Acta Medica Okayama

Volume 17, *Issue* 5 1963 *Article* 4

1963

Studies on the treatment of malignant tumors with fibroblast-inhibiting agent. II. Effects of chloro-quine on animal tumors

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Studies on the treatment of malignant tumors with fibroblast-inhibiting agent. II. Effects of chloro-quine on animal tumors*

Kiyoshi Hiraki and Ikuro Kimura

Abstract

Based on our original concept, a fibroblast-inhibiting agent, chloroquine, was used against various animal tumors. Among transplanted animal tumors, the drug was most effective on relatively connective tissue-rich Bashford and Brown-Pearce tumors, as reflected by prolongation of life span, inhibition of tumor growth, inhibition of lowering of liver catalase activity, improvement of iron metabolism, increase of tumor necrosis, inhibition of connective tissue formation, and decrease of acid mucopolysaccharide. On the other hand, it was of little advantage in Ehrlich, Yoshida and MH134 tumors which contain little connective tissue, except for a decrease of the amount of ascites and ascites tumor cells in the former two tumors. These results indicate that chloroquine suppress the growth of the tumors relatively rich in connective tissue. This effect of chloroquine appears to be due to the primary attack of the stromal connective tissue of tumors being followed by the degeneration of tumor cells, though its probable anti-tumor activity by the indirect effects through its anti-inflammatory and systemic humoral activities should be taken into consideration.

*PMID: 14164121 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 17, 239—252, (1963)

STUDIES ON THE TREATMENT OF MALIGNANT TUMORS WITH FIBROBLAST-INHIBITING AGENT

II. EFFECTS OF CHLOROQUINE ON ANIMAL TUMORS

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Received for publication August 10, 1964

Malignant tumors consist of the parenchymal tumor cells, stromal connective tissue and blood vessels. The latter two elements are undoubtedly playing an important role in the growth of the tumors. There are many people[1,2,3,4,5] who emphasized a close interrelationship of tumor cells and stromal elements, or who observed synbiotic growth of tumor cells and fibroblasts. We have also observed such an interrelationship in mixed culture of Yoshida sarcoma cells and fibroblasts a few years ago.[6]

The reason why we employ chloroquine against animal tumors is based on our unique concept that damage of the stromal connective tissue will lead to damage of the cancer cell. As reported previously, chloroquine is a potent fibroblast-inhibiting agent[7], and damage of the stromal tissue should indirectly bring about a profound deleterious effect upon the parenchymal tumor cells.

The present paper deals with the results obtained in the treatment of various animal tumors with a fibroblast-inhibiting agent, chloroquine.

MATERIALS AND METHODS

Animal tumors used were Bashford cancer, Ehrlich ascites and solid cancer, MH134 tumor maintained in inbred strains Strong A, C3H, RIII, and RF mice, Yoshida ascites and soild tumors carried in Wistar and random-bred rats, and Brown-Pearce carcinoma transplanted in albino male rabbits.

Either chloroquine diphosphate or chloroquine diorotate was administered daily by the intraperitoneal, intravenous, subcutaneous or oral route in the net amount of 6-15 mg. of chloroquine per kg. of body weight, beginning 24 hours after transplantation of tumors. Observations were performed on the survival of these animals, growth of tumors (average of two dimensions), weight of tumors, liver catalase activity (Euler-Josephson method[8]), organ iron content (Brückmann-Zondek method[9]), and histological changes as revealed by hematoxylin and eosin, PAS stain, toluidine blue, Hale reaction, Azan-Mallory stain, van Gieson stain, and silver impregnation. Ascites tumor cells were also

observed under the phase contrast microscope.

RESULTS

A higher survival rate was obtained in chloroquine-treated animals bearing Bashford and Brown-Pearce tumors in comparison to the control. No significant life prolongation was observed in animals with Ehrlich and Yoshida ascites tumors, and solid Ehrlich, Yoshida and MH134 tumors. Transplantation of Ehrlich ascites tumor cells after 24 hours' incubation with 2^{γ}, 20^{γ} and 200^{γ} per c. c. of chloroquine *in vitro* failed to modify the life span of the host animals. Furthermore, daily administration of chloroquine to animals for a week preceding or following the transplantation of Ehrlich ascites tumor cells had no life prolongation effect (Fig. 1, 2, 3, 4, 5).

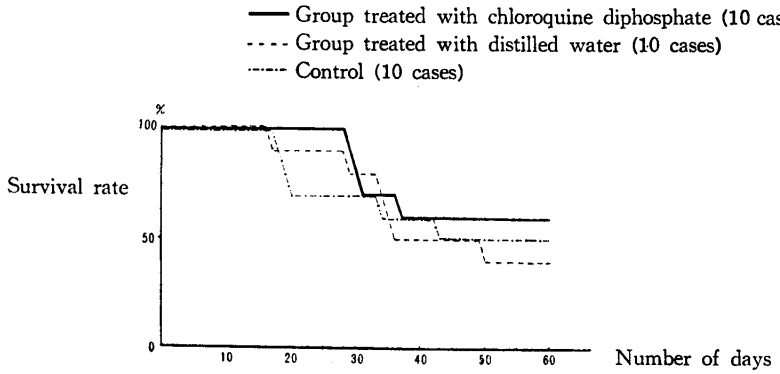

Fig. 1 Influence of chloroquine diphosphate on survival rate of mouse with Bashford cancer (Strong A strain ♂ mouse, continuous intraperitoneal injection of 25 mg./kg.)

