Opposing effects of quinacrine and chloroquine on the development of TA3 transplanted tumors in mice

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Both quinacrine and chloroquine had been used as antimalarial agents. Furthermore, antineoplastic and antiviral effects have been described for quinacrine, while chloroquine has been described to induce viral replication and promote tumor growth.
To search for differences in the growing rate of transplanted tumors, chloroquine or quinacrine were administered orally to AJ mice from 30 days previous to the inoculation of TA3 transplantable tumor cells, treatment being continued up to the end of the experiment. A control group, transplanted with tumor cells received tap drinking water. Marked differences between the three groups were found. Quinacrine had antitumoral effect, while chloroquine promoted a faster tumoral growth than controls. ($p<0.01$). Results suggest caution in the use of chloroquine, because it might have a similar promoting effect on human neoplasia.

Key terms: *antineoplastic treatment, chloroquine, quinacrine, transplantable tumors, tumor growth enhancement.*

INTRODUCTION

Quinacrine (mepacrine, atabrine), an acridinic derivative with antimalarial activity, has been used by hundreds of thousands of people, particularly by American soldiers during the Second World War, as a preventive agent for malaria. A review of the toxicology of this compound is found in medical reports of the United States Army (Steck, 1972). Chloroquine, one of the multiple 4-aminoquinoline compounds tested as antimalarial agents, proved to be better than quinacrine and, at the end of the Second World War, it replaced quinacrine as the preferred antimalarial drug.

Some experiments carried out in recent years have led to the suggestion that chloroquine stimulates tumor growth and activates viral replication. Thus, Gamburg (1986) administered chloroquine daily for one month to two-month-old hamsters, after inoculating them with an oncogenic virus (SV40) at the time of birth, the inoculated animals having a reduced latency period of tumor appearance of 6 weeks. Otherwise, Maheshwari *et al* (1990), investigating the role of interferon as prophylactic agent for malaria produced by *Plasmodium cynomolgi* B malaria in monkeys, suggested that chloroquine could have interfered in the treatment, since it inhibits the antiviral activity of interferon alpha/beta in the mouse and also that of polyinosinic-polycytidylic acid (poly I:C) against Semliki forest virus (SFV). These authors advised that interferon

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should not be used in cases of viral infections, cancer or malaria in zones where chloroquine was of common use, as chloroquine increases viral replication in the mouse. They suggested a potential relationship between malaria and certain viral infections that have recently increased, such as herpes zoster in children and AIDS, inferring that chloroquine could predispose to these viral infections.

Thomas *et al* (1990) studied the effects of chloroquine and hyperthermia on the development of C-1300 murine neuroblastoma, in Ajax white mice. The neuroblastoma was injected in four groups: 1, control; 2, hyperthermia; 3, chloroquine; 4, hyperthermia plus chloroquine. The treatment lasted for 14 days, animals being sacrificed on the 21st day. The parameters studied included mortality (6% in group 1; 25% in group 2; 50% in group 3; 40% in group 4) and dissemination of metastasis (0% in group 1; 60% in group 2; 90% in group 3; 90% in group 4).

The above experiments indicate that chloroquine has pharmacological actions that induce viral replication and increase tumor growth and dissemination.

In experimental carcinogenesis, the model used by McCormick (1988) included mammary tumors induced by N-methyl-N-nitrosamine (MNU). Oral quinacrine hindered the development of these mammary tumors induced by MNU at low doses, but this antitumoral effect was lost when the carcinogenic agent was administered at higher doses. Alade *et al* (1991) demonstrated that using two antimalarial substances, quinacrine and chloroquine, in a culture medium of leukemic monoblastic cells (line U937), only quinacrine was active as an antileukemic agent.

In previous studies (Guerrero *et al*, 1992), we have reported the antitumoral effects of quinacrine. Considering that chloroquine apparently induce opposite effects in the development of tumors, we designed an experiment to evaluate the behavior of a transplantable malignant tumor in animals receiving chronically either quinacrine or chloroquine before and after the tumor transplant.

