma, U.S.A.) and dimethylsulphoxide (M.W. 78.13 Merck, Germany).

I. Toxicity studies

Determination of the acute (LD_{100}) and median lethal (LD_{50}) doses of IAA was carried out in mice on i.p. administration according to the method of Reed and Muench (1938). The LD_{50} value of DMSO was previously reported (Bartasch *et al.*,1976) to be 1380 mg/100 g b.w. on i.p. administration in mice from which the $LD_{12.5}$ value was calculated.

II. Anti-tumor studies

The anti-tumor activities of IAA and/or DMSO were assessed on 32 mice bearing solid Ehrlich carcinoma that were classified into 4 equally-sized groups (Gr.1-4). Nine days after inoculating the EAC cells (when the tumor became palpable) the animals in Gr.(2-4) were i.p. injected with the $LD_{12.5}$ values of IAA and/or DMSO every other day for a total of 10 injections, then left untreated until the end of the experiment The animals in Gr.(1) received no treatment and served as a control group. The size of the solid tumor was measured with a Vernier caliper every 4 days and the tumor weight (g) calculated (Geran *et al.*, 1972). The cumulative mean survival time (cMST) of the animals, the tumor growth inhibition ratio (T/C%) and the tumor growth delay time (T-C value), which is based on the

median time (in days) required for tumors to reach a predetermined size, were also recorded (Bissery *et al.*, 1991).

III. Biochemical studies

Sixty mice bearing 9-day-old solid Ehrlich carcinoma were randomly allocated into 4 equally-sized groups as follows: Group II Tumor-bearing controls (Gr.II T): the animals received no treatment and served as negative controls. Group III Iodoacetate group (Gr.III IAA): each animal received a single i.p injection of the $LD_{12.5}$ value of IAA every other day for a total of 10 injections. Group IV Dimethylsulphoxide group (Gr.IV DMSO): each animal was treated every other day with the $LD_{12.5}$ value of DMSO for 20 days. Group V Combined group (Gr.V Combined): each animal was treated every other day with a mixture of IAA and DMSO ($LD_{12.5}$ each) for 20 days. For statistical comparison, a normal control group (Gr.I NC) consisting of 15 normal adult female mice was injected i.p. with sterile saline.

Blood collection and preparation of plasma and blood hemolysates

At the end of the experimental period (20 days), the animals were sacrificed by decapitation after an overnight fast. Because of the difficulties in obtaining sufficient volumes for the required assays, blood samples pooled from 3 mice were prepared. Plasma samples were stored at -20ºC in Eppendorf vials for biochemical analysis, while packed red blood cells were immediately washed twice with 20 volumes of phosphate-buffered saline (pH 7.4) and lysed in ß-mercaptoethanol stabilizing solution (Beulter, 1975).

Tissue sampling and preparation of liver homogenate

The livers from the sacrificed animals were quickly excised, rinsed from blood in isotonic saline and blotted dry with a piece of filter paper. For each specimen a 0.1g piece of liver tissue was placed in a test tube containing 2 ml of 30% potassium hydroxide solution for glycogen determination. Another accurately weighed piece of liver tissue was homogenized in ice-cold saline using a glass homogenizer. The homogenate was then diluted with the homogenization medium to ultimately yield a 10% (v/v) whole liver homogenate for determination of total protein, RNA and DNA concentrations. All operations were carried out in vessels immersed in an ice bath and dilutions of the stock whole liver homogenate were made as needed.

Biochemical assays

Levels of plasma glucose (Trinder, 1969) and Lactate dehydrogenase activity (LDH) activity (Allain *et al.*, 1973) were determined using commercial assay kits (Sclavo Diagnostics, and TECO Diagnostics, Italy, respectively). Glutathione peroxidase (GP_x) activity (Paglia and Valentine, 1967), Glucose-6-phosphate dehydrogenase (G6PDH) activity (Kornberg *et al.*, 1955) and catalase activity (CAT) (Beulter, 1975) were determined in fresh blood hemolysates. Liver glycogen, total protein, RNA and DNA concentrations were also assessed in the diluted whole liver homogenates (Dubois *et al.*, 1956; Markwell *et al.*, 1978; Dische and Schwartz, 1954; and Dische, 1957, respectively).