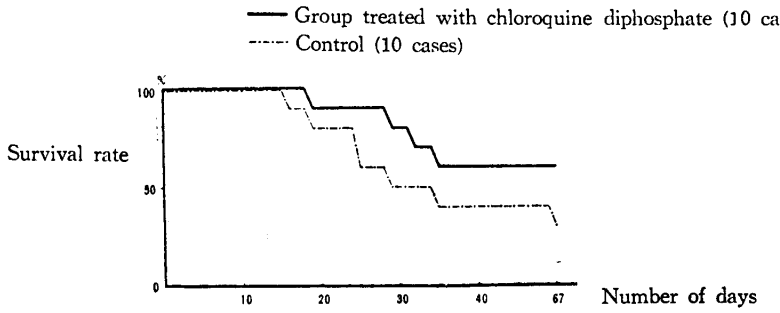

Fig. 2 Influence of chloroquine diphosphate on survival rate of rabbits with Brown-Pearce cancer (White rabbit ♂, continuous intravenous injection of 10 mg./kg.)

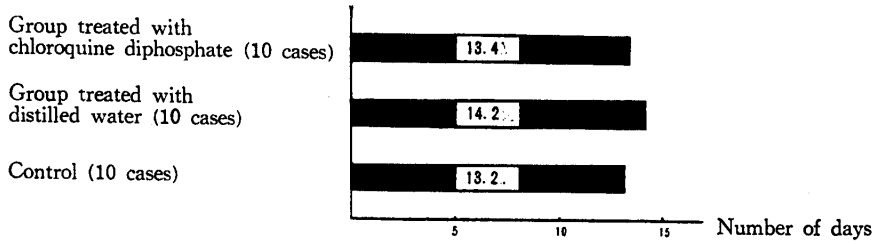

Fig. 3 Influence of chloroquine diphosphate on survival period of mouse with Ehrlich ascites cancer (Strong A strain ♀ mouse, continuous intraperitoneal injection of 25 mg./kg.)

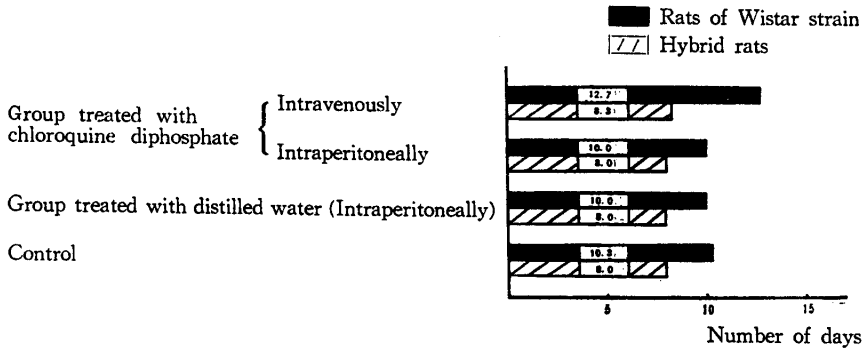

Fig. 4 Influence of chloroquine diphosphate on survival period of rats with Yoshida sarcoma (continuous injection of 10 mg./kg.)

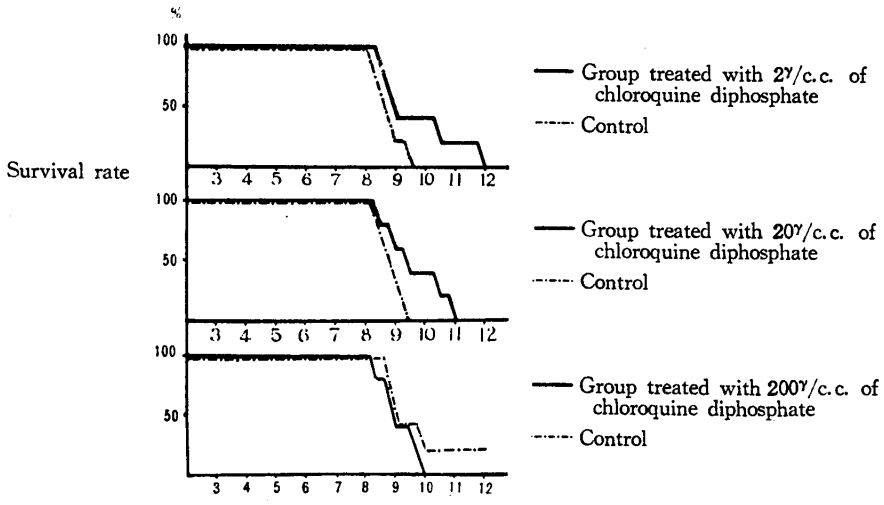

Fig. 5 Influence of chloroquine disphosphate on Ehrlich ascites tumor cells Survival rate of mouse after implanting cells treated with chloroquine *in vitro* (Strong A strain ♂ mouse)

Measurements of the size and weight of Bashford and Brown-Pearce tumors clearly indicated an inhibitory effect of chloroquine on the growth of these tumors. A decrease in both the amount of ascites and the number of tumor cells was demonstrated following intraperitoneal, oral or intravenous administration of chloroquine to animals bearing Ehrlich and Yoshida ascites tumors. But this was not the case with Ehrlich ascites tumor cells incubated with the agent *in vitro*. The drug showed no effect on the growth of solid Ehrlich, Yoshida and MH134 tumors (Figs. 6, 7, 8, 9, 10, 11, Photo 1).

