論 議

Lester 등(1958)은 steroid 가 微生物의 生長에 抑制作用을 나타냄을 最初로 報告하였다. 이어 Kawada(1959)와 Cox 및 MacLeod (1961)는 哺乳動物의 培養細胞에도 이와같은 效果가 있음을 알았고, Stone(1962), Stone 및 Kang (1962), Kang (1963)등은 HeLa 와 Chinese hamster 의 cell line 에서 progesterone, testosterone, 및 DOC(Deoxycorticosterone)등의 steroid 物質이 이들 細胞의 生長抑制作用은 물론 染色體의 數 및 構造的인 變化도 일으킴을 보았다.

本 實驗의 結果중 steroid 處理에 의한 染色體의 數 및 構造的인 異常率은 著者들의 이전 研究의 結果와 一致하며, X-線의 영향과 유사한 效果를 나타낸다는 著者들의 의견에 부합되고 있다.

培養細胞 染色體의 DNA 合成에 미치는 steroid 物質의 영향을 研究한 것은 Amaral (1967)의 報告가 처음이다. 그는 생쥐의 肝細胞에 cortisone 를 處理했을 경우 染色體의 DNA 合成이 抑制될뿐 아니라 倍數體가 다수 나타남을 보았다. 著者들은 사람의 腎臟細胞를 材料로 한 研究에서 progesterone 과 testosterone 은 染色體의 DNA 合成과 分裂活動을 抑制시킨다고 報告하였다. 이밖에 생쥐를 材料로 한 *in vivo* 의 實驗으로 estrogen 과 hydrocortisone 이 染色體의 DNA 合成樣相과 時期에 미치는 영향도 報告되고 있다(Beato 등, 1968; Frankfurt, 1968).

5-AU 處理에 의한 同時分裂 機作에 대해서 Smith 등(1963)과 Prensky 및 Smith(1965) 등은 5-AU 가 DNA 合成을 中斷시키며, DNA 合成에 이르지 못한 細胞週期의 다른 段階의 細胞들은 영향을 받지 않고 그대로 分裂을 계속하여 S-stage 까지 감으로 해서 S-stage 의 細胞가 축적되고, 다음 5-AU 를 제거하면 이 切斷이 해제됨으로써 부분적인 同時分裂細胞群을 이룬다고 하였다. 그러나 Jacob 및 Trosko(1965)는 5-AU 가 DNA 合成을 中斷시키는 것보다 다만 지연시킬 뿐이며, 同時分裂誘發機作은 G_2 의 細胞가 分裂을 더 계속하지 못하고 中斷되는 때문이라고 하였다.

本 實驗에 있어 5-AU 處理받은 細胞에서 標識된 分裂像의 頻度가 增加하는 것은 5-AU 에 의해 S-stage 의 細胞가 축적되는 결과로 해석되며, steroid 를 받은 細胞에서 그 率이 감소하는 것은 이같은 處理物質 때문에 DNA 合成이 지연되는 結果에서 온다고 본다.

사람의 培養細胞의 generation time 과 각 段階別 持續時間에 대해 Bender 및 Prescott(1962)는 白血球細胞에서 G_1=최하 24時間, S=최하 12時間, G_2=최고 6時間이라 했으며, Terakih(1965)는 羊膜細胞에서 S=16時間 내의, G_2=5-9時間, G_1=24時間 이상이라 報告하였다. 그러나 Gave(1966)는 白血球細胞의 G, S 그리고 G_2 의 平均 持續時間은 각각4.6, 9.6, 2.5時間이라 하였다.

本 實驗에 있어 對照區의 結果는 대체로 위와 일치하나 steroid 處理한 細胞에서는 G_2-stage 가 훨씬 연장되고 있으며, S-stage 는 별차 없음을 알 수 있다. 이것은 處理物質이 DNA 合成 持續時間에는 크게 영향을 끼치지 않고, 다만 抑制作用만 나타내는 때문이라고 생각된다.

要 約

5-AU 에 의해 同時分裂 促進된 사람의 胎兒 腎臟細胞를 材料로 steroid 에 의한 染色體 異常率. 時間經過에 따른 染色體異常率, DNA 合成樣相을 調査한 結果는 다음과 같다.