Statistical analysis

The statistical significance of the experimental biochemical results was determined by the Student's *t* test. The statistical significance of the frequency of survivals and cumulative mean survival time of treated animals was determined by the Chi-square test and Logistic *t* test, respectively (Murray, 1982). For all analyses, $p<0.05$ was accepted as a significant probability level.

RESULTS

Table I shows the anti-tumor activity of IAA and/or DMSO treatments against solid Ehrlich carcinoma in mice. Treatment of tumor-bearing animals with the $LD_{12.5}$ values of IAA (Gr.2), DMSO (Gr.3) or both compounds (Gr.4) recorded an increase in the life span (ILS %), which amounted to 145.78, 195.54 and 220.77, respectively, compared to tumor-bearing controls. On day 40, at which time all tumor-bearing control animals (Gr.1) were dead (MTW 8.75±1.63g), the mean tumor weights were markedly reduced to 4.92±0.53, 3.39±1.05 and 1.89±0.46 g, and the tumor growth inhibition ratio (T/C%) recorded 43.80, 61.30 and 78.40, respectively. The tumor growth delay time was 8, 16 and 24 days, respectively.

TABLE I

The anti-tumor activities of IAA and/or DMSO on solid Ehrlich carcinoma compared to tumor-bearing controls.

	Gr. 1 (C)		Gr. 2 (IAA)		Gr. 3 (DMSO)		Gr. 4 (Comb.)	
PTI (days)	M	MTW ± SD	M	MTW ± SD	M	MTW ± SD	M	MTW ± SD
9.0	0/8	0.56 ± 0.24	0/8	0.24 ± 0.15	0/8	0.29 ± 0.21	0/8	0.21 ± 0.09
13	0/8	1.30 ± 0.33	0/8	0.54 ± 0.20	0/8	0.48 ± 0.30	0/8	0.39 ± 0.16
17	0/8	2.49 ± 0.39	0/8	0.96 ± 0.32	0/8	0.63 ± 0.33	0/8	0.49 ± 0.17
21	0/8	4.20 ± 0.33	0/8	1.57 ± 0.44	0/8	0.92 ± 0.46	0/8	0.65 ± 0.26
29	0/8	6.78 ± 1.05	0/8	2.15 ± 0.67	0/8	1.67 ± 0.69	0/8	0.89 ± 0.37
33	1/8	7.98 ± 1.31	0/8	3.79 ± 0.80	0/8	2.11 ± 0.67	0/8	1.13 ± 0.43
37	6/8	8.33 ± 1.45	0/8	4.12 ± 0.46	0/8	2.68 ± 0.83	0/8	1.49 ± 0.42
40	8/8	8.75 ± 1.63	0/8	4.92 ± 0.53	0/8	3.39 ± 1.05	0/8	1.89 ± 0.46
43			0/8	5.12 ± 0.63	0/8	4.09 ± 1.25	0/8	2.31 ± 0.51
49			1/8	6.31 ± 0.92	0/8	4.97 ± 1.10	0/8	3.05 ± 0.67
55			5/8	7.11 ± 0.77	0/8	5.84 ± 1.05	0/8	4.05 ± 1.11
57			8/8	7.50 ± 0.57	0/8	6.38 ± 1.25	0/8	4.51 ± 1.08
60					0/8	6.92 ± 1.05	0/8	4.96 ± 0.96
66					1/8	7.90 ± 0.78	0/8	5.91 ± 0.98
72					5/8	7.99 ± 0.97	0/8	6.86 ± 0.97
74					8/8	8.03±0.57	0/8	7.09 ± 0.88
78							3/8	7.21 ± 1.01
82							8/8	7.35 ± 0.64
MST (days)	35.67		52		69.75		78.75	
ILS (%)	-		145.78		195.54		220.77	
T/ C (%)	-		43.80		61.30		78.40	
T-C (days)	-		8		16		24	

MST = mean survival time

$$\text{Increase of life span (ILS\%)} = \frac{\text{mean survival time of test}}{\text{mean survival time of control}} \times 100$$

$$\text{Tumor inhibition ratio (T/C\%)} = \frac{\text{mean tumor weigth of control - mean tumor weight of test}}{\text{mean tumor weight of control}} \times 100$$

(T-C) value = tumor growth de lay time
PTI = post tumor inoculation
M = mortality
MTW = Mean tumor weigth