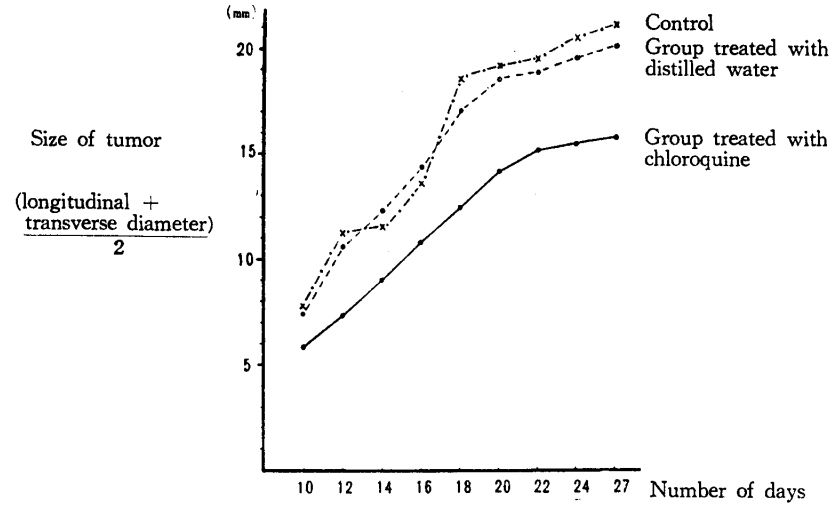

Fig. 6 Influence of chloroquine diphosphate on growth of Bashford cancer (Strong A strain ♀ mouse, 25 mg./kg. intraperitoneal injection)

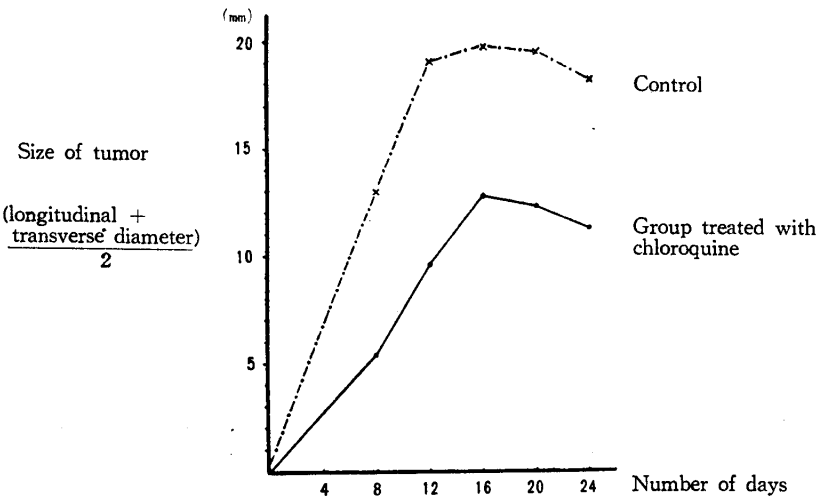

Fig. 7 Influence of chloroquine diphosphate on growth of Brown-Pearce cancer (White rabbit ♀, 10 mg./kg. intravenous injection)

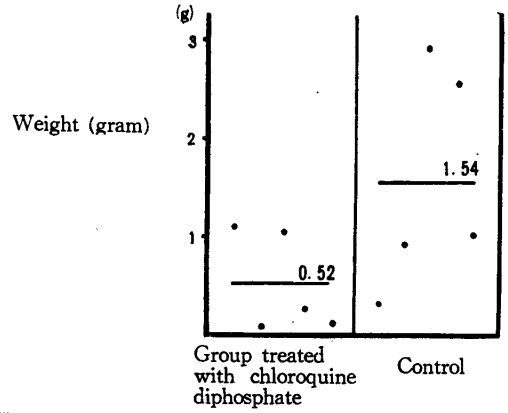

Fig. 8 Influence of chloroquine diphosphate on weight of Bashford cancer (Strong A strain ♂ mouse, 25mg./kg. intraperitoneal injection)

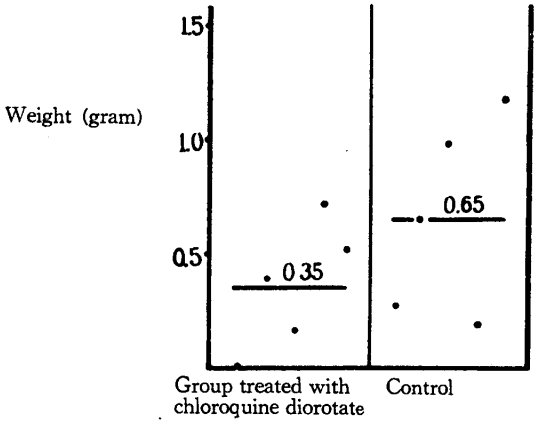

Fig. 9 Influence of chloroquine diorotate on weight of Bashford cancer (Strong A strain ♂ mouse, 30 mg./kg. intraperitoneal injection)