METHODS

Male AJ mice bred at the Department of Experimental Medicine, University of Chile, were used. The TA3 tumor used corresponds to a mammary carcinoma of ascitic growth, that is maintained by transfer every 7 days in the peritoneal cavity of the same mice strain. Transfers were made from a cell suspension of the ascitic tumor, in physiological saline with penicillin 100 units/ml and streptomycin 100 mg/ml, with a previous recount in the hemocytometric chamber. They were diluted to obtain a final concentration of 10,000,000 cells per ml. Each animal was inoculated with 0.1 ml intramuscularly. Each group was composed of 10 male AJ mice, weighing between 20-25 g each. All animals were inoculated with 1,000,000 tumor cells in the right thigh. Surviving mice were sacrificed on the 30th day after tumoral inoculation.

The first group of mice served as control and these animals received common pelleted food and tap drinking water. The second group of mice received drinking water *ad libitum* with quinacrine in a concentration of 100 mg/l from 30 days prior to tumoral inoculation up to the end of the experiment. The third group of mice received drinking water with a solution of 100 mg/l of chloroquine from 30 days prior to inoculation up to the end of the experiment.

Quinacrine and chloroquine were purchased from Sigma (St Louis, USA).

Evolution of the tumors was recorded measuring the maximal and minimal diameters every 3 to 4 days. Final results were analyzed by Student's "t" tests, with Bonferroni's correction. Statistical analyses of survival were made according to Kaplan-Meier survival probability tests (Matthews and Farewell, 1985).

RESULTS

The growth curve of tumors in the different groups is shown in Figure 1. Tumors in the control group presented a Gompertzian type of growth (decreasing logarithmic growth), increasing from an average of 8.8 mm on the 3rd day up to 28.1 mm on day 27th. Two out

TABLE I

Kaplan-Meier actuarial survival curves.

Survival probability test in 3 groups of animals injected with TA3 tumor and drinking tap water alone (control) or added with either chloroquine or quinacrine

DAY	DEATH NUMBER r	TOTAL NUMBER N	(N-r)/N	TEST Pr (T > t)	SE
		CONTROL			
15	0	10	1.0	1.0	–
18	0	10	1.0	1.0	–
21	0	10	1.0	1.0	–
24	0	10	1.0	1.0	–
27	2	8	0.75	0.75	0.19
		CHLOROQUINE			
15	0	10	1.0	1.0	–
18	1	9	0.89	0.89	0.10
21	2	7	0.71	0.64	0.15
24	0	7	0.71	0.64	0.15
27	7	0	0	–	–
		QUINACRINE			
15	0	10	1.0	1.0	–
18	0	10	1.0	1.0	–
21	0	10	1.0	1.0	–
24	0	10	1.0	1.0	–
27	0	10	1.0	1.0	–

TABLE II

Growth of TA3 tumors after 27 days of inoculation

Group	n	Mean diameter ± SEM (mm)	Values of "*t*" *vs* a	*vs* b
a. Control	8	28.1 ± 0.55		
b. Quinacrine	10	10.0 ± 1.56	9.96*	
c. Chloroquine	4	31.1 ± 0.43	3.53**	9.46*

* $p < 0.001$
** $p < 0.004$

of 10 animals died within this period (day 25th).

Animals receiving chloroquine had a more rapid development of their tumors (Fig 1) and a shorter survival time than controls (Table I). On day 27th the mean diameter of the 4 surviving animals was 31.1 mm.

Mice receiving quinacrine had a slower rate of tumor growth (Fig 1) and presented complete tumoral regression in 50% of the cases. At the end of the experiment, the quinacrine treated group had statistically significant smaller tumors than either the chloroquine treated or control groups (Table II) and all animals survived.