Table II illustrates the statistical significance of the percentage of survivors and the cumulative mean survival time (cMST) of tumor-bearing mice treated with IAA (Gr.2), DMSO (Gr.3) or combined agents (Gr.4), compared to tumor-bearing controls. On day 40, the percentage of survivors was 100% ($p< 0.001$) in all treated groups (Gr.2&3&4), whereas on day 57, when all of the Gr.(2) animals were completely depleted, the percentage of survivors in Gr. (3&4) was 100% ($p<0.001$). On day 74, at which time all of the Gr. (3) animals were dead, the percentage of survivors in Gr.(4) was 100% ($p< 0.001$). A highly significant increase of 43.61, 85.30 and 109.78% ($p< 0.001$) was recorded in the cMST of Gr.(2, 3, & 4), respectively, compared to Gr.(1). Also, an increase of 29 and 46.07% ($p<0.001$) was recorded in the cMST of Gr.(3&4), respectively, compared to Gr.(2), while it was only 13.24 % ($p< 0.05$) in Gr.(4) compared to Gr.(3).

Table III shows the changes in some blood and liver analytes in tumor-bearing mice treated with IAA and/or DMSO. Significant elevations were recorded in the levels of plasma LDH (195.47%, $p<0.001$), erythrocytes GP_x (69.98%, $p<0.001$), G6PDH (46.63%, $p<0.001$) and CAT activities (28.60%, $p<0.05$), with a concomitant reduction in the plasma glucose level (51.36%, $p<0.001$) of tumor-bearing animals (Gr.II T), compared to normal controls (Gr.I NC). Treatment of tumor-bearing animals with 10 injections of IAA (Gr. III) produced significant decreases in the levels of plasma glucose (23.05%, $p<0.01$), erythrocytes GP_x (26.74%, $p<0.01$), and G6PDH activities (22.78%, $p<0.05$) associated with a significant increase in the level of plasma LDH activity (41.10%, $p<0.01$), compared to normal controls. Treatment with 10 injections of DMSO (Gr.IV) increased significantly the activities of erythrocytes GP_x and G6PDH (17.65%, $p<0.05$ and 114.65%, $p<0.001$, respectively), while the levels of plasma glucose and LDH activity were not significantly changed. Combined treatment of tumor-bearing animals (Gr.V) gave rise to significant elevations in the levels of plasma LDH (37.63%, $p<0.01$)

TABLE II

Effect of IAA and/or DMSO on the frequency of survivors and the cumulative mean survival time of tumor-bearing animals compared to tumor-bearing controls.

	Frequency of Survivors[a]			Cumulative Survival Time		
Groups	Survivors n (%)	Deaths n (%)	P [b]<	Means ± SD (days)	Change %	P [c]<
On day 40						
Gr.1 (T)	0 (0)	8 (0)	-	20.75 ± 8.92	-	-
Gr.2 (IAA)	8 (100)	0 (0)	0.001	29.80 ± 13.54	43.61	0.001
Gr.3 (DMSO)	8 (100)	0 (0)	0.001	38.44 ± 18.24	85.30	0.001
Gr.4 (Comb.)	8 (100)	0 (0)	0.001	43.53 ± 21.05	109.78	0.001
On day 57						
Gr.2 (IAA)	0 (0)	8 (0)	-	29.80 ± 13.54	-	-
Gr.3 (DMSO)	8 (100)	0 (0)	0.001	38.44 ± 18.24	29.00	0.001
Gr.4 (Comb.)	8 (100)	0 (0)	0.001	43.53 ± 21.05	46.07	0.001
On day 74						
Gr.3 (DMSO)	0 (0)	8 (0)	0.001	38.44 ± 18.24	-	-
Gr.4 (Comb.)	8 (100)	0 (0)	0.001	43.53 ± 21.05	13.24	0.05

(a) Percentage of survivors on day 40, 57, and 74 post tumor inoculation.
(b) Probability value was obtained by use of Chi-square analysis.
(c) Probability value was obtained by use of logistic t-test.

TABLE III

Effects of IAA and/or DMSO (10 injections) on the levels of plasma glucose, lactate dehydrogenase and erythrocytes glutathione peroxidase (GP_x), glucose-6-phosphate dehydrogenase (G6PDH) and catalase (CAT) activities and hepatic total protein, RNA and DNA contents in tumor-bearing animals compared to normal controls.