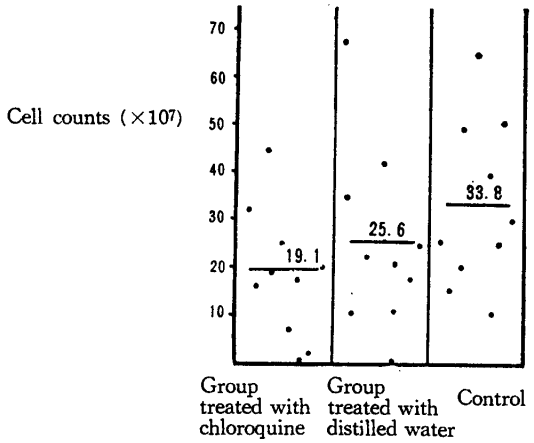

Fig. 10 Influence of chloroquine diphosphate on ascitic cell counts in mouse with Ehrlich ascites cancer at death (Strong A strain ♀ mouse, 25 mg./kg. continuous intraperitoneal injection)

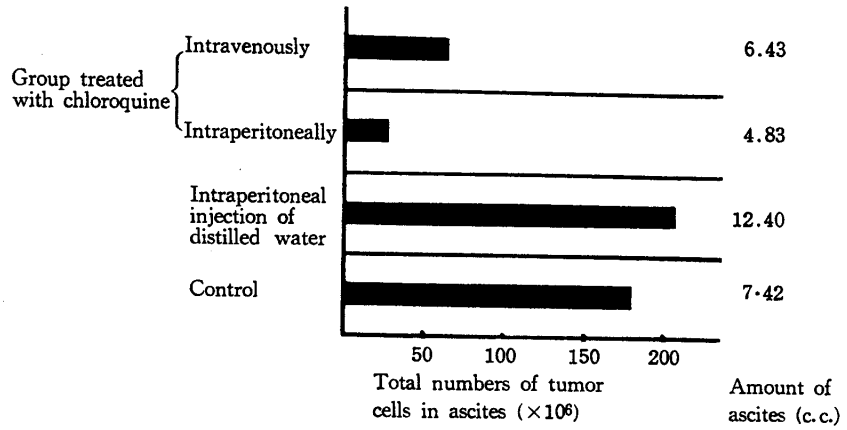

Fig. 11 Influence of chloroquine diphosphate on ascites tumor cells of rats with Yoshida sarcoma (♀ Hybrid rats, killed 1 week after 25 mg./kg. continuous treatment)

The liver catalase activity of animals bearing Bashford tumor was found to be increased after intervals of 1, 2 and 3 weeks' chloroquine treatment. On the other hand, such inhibition of lowering of liver catalase activity could not be seen with Ehrlich ascites tumor nor with Brown-Pearce tumor whose growth failed to be inhibited with chloroquine. It was our impression that in animals bearing tumors whose growth was inhibited by chloroquine, the liver catalase activity was increased (Figs. 12, 13, 14).

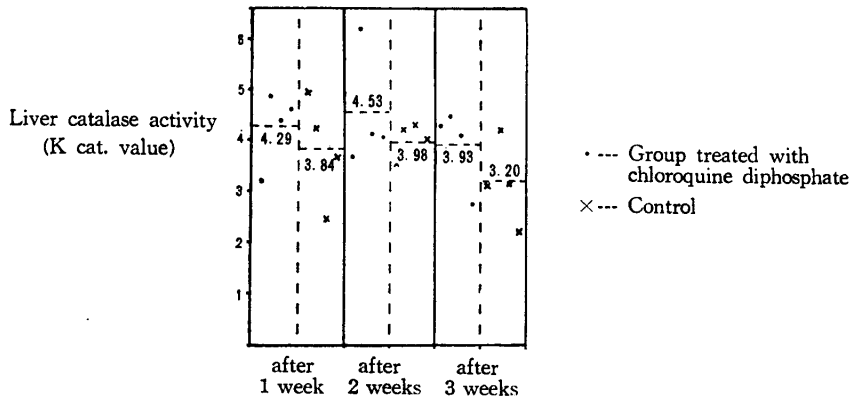

Fig. 12 Influence of chloroquine diphosphate on liver catalase activity of mouse with Bashford cancer (♀ Strong A strain, 25 mg./kg. continuous intraperitoneal injection)

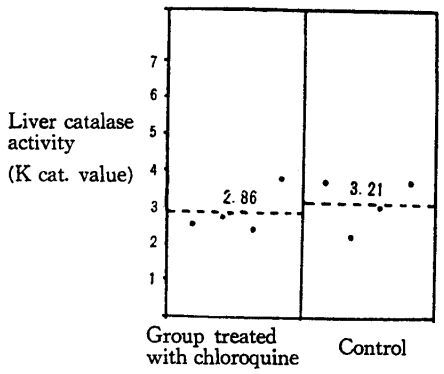

Fig. 13 Influence of chloroquine diphosphate on liver catalase activity of mouse with Ehrlich ascites cancer (♀ RIII strain, 25 mg./kg. continuous intraperitoneal injection)