1. 5-AU處理區에서 細胞當 染色體 異常率은 0.131 로 對照區에 比해 3倍이상이나 된다. 또한 5-AU+progesterone 과 5-AU+testosterone 處理區에서는 細胞當 染色體異常率이 각각 0.340 과 0.452 이다.

2. 5-AU 處理區에서 異常染色體를 지니는 細胞는 0.8%로 時間變化에 무관하게 전체 期間에 걸쳐 존재한다. 5-AU+progesterone 과 5-AU+testosterone 處理區에서는 2.2%, 4.3% 의 異常染色體數가 觀察되고, 時間이 지남에 따라 增加한다. 또한 染色體 異常率은 5-AU+progesterone 處理區에서는 12時間과 18時間에 가장 높았고, 5-AU+testosterone 處理區에서는 時間變化에 따라 감소하고 5-AU 處理區에서는 유의한 차이가 없다.

3. 5-AU 는 標識分裂像의 出現頻度와 標識强度를 增加시키는데, 이는 5-AU 에 의해 S-stage 의 細胞가 축적되는 결과로 생각된다. 그러나 steroid 는 標識分裂像의 出現頻度를 감소시키고 DNA 合成時期를 지연시키고 있다. 또한 性染色體의 DNA 合成樣相이 細胞週期의 각 段階에 따라 다르며, 이는 5-AU 와 steroid 의 二重處理로 DNA 合成時期를 不規則하게 만든 때문이다.

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Radioprotective Effect of Methylene Blue

1. Effect of Methylene Blue on Lactic Dehydrogenase Level and Lactic Dehydrogenase Isoenzymes of Rats Exposed to Gamma-Irradiation

Sang Yul Nam and Seung Han Chang

(Department of Biology, Kyung Hee University, Seoul, Korea)

Methylene Blue의 放射線 防禦效果

1. Methylene Blue가 γ線에 照射된 흰쥐의 乳酸脫水素酵素의 含量과 乳酸脫水素酵素의 同位酵素에 미치는 影響

南 相 烈·張 承 漢

(慶熙大·文理大·生物學科)

(Received November 29, 1969)

摘 要

成熟한 albino 雄性 흰쥐를 對照群과 實驗群으로 나누어 對照群에는 0.9% 生理食鹽水를, 實驗群에는 methylene blue(38 mg/kg, 食鹽水에 溶解, pH 7.4)를 各各 腹腔注射處理하였다. 約 30分後 兩群 共히 總線量 360 rads인 ^{60}Co의 γ線을 一時 全身照射하였다. 照射後 212時間에 걸쳐 血淸總乳酸脫水素酵素의 含量(Cabaud and Droblewski 1958)과 血淸을 비롯하여 肝, 心臟, 精巢組織의 乳酸脫水素酵素의 同位酵素푸랙숀(Preston *et al.*, 1965)을 各各 測定하여 methylene blue의 放射線에 對한 防禦效果를 硏究調査하였다. 一般的으로 血淸總乳酸脫水素酵素의 含量은 兩群共히 γ線照射後 初期에 增加하나 特히 methylene blue 實驗群인 處理群은 對照群인 生理食鹽水群에 比하여 15 및 64時間區에서 增加의 遲滯性을 나타내었다. 血淸과 肝, 心臟 및 精巢組織의 乳酸脫水素酵素의 同位酵素는 兩群共히 γ線照射後 40과 116時間區에서 陽極쪽의 同位酵素푸랙숀含量은 減少한다. 照射後 methylene blue 處理群의 血淸과 肝 및 精巢組織 乳酸脫水素酵素의 同位酵素패턴에서는 對照群과 別差異가 없었으나 特히 methylene blue 處理群의 心臟組織은 對照群에 比하여 若干의 增減의 遲滯性을 나타내었다. 兩群共히 照射後 血淸乳酸脫水素酵素含量의 增加는 同位酵素푸랙숀 1의 增加에 基因된다고 思料되며 이상의 結果로 미루어보아 總乳酸脫水素酵素 活性과 心臟組織의 乳酸脫水素酵素의 同位酵素패턴에 있어서 methylene blue가 放射線防禦效果가 있는 것으로 思料된다.