	Plasma[a]		Erythrocytes[a]			Liver[b]			
Groups	Glucose mg/dl	LDH IU/L	GP_x U/g Hb	G6PDH U/g Hb	CAT K/g Hb	Glycogen mg/g tissue	T. Protein mg/g tissue	RNA mg/g tissue	DNA mg/g tissue
Gr.I NC									
Mean ± SD	114.10 ± 11.9	1964 ± 138.2	130.9 ± 13.1	9.35 ± 0.63	175.6 ± 34.6	13.84 ± 1.68	197.1 ± 10.32	10.19 ± 1.28	3.95 ± 0.49
Gr. II T									
Mean ± SD	55.5 ± 7.0	5803 ± 610	222.5 ± 18.2	13.71 ± 1.39	225.8 ± 29.4	8.34 ± 1.94	184.50 ± 20.6	11.94 ± 0.86	4.5 ± 0.69
Change %	-51.36	195.47	69.98	46.63	28.60	-39.74	-6.40	17.17	13.92
P<	0.001	0.001	0.001	0.001	0.05	0.001	N.S.	0.01	N.S.
Gr. III IAA									
Mean ± SD	87.8 ± 4.9	2771 ± 396	95.9 ± 16.9	7.22 ± 1.58	204.8 ± 21.5	14.56 ± 3.21	169.1 ± 12.3	19.0 ± 1.06	3.19 ± 0.75
Change %	-23.05	41.10	-26.74	-22.78	16.63	5.20	-14.20	-11.70	-19.24
P<	0.01	0.01	0.01	0.05	N.S.	N.S.	0.00	0.05	0.02
GrIVDMSO									
Mean ± SD	118.0 ± 19.7	2068 ± 210	154.0 ± 15.4	20.06 ± 0.73	199.6 ± 15.3	16.42 ± 4.25	211.6 ± 12.9	9.14 ± 0.93	2.97 ± 1.14
Change %	3.42	5.30	17.65	114.65	13.67	18.64	7.36	-10.30	-24.81
P<	N.S.	N.S.	0.05	0.001	N.S.	N.S.	0.02	0.05	0.05
Gr. V Comb.									
Mean±SD	105.9 ± 11.4	2703 ± 433	120.9 ± 20.2	14.79 ± 0.70	186.0 ± 12.4	19.79 ± 5.614	164.2 ± 11.5	8.19 ± 1.50	3.19 ± 0.61
Change %	-7.18	37.63	-7.64	58.18	5.92	43	-16.70	-19.63	-19.24
P<	N.S.	0.01	N.S.	0.001	N.S.	0.01	0.001	0.01	0.01

[a] Results are means±SD of 5 values, each consisted of 3 pooled mice
[b] Results are means±SD of 10 mice

and erythrocytes G6PDH activities (58.18%, $p<0.001$). The level of erythrocyte CAT activity was not significantly affected in all treated groups. In connection to liver parameters a significant reduction in glycogen concentration (39.74%, $p<0.001$), a significant increase in RNA concentration (17.17%, $p<0.01$) and a non-significant change in the levels of total protein and DNA were recorded in tumor-bearing animals (Gr. II), compared to normal controls. Treatment of tumor-bearing animals with 10 injections of IAA (Gr.III) produced significant decreases in total protein (14.20%, $p<0.01$), RNA (11.70%, $p<0.05$) and DNA (19.24%, $p<0.02$) concentrations, whereas hepatic glycogen concentration was not significantly changed, compared to normal controls. Treatment with 10 injections of DMSO (Gr. IV) showed a significant elevation in total protein concentration (7.36%, $p<0.02$), and in contrast a significant reduction in RNA and DNA concentrations (10.30 and 24.81%, $p<0.05$, respectively). Combined treatment (Gr.V) caused a significant elevation in glycogen concentration (43%, $p<0.01$) and significant decreases in total protein (16.70%, $p<0.001$), RNA and DNA concentrations (19.63 and 19.24%, $p<0.01$, respectively) compared to normal controls.