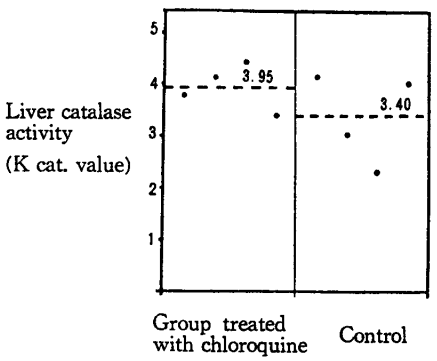

Fig. 14 Influence of chloroquine diorotate on liver catalase activity of mouse with Bashford cancer (♂ Strong A strain, 30 mg./kg. continuous intraperitoneal injection)

Non-hemin iron contained in the tumorous and non-tumorous tissues of animals bearing Bashford and Brown-Pearce tumors was reduced following treatment with chloroquine. In the case of Ehrlich tumor, the iron content of the ascites was decreased while that of the liver increased. This appeared to be indicative of a favorable effect of chloroquine on the iron metabolism even in tumors which were unresponsive to the agent (Figs. 15, 16).

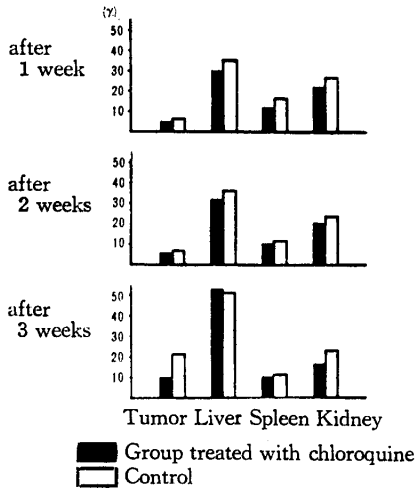

Fig. 15 Influence of chloroquine diphosphate on iron metabolism of mouse with Bashford cancer (total amount of non-hemin iron) (♀ Strong A strain, 25 mg./kg. continuous intraperitoneal injection)

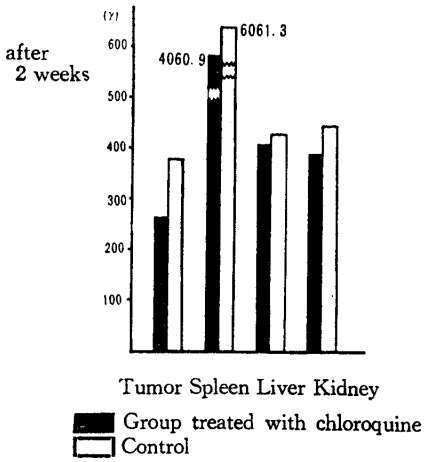

Fig. 16 Influence of chloroquine diphosphate on iron metabolism of rabbits with Brown-Pearce cancer (total amount of non-hemin iron) (♂ white rabbits, 10 mg./kg. continuous intravenous injection)

Histologically, chloroquine induced an increase of necrotic areas, poor formation of fibroblasts and collagenous fibers in the periphery of tumors, decrease of PAS positive granules, and decrease of large metachromatic granules. The last finding was regarded as showing a decrease of acid mucopolysaccharide. Solid Ehrlich and Yoshida tumors, being poor in connective tissue element, did not show up any appreciable changes (Photos 2, 3).

Cytological examination by phase contrast microscopy of Ehrlich and Yoshida ascites tumor cells removed after 5 days' intraperitoneal injection of chloroquine revealed an increase of cytoplasmic lipoid granules and enlargement of vacuoles in the Golgi field, although these changes were slight. Similar changes were also observed in mixed culture of fibroblasts and cancer cells in medium containing chloroquine (Photos 4, 5, 6, 7).

Metastasis of Brown-Pearce cancer in treated rabbits were significantly reduced when examined at their tumor death, and this was more apparent in the thoracic organs (Table 1).

Table. 1 Influence of chloroquine diphosphate on metastasis at death of rabbits with Brown-Pearce cancer (♂ white rabbits, 10 mg./kg. continuous intravenous injection)

		Control							Group treated with chloroquine			
Number of rabbits Survival period (days)		(23) 16	(41) 19	(21) 25	(25) 25	(37) 29	(39) 35	(35) 67	(24) 19	(30) 29	(26) 32	(43) 34
Organs investigated	Skin	++	++			+	+			+		+
	Brain										+	
	Eyes	+					++				++	
	Heart		+	+		+						
	Lung	++	++	+		+	+	++		+	++	
	Pleura	+	+	++	+	+	+				+	+
	Diaphragm	++	+	++	++	++	++		++	+	++	+
	Peritoneum	+		+	+	+	+	++	++	+		++
	Stomach & Intestine					+						
	Liver		+						+	++		
	Kidney	+	++	+	++	+	++		+	+++	++	+
	Omentum	++	+++	+++	++	++			++	+++		++
	Lymph node mesenteric	+	+	+		+	+		++			
	retroperitoneal		+	++	+	++	++		+	++	+	++
	(Ascites)	++			++				++			

DISCUSSION

Treatment of malignant tumors with chloroquine is initiated on the basis of our original idea to inhibit the growth of the stromal connective tissue of tumors by a powerful fibroblast-inhibiting action of the drug and to expect the secondary retardation of tumor growth.