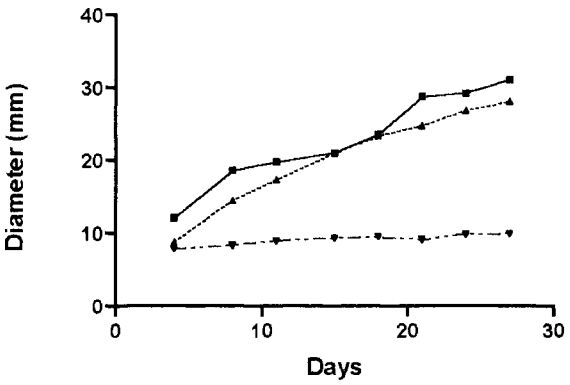

Fig 1. Mean diameter of TA3 tumors transplanted into mice receiving tap water alone (control, triangles) or added with either chloroquine (squares) or quinacrine (inverted triangles) from 30 days before inoculation to 27 days after tumor transplant. n = 10 in each group, up to 15th day (see survivals in text).

DISCUSSION

The AJ male mouse is an animal in which experimentation has been carried out in our laboratories for many years. Transplanted TA3 tumors grow very fast in 100% of the animals; they metastasize the lungs and kill all mice within 28 to 35 days (Guerrero *et al*, 1992). The control group of this series of experiments confirms the evolution of this transplanted tumor.

Mice drink daily an average of 5 ml of tap water. As the solutions contain 0.1 mg of drug per ml, the ingestion of quinacrine or chloroquine would be 0.5 mg/day. This dose did not produce any evident toxic effect in mice.

In this series of experiments, we demonstrate the preventive effect of chronically administered quinacrine upon the development of transplantable TA3 tumors in mice. In previous studies (Guerrero *et al*, 1992; Dabancens *et al*, 1994), we have demonstrated the antitumoral effects of quinacrine administered simultaneously to tumor inoculation. Possible mechanisms of these effects include inhibition of phospholipase A2 and prostaglandin synthesis, and chelating action of quinacrine over bivalent cations.

Contrarily to the above, chloroquine accelerates the growth of the transplanted tumor and shortens the survival period of the inoculated animals. In this sense, chloroquine can be classified within the category of promoters or enhancers of neoplasia. Our results are in accordance with those of Thomas *et al* (1990) and Gamburg (1986), who have demonstrated a tumor promoting effect of chloroquine.

In summary, present results indicate that two antimalarial drugs (quinacrine and chloroquine) have opposite actions in a same tumor model. These drugs might also behave in opposite direction in human neoplastic diseases.

REFERENCES

ALADE SL, BROWN RE, WORMLEY S, WU MC (1991) Cytostatic and tumoricidal effects of quinacrine on a monoblastic cell line (U937) expressing P-glycoprotein. Meet Am Assoc Cancer Res 32: A2247

DABANCENS A, ZIPPER J, GUERRERO A (1994) Quinacrine and copper, compounds with anticonceptive and antineoplastic activity. Contraception 50: 243-251

GAMBURG L (1986) Effects of khingamin on the development of SV40 virus-induced and transplantable tumors in hamsters. Eksp Onk 8: 45-47

GUERRERO A, ZIPPER J, DABANCENS A (1992) Actividad antineoplásica ejercida por quinacrina oral sobre tumores malignos transplantables en ratón. Rev Chil Cancerol 2: 119-122

MAHESHWARI RK, SRINKANTAN V, BHARTIYA D, PURI SK, DUTTA GP, DHAWAN BN (1990) Effects of interferon in malaria infection. Immunol Lett 25: 53-57

MATTHEWS DE, FAREWELL V (1985) Using and understanding medical statistics. Basel: Karger.

McCORMICK DL (1988) Anticarcinogenic activity of quinacrine in the rat mammary gland. Carcinogenesis 9: 175-178

STECK EA (1972) Chemotherapy of protozoan disease. Vol III. Washington, DC: Walter Reed Army Institute of Research, US Government Printing Office

THOMAS R, VANE DW, GROSFELD JL, FAUGHT PR (1990) The effect of chloroquine and hyperthermia on murine neuroblastoma. J Pediatr Surg 25: 929-932