DISCUSSION

In the present study, the acute LD_{100} and LD_{50} values of IAA on i.p administration

are reported to be 11.59 and 7.35 mg/100 g b.w. respectively in adult female Swiss albino mice. Treatment of tumor-bearing mice with the $LD_{12.5}$ values of IAA (1.84 mg/100 g b.w.) and DMSO (350 mg/100 g b.w.) exerted a significant effect in the retardation of the tumor growth. This is demonstrated by significant increases in the percentage of survivors and the cumulative mean survival time on day 40 ($p<0.001$), compared to tumor-bearing controls, and the marked decrease in the mean tumor weights. It merits note that a more pronounced effect was observed in the combined treatment represented by higher increases in the life span of animals (220.77%) and the tumor inhibition ratio (78.40%), compared to tumor-bearing controls, in addition to higher increases in the percentage of survivors and the cMST, compared to single IAA and DMSO treatments (Tables I and II). Our findings are in line with the main concept of cancer research that evaluation of any tested substance depends on extension of the survival time of cancer patients, and that an increase in the life span of drug -treated tumor-bearing mice ≥125% is considered indicative of presumptive drug activity (Rose *et al.*, 1984 and Buc-Calderon *et al.*, 1989) .

Biochemical studies were carried out to assess the effects of the metabolic inhibitor IAA and the free radical scavenger DMSO on the glucose metabolism and the antioxidant defense system of tumor-bearing animals and, in turn, their involvement in retardation of the tumor growth. Hypoglycemia and depleted liver glycogen content, which were previously reported in tumor-bearing animals (Balint, 1991; Killington *et al.*, 1991; Rawall *et al.*, 1991; and Fahim *et al.*, 1993), are greatly modulated by IAA treatment. This observation could be explained in terms of the IAA inhibitory effects on the anaerobic glycolysis (Bickis and Quastel, 1965), the pentose phosphate pathway (Badwey and Karnovsky, 1980) and the glycogen phosphorylase activity (Takeuchi and Glenner, 1961). The amelioration of the plasma glucose level and the significant increase in the liver glycogen concentration in the combined group could be ascribed primarily to the ability of the polar solvent DMSO to cross cell membranes and thus potentiate the hyperglycemic effect of IAA. This indicates that IAA and DMSO act synergistically to suppress the growth of solid tumor by depriving the cancer cells of the energy fuels, and by decreasing the pentose pool necessary for DNA and RNA syntheses in rapidly proliferating cells.

Results presented in Table III demonstrate a significant elevation in the levels of erythrocytes GP_x, G6PDH and CAT activity after 29 days of tumor implantation. Our results are in accordance with those of Navarro *et al.* (1999), who affirmed significant elevations in erythrocytes GP_x, G6PDH and CAT activities in mice bearing growing Ehrlich ascites tumor cells, which confirms the oxidative stress on the host in response to the continual generation of free oxygen radicals by the increasing tumor load. Treatment with 10 injections of IAA caused significant reductions in erythrocytes GP_x and G6PDH activities, and a non-significant change in the level of erythrocyte CAT activity, compared to normal controls (Table III). The biochemistry of the erythrocyte is relatively simple, with its metabolism dependent primarily on glycolysis and the PPP. The first oxygen free radical to be generated is the superoxide anion ($^{\bullet}O_2^-$), which is responsible for the formation of the subsequent oxygen free radicals by dismutation into H_2O_2, which is detoxified by catalase or transformed into $^{\bullet}OH$ radical by Fenton's reaction (Burrell and Blake, 1989). IAA is also known as a sulfhydryl inhibitor, i.e. an electrophile that inhibits the G3PDH enzyme by conjugation with the (-SH) group essential for the enzyme activity. However, other thiols, such as glutathione, also react with IAA at high concentrations causing depletion of the intracellular pool of glutathione (Nicotera and Orrenius, 1986). Taken together, the significant reduction in blood GP_x and G6PDH by IAA treatment could be infered.