Our experiments have shown that, although chloroquine is not superior to other anti-tumor agents in the treatment of animal tumors, yet it does act as to inhibit the growth of these tumors. The favorable drug effects are prolongation of life span of animals bearing Bashford and Brown-Pearce tumors, inhibition of their growth, improvement of the liver catalase activity and iron metabolism, increase of tumor necrosis, inhibition of connective tissue formation, and a finding suggestive of influencing the metabolism of acid mucopolysaccharide. Its effects are absent or slight in Ehrlich, Yoshida and MH134 tumors. In general, the drug is more effective on tumors rich in the stromal connective tissue.

The growth of malignant tumors must be accompanied by the growth of the stromal tissue, whose connective tissue and blood vessels support cancer cells by fulfilling their nutritional requirements. An important role of the storma in malignant tumors was demonstrated already in 1925 by the experiments of Fischer[1] in which it was noted that fibroblasts accelerated the growth of Raus sarcoma cells in tissue culture. Similar observations were made by other investigators.[3,4,5,6] We have come to think, therefore, that inhibition of the growth of stromal fibroblasts of tumors might indirectly inhibit the growth of tumor cells. The fact that chloroquine increased tumor necrosis and induced considerable changes of the stromal tissue is certainly suggestive of a drug action such as conceived by us.

It has been reported that mucopolysaccharide increases in malignant transformation, and some observed a close relationship between the growth of tumors and this substance.[10,11] We have obtained findings suggestive of a decrease of acid mucopolysaccharide as well as a decrease of connective tissue elements in cancerous tissue following administration of chloroquine. Although it remains unknown whether a decrease of acid mucopolysaccharide is the cause of inhibition of tumor growth, it appears reasonably safe to regard that chloroquine has something to do with the metabolism of the mucopolysaccharide of malignant tumors. At any rate, these rather characteristic changes will provide clues to the understanding of the drug action of chloroquine in malignant tumors.

On the other hand, experiments were initiated apart from our ideas, in which anti-tumor effects of quinacrine, quinoline and quinone derivatives were tested, and their favorable effects were shown.[12,13,14,15] In view of these results, it may be necessary to consider a direct effect of chloroquine upon tumor

cells. However, our observation that Ehrlich ascites tumor cells transplanted intraperitoneally after association *in vitro* with chloroquine grew vigorously as well, and despite an increase of tumor necrosis, cancer cells showed little change in histologic and cytologic examinations appear to indicate that the direct effect of chloroquine is slight, if any.

SUMMARY

Based on our original concept, a fibroblast-inhibiting agent, chloroquine, was used against various animal tumors.

Among transplanted animal tumors, the drug was most effective on relatively connective tissue-rich Bashford and Brown-Pearce tumors, as reflected by prolongation of life span, inhibition of tumor growth, inhibition of lowering of liver catalase activity, improvement of iron metabolism, increase of tumor necrosis, inhibition of connective tissue formation, and decrease of acid mucopolysaccharide. On the other hand, it was of little advantage in Ehrlich, Yoshida and MH134 tumors which contain little connective tissue, except for a decrease of the amount of ascites and ascites tumor cells in the former two tumors.

These results indicate that chloroquine suppress the growth of the tumors relatively rich in connective tissue. This effect of chloroquine appears to be due to the primary attack of the stromal connective tissue of tumors being followed by the degeneration of tumor cells, though its probable anti-tumor activity by the indirect effects through its anti-inflammatory and systemic humoral activities should be taken into consideration.

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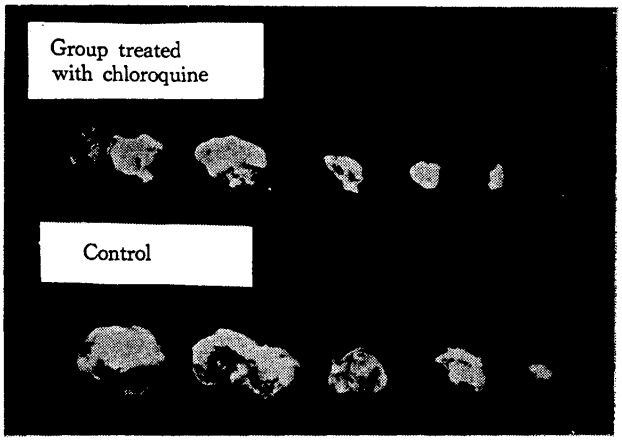

Photo. 1 Influence of chloroquine diphosphate on growth of Bashford cancer (Removed tumors)

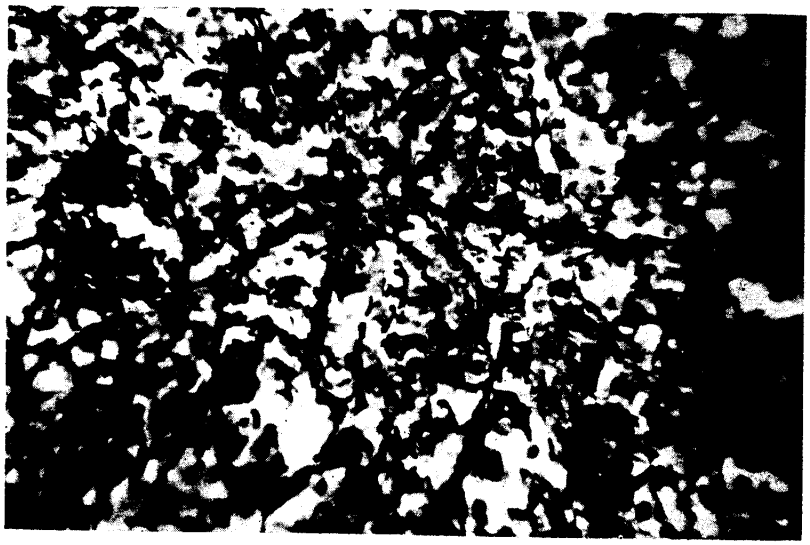

Photo. 2. Bashford cancer in untreated mouse (Silver impregnation)