INTRODUCTION

The role of sulfhydryl groups as protective compounds against radiation injury has been extensively investigated. Methylene blue has been shown recently to have a radioprotective action in mice and rats (Chung and Nam, 1967; Kim and Nam, 1967; Chang and Nam, 1968; Nam and Koh, 1969). Chung and Nam

* This work was supported in part by a research grant from the Ministry of Education of Korea and part of this study was reported in the International Symposium on Biological Aspects of Radiation Protection in Kyoto, Japan, October 14, 1969.

(1967) have reported that methylene blue greatly reduced the sensitivity of mice to sublethal dose of X-rays, provided that methylene blue was given before the exposure. Exposure of living organisms to ionizing radiation results in severe metabolic alteration and one of the biochemical indicators in mammalian radiation injury is an increase in the enzyme lactate dehydrogenase (LDH) activity of serum and plasma after irradiation with gamma or X-rays(Hawrylewicz, 1961; Dalrymple *et al.*, 1965; Hawrylewicz and Blair, 1965; Hori *et al.*, 1968). The data are of limited value, since other physical stresses also increase serum LDH activity(Garbus *et al.*, 1964; Blatt *et al.*, 1965; Altland *et al.*, 1968; Mager *et al.*, 1968). The LDH enzyme system plays a principal role in the glycolytic cycle for the conversion of stored energy. Also, gamma-irradiation and proton irradiation produce abnormal serum LDH isoenzyme patterns (Hawrylewicz and Blair, 1965; Blair and Hawrylewicz, 1965; Hawrylewicz and Blair, 1965). The serum and various tissue isoenzymes appear to be a reflection of all the tissue-specific isoenzyme patterns. Two basic types of LDH have been identified: H and M (Kaplan, 1962; Kaplan, 1963). The H and M molecules are tetramers, and their hybrids account for other isoenzymes. The present report describes the radiation effect of methylene blue on LDH level in serum, and LDH isoenzyme patterns in serum and various tissues of rats.

MATERIALS AND METHODS

Laboratory-conditioned male rats (albino strain) weighing 110 to 150 gm were used. They were housed in a temperature and humidity-controlled room (23±1°C, 62 ±4%), and fed *ad libitum* on water and commercial diet. In these experiments, rats were divided into a control group and a group treated with purified methylene blue provided by the Wako Pure Chemical Industries, Ltd., Osaka, Japan. Each rat in the control group received an intraperitoneal injection of 0.9% saline (7ml/kg body weight), while each rat in the other group was injected intraperitoneally with an equal volume of an aqueous solution of methylene blue at a dosage of 38 mg/kg.

The rats in both the control and methylene blue groups were given 360 rads of whole body gamma-irradiation beginning 28 to 32 minutes after the intraperitoneal injection. Irradiation was done with gamma-rays of ^{60}Co. The rats in both groups were exposed to gamma-irradiation in less than 4 minutes. Irradiation was done at the Radiation Research Institute, Seoul, Korea. The control and methylene blue-treated rats were restrained and irradiated at one time in a wooden cage (18×18×7cm) and slowly rotated under the beam to insure uniform exposure.

After irradiation, the control and methylene blue-treated rats were kept under the above mentioned condition up to the time of sacrifice; 15, 40, 64, 92, 116, and 212 hours. Blood samples were obtained with tubes from severed left saphenous vein. Serum was separated by centrifugation at 3,000 rpm for 15 minutes and kept in a refrigerator until enzyme-isoenzyme analysis.

Total serum LDH activity was determined at 550mμ with a Spectronic 20 spectrophotometer by a colorimetric method of Cabaud and Wroblewski(1958) utilizing commercial reagents supplied by the Sigma Chemical Company. The LDH activity was expressed as units per ml.

For tissue extracts, liver, heart, and testis tissues were removed, and washed twice with Krebs-Ringer phosphate solution. The tissues were transferred to 20× (W/V) cold buffer (phosphate, pH 7.5, 0.05M, 0 to 2°C) and homogenized in a tissue homogenizer. The homogenate was centrifuged for 30 minutes at 12,000× *G* to remove particulate matter. Isoenzymes of the serum and various tissue extract samples were subjected to separation by electrophoresis on cellulose acetate (Sepraphore Gelman Instrument Co.) by a method of Preston *et al.* (1965). Samples were electrophoresed for 70 minutes at 0.8 mA per cm. Cellulose acetate strip samples were stained with nitro-Bt tetrazolium using sodium lactate as substrate and β-nicotinamide adenine dinucleotide (β-NAD) as a cofactor following the method described by Preston *et al.* (1965). Relative isoenzyme activity levels were determined by densitometry using a densitometer (Analytrol, Beckman Model RB).