In contrast, DMSO treatment resulted in a significantly sharp increase in the erythrocytes G6PDH activity and a slight increase in GP_x activity, while the erythrocyte CAT activity was not significantly changed, compared to normal

controls (Table III). Our results are in line with those of Ahmed *et al.* (1993), who reported that DMSO increases the activity of the oxidative segment of the PPP by two-fold, and the activity of two NADPH-producing enzymes; G6PDH and 6-phosphogluconate dehydrogenase by 3-6 fold in human promyelocytic leukemic cell line HL60. It has also been reported that DMSO could protect biological systems from the damaging effects of free radicals by its capacity to be oxidized (McGregor, 1967). Protection against free radicals and peroxides is largely dependent upon the availability of reduced glutathione (GSH), which in turn requires NADPH for its continual regeneration (Gessner *et al.*, 1990). Taken together, together, it could be interpreted that the significant elevations in erythrocytes GP_x and G6PDH activity provide a greater protection against the induced tumor. In the combined group, the counteracting effects of IAA and DMSO could account for the insignificant variations in the activity of blood GP_x and CAT enzymes. However, the persistent increase in blood G6PDH activity could be ascribed to the direct effect of DMSO on the oxidative segment of the PPP.

Untreated tumor-bearing mice (Gr.IIT) showed non-significant changes in the hepatic total protein and DNA concentrations associated with a significant elevation in the hepatic RNA concentration after 4 weeks, compared to normal controls (Table III). Comparable results were previously reported and explained (Lundholm *et al.*, 1978, 1979; Bengtsson and Andersson, 1981; and Ternell *et al.*, 1985). The significant decreases in the levels of hepatic total protein, RNA and DNA in response to IAA treatment are in accordance with the findings of Glazer and Weber (1971), who reported a significant reduction in the total protein, RNA and DNA synthesis of newborn rat brain cortex slices after the addition of IAA due to one site of action for IAA on DNA synthesis, which may involve some processes concerned with the formation of DNA precursors, such as the sulfhydryl-sensitive ribonucleotide reductase complex. Another explanation for this observation was documented by Kosower and Kosower (1978) that IAA conjugates with the –SH group of glutathione, which has been postulated as a potential regulator for protein synthesis, DNA synthesis and cell proliferation. This suggestion is in line with our findings and accounts for the significant decreases in the levels of erythrocytes GP_x and G6PDH activity (Table III).

On the other hand, treatment with 10 injections of DMSO (Gr.IV) significantly decreased the hepatic RNA and DNA concentrations, and in contrast, increased the level of hepatic total protein (Table III). Our data are in agreement with the results obtained from the *in vitro* studies of Higgins and Donnell (1982) and Higgins (1984), who tested the effect of the differentiation-inducing agent (DMSO) on the protein composition of murine hepatic tumor cell line (BW 77-1) and found that DMSO increased total cellular protein, especially the albumin and transferrin fractions. In addition, Higgins (1986) affirmed that suppression of murine liver tumor cells (BW 77-2 and Hepa-1/A1) proliferation by 3% DMSO (0.26 g/100 g b.w.) persists only in the continuous exposure assay and that resumption of cell division is readily observed following the removal of DMSO from the culture medium. During DMSO treatment, 88% of the tumor cells were accumulated in the G_1 phase of the cell cycle, compared to 48% of G_1 cells in the control cultures, and exhibited a substantial shift to lower mean cellular RNA content. Low RNA, non-replicating hepatic tumor cells in DMSO-treated cultures were designated as being in the "Q_i" substate (Quiescent phase). Accordingly, this points to the importance of the continuous exposure of DMSO in the tumor treatment, which accounts for the partial suppression of cell proliferation in the intermittent treatment modality implemented in the present study due to incomplete cell arrest in the quiescent substate. The more notable reduction in the liver total protein concentration in response to the combined treatment (Gr.V) might be due to the IAA and DMSO synergism (Table III).

CONCLUSIONS

In conclusion, treatment with IAA or DMSO significantly retards the growth of solid Ehrlich carcinoma, which is more pronounced in the combined treatment modality demonstrated by the higher increase in the cumulative mean survival time, tumor growth inhibition ratio, and the highest reduction in the mean tumor weight, compared to tumor-bearing controls. Results obtained from this study suggest that the retardation of the tumor growth rate by IAA and DMSO takes place by two different modes of action. The IAA inhibits the main pathways of energy production, i.e. glycolysis, pentose monophosphate pathways and glycogenolysis. On the other hand, DMSO suppresses the tumor growth not only by arresting the tumor cells in the quiescent phase as previously reported, but also by inducing the host defense system through the induction of the antioxidant enzyme system to increase the disposal of overproduced reactive free oxygen radicals. This suggests that IAA and DMSO could be used as adjuncts to chemotherapy or surgery for the management of cancer.

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