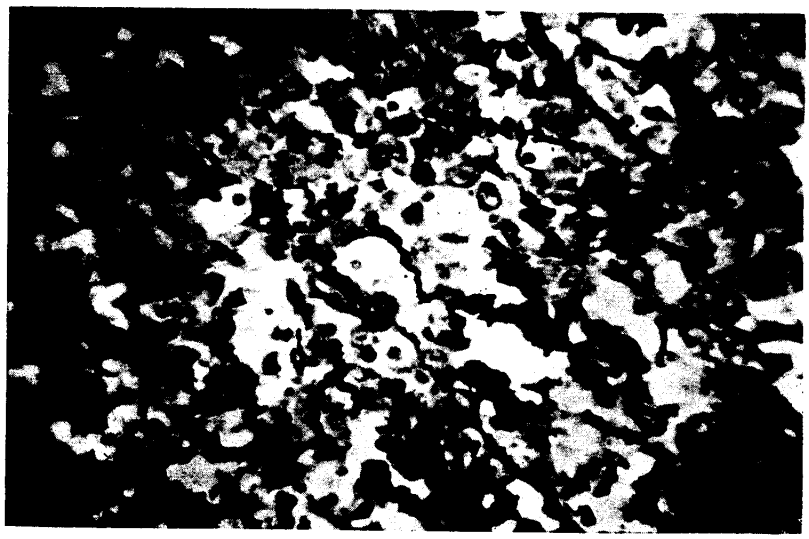

Photo. 3 Bashford cancer in mouse given chloroquine diphosphate (Silver impregnation)

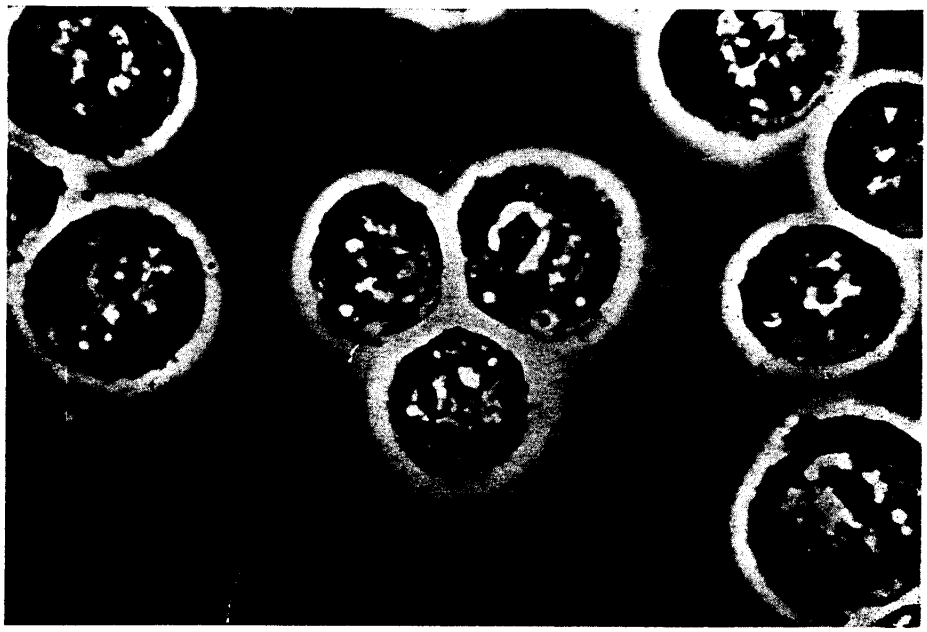

Photo. 4 Ehrlich ascites cancer cells in untreated mouse (Phase contrast microscopy)