RESULTS

Total Serum LDH

The changes in serum LDH activity of the control, physiological saline-treated and methylene blue-treated rats were followed up to 212 hours after exposure to 360 rads. These experiments verified the observation that whole-body gamma-irradiation causes a significant increase in serum LDH level in both the control and methylene blue-treated groups. The radiation-induced elevation of serum LDH level may be attributed mainly to the release of enzyme from the radiosensitive cells.

As is shown in Table 1 and Fig. 1, the serum LDH levels of the control-nonirradiated rats and the methylene blue-treated rats without irradiation were an average of 266.90 units and 267.50 units, respectively. The LDH levels of the control and methylene blue-treated rats began to increase at 15 hours after exposure, decreased slightly at 40 hours and continued to increase up to a maximum level at 64 hours after exposure. Then it returned nearly to the initial level in 212 hours after exposure. The control group exhibited a rise of LDH to an average of 283.65 units at 15 hours and 329.50 units at 64 hours, while the methylene blue-treated group 272.23 units at 15 hours and 313.75 units at 64 hours postirradiation, respectively. Methylene blue showed a marked delay in serum LDH rise at 15 and 64 hours after exposure, but no significant difference in serum LDH level between the control and methylene blue-treated groups.

Table 1. Effect of methylene blue (38 mg/kg) administration in LDH activity in serum of rats after whole body Co-60 gamma irradiation with 360 rads

Treatment	Hours after irradiation	No. of rats	Serum LDH activity level (units)	
			Mean	SE
NaCl 360 rads	0	15	266.90	2.03
	15	9	283.65	6.12
	40	9	281.27	4.77
	64	10	329.50	4.50
	92	10	296.20	5.40
	116	10	296.20	5.41
	164	9	276.50	5.37
	212	7	263.70	3.24
Methylene blue 360 rads	0	15	267.50	2.14
	15	9	272.23	4.27
	40	9	271.23	4.21
	64	9	313.75	6.77
	92	10	281.27	4.77
	116	8	283.65	7.12
	164	9	270.10	6.42
	212	7	258.20	4.68

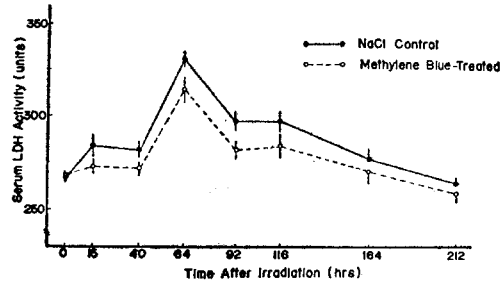

Fig. 1. Influence of methylene blue in the serum LDH level of rats at various time intervals after Co-60 gamma irradiation with 360 rads. Each point represents average value and the vertical bars indicate the standard error of the mean.

Serum and Tissue LDH Isoenzymes

The electrophoretic resolution patterns of each serum and tissue LDH enzyme samples indicated a nonuniform response to irradiation with respect to the isoenzyme fractions. Mean values of levels for serum and various tissue LDH isoenzyme and standard errors of the mean are included in Table 2. Mean values are shown graphically in Figs. 2, 3, 4 and 5 for visual comparison. Electrophoresis of the serum of normal rat shows three bands indicating 3 LDH isoenzymes. The most prominent band (band 5) corresponds with the major LDH isoenzyme in rat liver (Garbus *et al.*, 1964). Less conspicuous is band 1 (nearest anode), which corresponds to the major isoenzyme found in the heart and testis, and band 2 which is considered as a mixture derived from aggregation of the subunits of 1 and 5 (Markert, 1963) and is found in variable amount in most organs studied.

The immediate rise in total serum LDH activity was manifested by a marked increase in the band 1, H

Table. 2. Effect of methylene blue administration in serum and tissue LDH isoenzyme distribution of rats after whole-body Co-60 gamma irradiation with 360 rads

Treatment	Hours after irradiation	No. of Rats	No. of LDH bands	LDH isoenzyme patterns (%)							
				Serum		Heart		Liver		Testis	
				Mean	SE	Mean	SE	Mean	SE	Mean	SE
NaCl control	0	15	1	36.90	3.60	27.13	4.95	14.20	1.50	38.80	6.96
			2	19.10	5.53	27.64	6.25	13.50	3.11	30.36	3.46
			3	—	—	15.93	2.84	18.85	1.02	7.47	2.12
			4	—	—	17.20	8.69	20.20	1.50	15.31	4.26
			5	44.00	8.10	12.10	3.51	36.25	10.38	8.06	2.87
Pre-irradiation	15	9	1	51.63	5.30	60.87	7.43	26.00	8.82	44.30	6.71
			2	29.97	3.90	29.06	6.35	24.95	0.85	37.35	4.87
			3	—	—	6.10	1.70	14.55	0.65	6.60	3.71
			4	—	—	1.57	0.63	7.40	2.91	4.00	1.11
			5	18.30	1.40	2.40	0.87	27.10	7.42	7.75	6.77
	40	9	1	63.57	5.45	57.47	4.21	35.83	8.87	53.70	8.72
			2	24.20	2.21	34.93	9.78	26.37	2.82	37.90	5.43
			3	—	—	3.80	1.34	18.50	1.49	2.30	1.19
			4	—	—	1.73	0.53	4.73	1.20	1.77	0.82
			5	12.23	3.48	2.07	0.90	14.57	8.12	4.33	1.62
	64	10	1	53.95	7.57	48.17	7.41	15.50	5.95	55.20	3.68
			2	25.80	0.20	31.56	2.36	10.00	0.59	38.70	5.16
			3	—	—	13.20	5.20	11.97	7.56	3.34	0.56
			4	—	—	3.77	1.44	9.47	4.38	1.63	1.14
			5	20.25	7.78	3.30	1.35	53.06	8.78	6.13	0.09
	92	10	1	33.85	14.49	39.90	2.11	5.30	1.70	46.85	0.35
			2	17.50	0.80	33.95	2.35	3.50	0.81	33.80	4.21
			3	—	—	16.40	0.33	8.20	0.36	13.70	3.11
			4	—	—	4.65	1.55	37.45	2.86	2.75	0.05
			5	48.65	13.68	5.10	3.31	45.55	6.88	2.90	1.50
	116	10	1	51.25	13.79	47.65	0.65	6.65	0.59	54.45	8.00
			2	26.90	3.82	41.45	1.75	8.70	1.91	27.50	15.14
			3	—	—	8.70	2.61	45.00	8.77	12.90	8.44
			4	—	—	1.25	0.15	28.30	0.24	2.40	9.01
			5	21.85	7.84	0.95	0.35	10.35	8.14	2.75	2.16
	164	9	1	44.95	8.15	26.70	6.48	23.00	9.17	38.90	11.65
			2	34.15	5.42	34.90	8.14	18.30	5.94	55.50	14.51
			3	—	—	35.90	7.10	22.50	6.71	4.40	0.43
			4	—	—	1.50	0.54	10.10	2.45	0.60	0.21
			5	20.90	4.12	1.00	0.63	26.10	9.15	0.60	0.26
	212	7	1	43.20	5.14	43.10	4.15	13.10	8.14	50.00	10.14
			2	17.00	3.21	39.10	4.18	21.30	7.16	40.60	8.15
			3	—	—	10.10	2.18	26.20	6.18	3.50	1.45