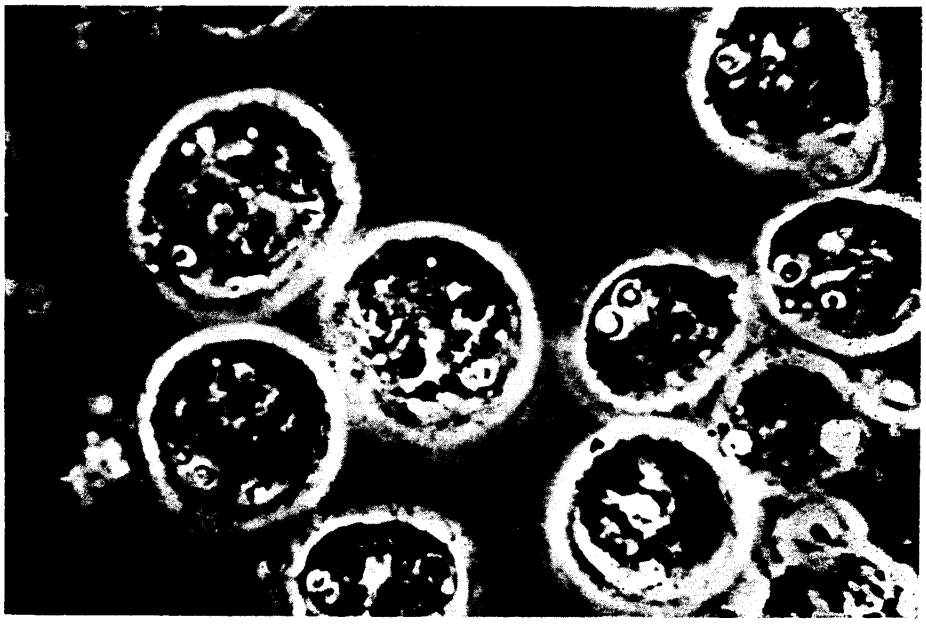

Photo. 5 Ehrlich ascites cancer cells in mouse treated with chloroquine diphosphate (Phase contrast microscopy)

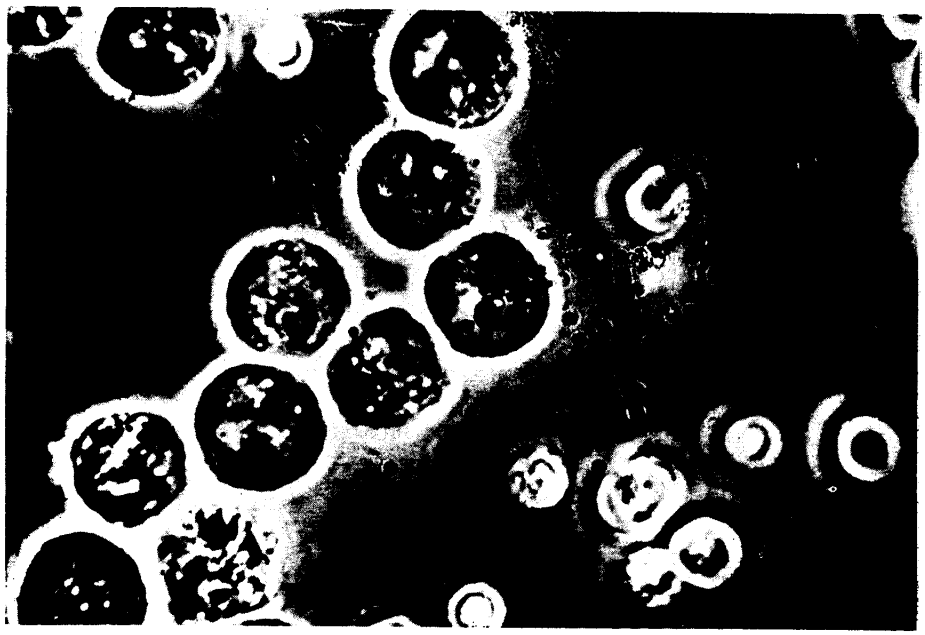

Photo. 6 Yoshida sarcoma cells in untreated rat (Phase contrast microscopy)

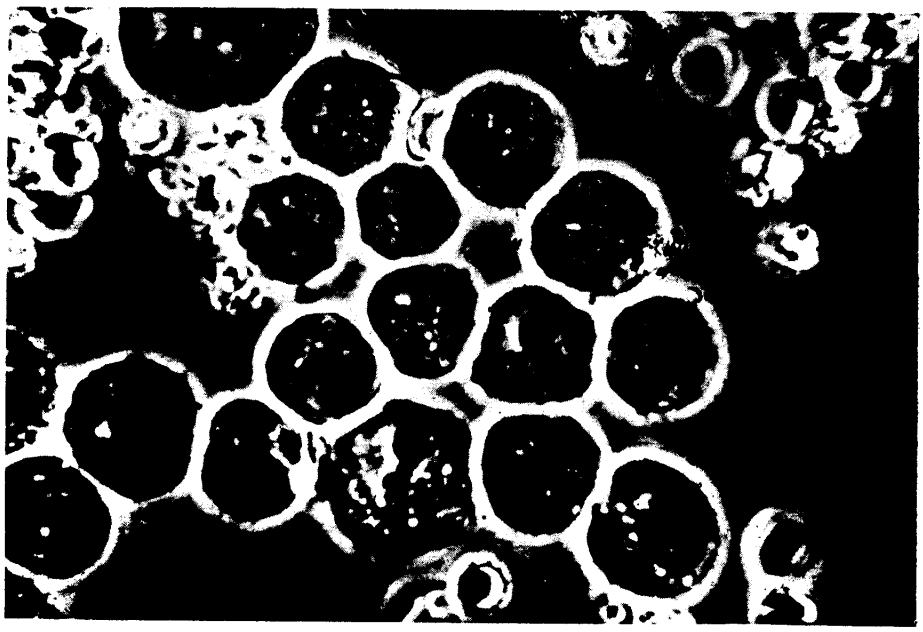

Photo. 7 Yoshida sarcoma cells in rat treated with chloroquine diphosphate (Phase contrast microscopy)