			4	—	—	3.60	1.10	8.20	4.14	1.80	0.94
			5	33.80	4.84	4.10	0.84	31.20	6.14	4.10	1.07
			1	38.80	8.86	26.50	1.60	13.23	0.41	38.40	7.47
			2	17.60	4.19	27.00	3.30	14.73	3.17	29.90	6.02
	0	15	3	—	—	18.60	1.99	18.07	2.43	7.37	3.10
			4	—	—	13.75	0.95	19.33	7.78	15.77	8.35
			5	43.60	9.66	14.15	1.18	34.63	3.75	8.57	6.92
			1	54.55	11.28	43.73	6.05	19.10	9.13	54.10	3.28
			2	33.65	6.07	35.80	3.62	19.25	2.55	41.76	1.50
	15	9	3	—	—	8.74	2.71	14.20	9.13	1.60	0.36
			4	—	—	5.33	9.66	15.15	4.87	1.27	4.27
			5	16.80	10.23	6.40	7.15	32.30	2.30	1.27	0.27
			1	51.85	4.36	45.73	7.42	57.25	7.71	54.30	2.98
			2	37.30	11.43	37.50	4.58	31.30	0.40	26.18	3.17
	40	9	3	—	—	5.70	1.49	2.05	0.64	7.90	3.20
			4	—	—	3.83	1.05	2.05	0.64	1.95	0.19
			5	10.95	7.07	7.23	2.54	7.35	4.96	9.67	4.54
			1	35.97	14.14	30.87	5.08	3.63	2.29	31.23	1.05
			2	19.73	8.16	32.23	2.35	7.04	5.95	33.60	3.91
Methylene blue-treated Pre-irradiation	64	10	3	—	—	16.93	2.45	8.80	2.23	12.44	1.90
			4	—	—	12.67	4.26	13.70	1.65	14.43	0.60
			5	44.30	12.14	7.30	5.09	66.83	6.69	8.30	2.53
			1	53.40	0.70	33.85	9.98	13.15	4.42	50.90	5.62
			2	10.80	0.45	35.90	5.11	11.40	1.81	37.95	6.37
	92	10	3	—	—	18.00	1.70	12.35	7.55	4.90	1.11
			4	—	—	6.10	0.45	17.45	0.75	4.55	0.15
			5	35.80	0.07	6.15	4.16	45.65	14.35	1.70	0.50
			1	55.25	6.83	45.90	5.11	8.00	4.54	51.60	3.82
			2	25.35	1.15	39.05	9.08	22.80	11.67	37.85	1.55
	116	8	3	—	—	9.05	3.06	24.30	10.11	7.37	4.56
			4	—	—	2.30	0.14	16.00	9.48	2.35	1.35
			5	19.40	8.52	3.70	2.01	28.90	12.14	0.85	0.55
			1	44.70	11.73	45.30	11.45	2.60	0.45	40.30	8.94
			2	31.75	4.11	32.10	9.40	4.20	0.46	30.90	7.54
	164	9	3	—	—	5.00	1.14	52.80	9.45	26.80	3.11
			4	—	—	1.60	0.18	23.60	4.54	0.70	0.45
			5	23.55	0.45	16.00	8.76	16.80	2.16	1.30	0.75
			1	35.00	11.37	50.20	12.56	2.60	0.14	49.10	9.45
			2	23.70	12.14	36.40	12.76	6.10	1.45	44.90	11.11
	212	7	3	—	—	8.00	0.54	9.70	4.53	4.20	0.94
			4	—	—	3.00	1.62	21.10	7.94	0.90	0.45
			5	41.30	9.84	2.40	1.00	60.50	10.15	0.90	0.37

LDH fraction after exposure (Figs. 1 and 2). Of particular significance is the observation that about 40 hours after exposure the band 5, M LDH fraction markedly decreased on both the control and methylene

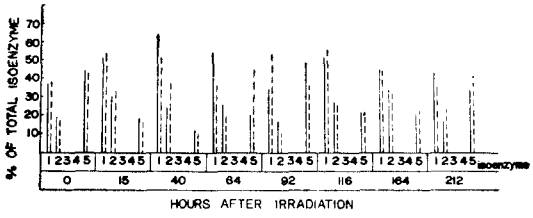

Fig. 2. LDH isoenzyme activity level in the serum analysis after Co-60 gamma-irradiation (360 rads). Distribution of isoenzyme expressed as percentage of total LDH activity. Band 1 is the more electrophoretically mobile(ano dal) isoenzyme. Band 5 is the least mobile (cathodal) isoenzyme. Soild lind indicates NaCl control rats; broken line methylene blue treated rats.

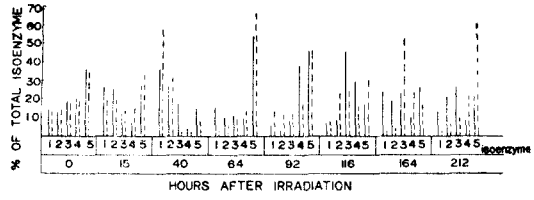

Fig. 3. LDH isoenzyme activity level in the liver analysis after Co-60 gamma-irradiation (360 rads). Distribution of isoenzyme expressed as percentage of total LDH activity. Band 1 is the more electrophoretically mobile (anodal) isoenzyme. Band 5 is the least mobile (cathodal) isoenzyme. Solid line indicates NaCl control rats; broken line methylene blue treated rats.

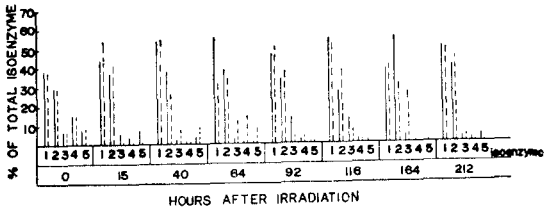

Fig. 4. LDH isoenzyme cativity level in the heart analysis after Co-60 gamma-irradiation (360 rads). Distribution of isoenzyme expressed as percentage of total LDH activity. Band 1 is the more electrophoretically mobile(anodal) isoenzyme. Band 5 is the least mobile (cathodal) isoenzyme. Solid line indicates NaCl control rats; broken line methylene blue treated rats.

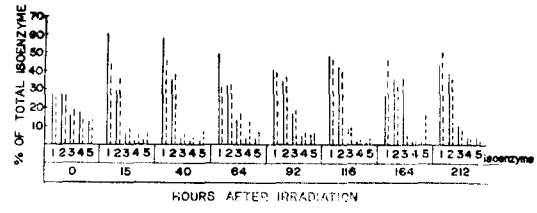

Fig. 5. LDH isoenzyme activity level in the testis analysis after Co-60 gamma-irradiation (360 rads). Distribution of isoenzyme expressed as percentage of total LDH activity. Band 1 is the more electrophoretically mobile (anodal) isoenzyme. Band 5 is the least mobile (cathodal) isoenzyme. Solid line indicates NaCl control rats; broken line methylene blue treated rats.

blue groups.

Band 1 is the leading anodal isoenzyme, while band 5 is the least mobile or cathodal counterpart in liver, heart, and testis tissues of normal rats. Mean values for band 1 in both the control and methylene blue groups after exposure were significantly increased in serum, liver, heart, and testis nearly at 40 and 116 hours. On the other hand, band 5 decreased in serum, liver, heart, and testis at 40 and 116 hours. Furthermore, the degree of alteration of bands 1 and 5 were directly related to the radiosensitivity of the organs. Mean values of activity for bands 2, 3, and 4 from serum, liver, heart, and testis were not significantly altered when the control and methylene blue-treated rats were compared after exposure. Especially, methylene blue showed a marked delay in heart LDH isoenzyme patterns in alteration after exposure.

DISCUSSION

Our findings indicate that methylene blue exerts a radioprotective action in serum LDH activity and heart tissue LDH isoenzyme activities. It should be noted that methylene blue must be given immediately before the exposure in the study of the time course of methylene blue protection against total body X-irradiation in mice (Chung and Nam, 1967).

The results obtained in both the control and methylene blue-treated rats confirmed that the single exposure to whole-body gamma-irradiation resulted in an increase in LDH activity in the serum of rats at 15 and 64 hours after exposure, and that the increased level of serum LDH returned to the initial level by 212 hours after exposure. Such transitory changes in serum LDH level were observed in *Rhesus* monkeys (Hawrylewicz and William 1966) and in rabbits (Albaum, 1960). Hori *et al.* (1968) reported the rise of plasma LDH level in mice in several hours after exposure to 600 R. Thymus and spleen exhibited a marked loss of LDH by exposure to 600 R, whereas liver, kidney and testis showed no significant changes between normal and irradiated mice (Hori *et al.*, 1968). Albaum (1960) found increased enzyme activities of glutamic-oxalacetic transaminase, LDH, enolase, and pyruvic kinase in the serum of rabbit in 24 hours following X-irradiation.

The immediate increase in serum LDH activity implies that extensive cellular damage has occurred. One may speculate that elevation of serum LDH level following whole-body exposure of rats might be due to the liberation of enzymes from radiosensitive tissues into the blood stream. It has been suggested that LDH enzyme is loosely held to the mitochondrial membrane (Brody and Engel, 1964) and readily removed in the process of cellular membrane damage. Therefore, the rise in total serum LDH activity may represent cell damage.

Kaplan and Ciotti (1961) reported that the distribution of LDH isoenzymes was variable in different tissues of a single species of animals and also showed variations in the same tissues of different species. The isoenzyme pattern is controlled not only by genetic determinants but also by physiological or metabolic requirements of individual organ and tissue (Allan, 1961).

The isoenzyme patterns found in this study agree well with the patterns determined by others (Kaplan and Ciotti, 1961; Allan, 1961) on a variety of tissues and species. The enzyme functions under widely varying physiological and biochemical conditions, and the differences are reflected in the different isoenzyme patterns associated with various organs(Vesell and Bearn, 1961). Markert (1963) has shown that the intermediate isoenzymes are a result of the substitution of genetically determined subunits into the H and M isoenzymes resulting in molecules of different electrophoretic mobilities and kinetic properties.

Wroblewski *et al.* (1960) have assumed that an increase in a particular serum isoenzyme is the result of leakage of the isoenzyme from cells of a damaged tissue and is a reaction to pathogenic situation. The distribution of tissue LDH enzyme patterns is influenced by diseased states (Wroblewski and Gregory, 1961). Hawrylewicz and William (1966) have suggested that increased cell permeability due to physiological stress may cause altered serum isoenzymes. The rise in serum of the H-type, band 1 of LDH isoenzyme in both the control and methylene blue-treated rats may reflect the sensitivity of particular tissues after exposure. Testis tissues in both groups appear to continuously loose the M-type, band 5 after exposure. It should be noted that exposure to 6,000 rads gamma-radiation in *Rhesus* monkeys caused marked alteration in the serum and tissue LDH enzyme activity, and that the increase of serum activity is a reflection of an immediate decrease in the H-LDH isoenzymes, bands 4 and 5(Howrylewicz and Blair, 1965).

In the present study, it is assumed that the increase of serum activity is a reflection of an increase in the H-type band 1. These results suggested that there is a difference in the degree of response as a function of time interval, type of radiation and dose. The extent of increase, the rate of increase and the extent of alteration of the isoenzyme ratios are functions of dose, energy level and type of radiation (Hawrylewicz and Blair, 1965).

The results obtained in this study support the concept that the methylene blue exerts a radioprotective action in serum LDH and heart tissue LDH isoenzyme activities. The mechanism by which methylene blue protects is unknown. However, it was hoped that some insight into the mechanism of the methylene blue protection might be forthcoming from a consideration of the influence of certain related substance of sensitivity to radiation.

SUMMARY

The protective action of methylene blue against

gamma-irradiation was studied with rats. Albino rats were given 360 rads of whole-body gamma-irradiation following an intraperitoneal injection of physiological saline or methylene blue. Male rats given methylene blue (38mg/kg) and the control rats given saline were alive following gamma-irradiation. Serum lactic dehydrogenase (LDH) activity, and LDH isoenzyme patterns in serum and various organs were determined at various time intervals after the exposure.

1) The serum LDH level in both the control and methylene blue-treated rats was increased during the initial phase, but returned to the initial level thereafter.

2) Methylene blue showed a marked delay in the rise of serum LDH at 15 and 64 hours after exposure.

3) The exposure in the control and methylene blue-treated rats resulted in an increase in the relative amount of the more electrophoretically mobile-anodal isoenzyme (band 1) and a decrease in the least mobile-cathodal isoenzyme (band 5) in serum, liver, heart and testis nearly at 40 and 116 hours, respectively.

4) Isoenzyme patterns in serum, liver and testis after exposure were not significantly different between the control and the methylene blue treated rats.

5) Methylene blue showed a slight delay in alteration of heart tissue LDH isoenzyme patterns after exposure.

6) The increase of serum LDH level after exposure is a reflection of an immediate increase in the H type, band 1 of LDH isoenzymes.

7) It is concluded from this study that methylene blue has a remarkable radioprotective action in the serum LDH activity and in the heart tissue LDH isoenzyme patterns.

ACKNOWLEDGEMENT

We would like to thank Mr. Soon Bae Chun and Mr. Hyung Suk Kim, Kyung Hee University for their excellent technical assistances.

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