

Quinacrine and copper, compounds with anticonceptive and antineoplastic activity

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Changes in the evolution of a malignant transplantable tumor in mice to whom quinacrine, copper and zinc were supplied in drinking water are reported. Male AJ mice were inoculated in the right thigh with 1,000,000 TA3 or TA3 MTXR tumoral cells. Three experiments were designed with different types of tumors and different schedules of quinacrine and cations administered in drinking water. The animals that received quinacrine or quinacrine plus copper in drinking water had significantly smaller tumors, and some groups had a high rate of complete tumor regression (up to 60%). Quinacrine and copper have synergistic antineoplastic activity. Zinc salts do not improve the antitumoral effect of quinacrine. The relevant fact of this experiment lies in the fact that a large number of women using IUDs with copper could occasionally be treated with quinacrine.

Keywords: Quinacrine; copper; zinc; transplantable tumor; tumoral regression

Introduction

Copper

In 1969, one of the authors published an article¹ that started the age of medicated intrauterine contraceptives. This led to extensive research on the physiology of this metallic oligoelement. Among all the studied metals, copper is the least toxic and the most effective. In the intrauterine

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medium, copper is released as oxide in the order of micrograms per **day**,² where it has strong antiespermatic activity, hindering the endometrium from implantation. Today, Cu **IUDs** are used by millions of women. Its half-life of use is approximately 6 years, which makes it the most effective contraceptive, excluding sterilization.

Quinacrine

In a paper published in 1973,³ the instillation of quinacrine in different concentrations, from 10 to 40 **mg/ml**, was studied in rat uterus. Implantation was impaired in the treated uterus. When the concentration of quinacrine was increased to **50 mg/ml** or more, an obstructive granuloma in the uterine cavity was produced. This granuloma could also be obtained at the tubal-uterine ostium level in women, when quinacrine pellets of 250 mg were inserted in the uterine cavity. This research developed a non-surgical sterilization technique* that was performed in large numbers in some **countries**.⁵ Research to study the anti-carcinogenic effect of quinacrine was begun. Copper and zinc are basically enzymatic cofactors in the genital tract. Their concentration in the endosalpinx and endometrium of women has been studied by Patek and **Hagenfeldt**.⁶ Quinacrine binds to DNA mainly in tissues with low concentration of **Zn**,⁷ as in the case of the endosalpinx in women, where it produces an obstructive granuloma.

Petering and Van **Giessen**,⁸ found that the antitumoral action of certain chelating agents has its origin in the metal-sequestering action of these compounds, especially KTS compound (**3-ethoxy-2-oxobutyraldehyde bis-thiosemicarbazone**), which is active in vivo against several transplantable tumors in mice; mammary carcinoma, sarcoma 180, etc. The antitumoral activity of KTS is found when the copper supplemented in diet reaches 160 **µg** per mouse daily. KTS owes its antitumoral activity to the formation of a copper **chelate** (Cu-KTS), highly basic and of great stability, which is the active antitumoral agent.

Quinacrine (Mepacrine-Atebrine) is an acridinic derivative used as an antimalarial agent in the human. It was used by hundreds of thousands people, particularly by American soldiers during the Second World War, as a preventive agent.

Quinacrine has been used orally, intramuscularly and intravenously. Concentrations of 25 to 100 **ng/ml** are reached in the plasma during antimalarial treatment. The oral dose for suppression is 0.1 g/day. The toxic iatrogenic actions are generally moderate. These actions disappear with drug withdrawal. A complete review of the toxicology of the compound is found in medical reports of the US **Army**.⁹

Quinacrine works in the human by destroying the **erythrocytic** forms of the Plasmodium vivax and Plasmodium falciform. Quinacrine binds in the blood to the **leucocytes**, red cells and plasma in different proportions, 200 - 2 - 1, **respectively**.¹⁰

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Quinacrine inhibits phospholipase **A2**. This inhibition restricts **arachi-****donic** acid production, and consequently reduces the substrate for **cyclo-****oxygenase** or **lipo-oxygenase**. This is one of the mechanisms of quinacrine action in mice, particularly in experimental carcinogenesis induced by N-Metil-Nitrosourea (MNU) in the mammary gland. In the experimental model used by **McCormick**,¹¹ oral quinacrine hindered the development of mammary tumors induced by MNU at low doses, but it lost that effect when the carcinogenic agent was administered at higher doses. **Alade et al.**¹² demonstrated that using two antimalarial substances, quinacrine and chloroquine, in a culture medium of **leucemic** monoblastic cells (line **U937**), only quinacrine was active as an antileucemic agent. There is no published evidence that quinacrine could be a carcinogenic agent.

Guerrero et al.¹³ demonstrated an antitumorigenic effect of quinacrine over transplantable tumors in mice. The present study was designed to establish the influence of Cu-cations on the anticarcinogenic effect of quinacrine.

Material and methods

Male AJ mice weighing between 20-25 **g** each, bred at the Departement of Experimental Medicine, were used. The **TA3** tumor corresponds to a mammary carcinoma of ascitic growth that is propagated by transfer every 7 days in the peritoneal cavity of the same strain. The TA3 **MTXR** variant resistant to methotrexate was developed by one of the authors (AG). The transfers are made from a cell suspension of the ascitic tumor in physiological serum with penicillin 100,000 units/ml and streptomycin 100 **mg/ml**. The cells are diluted in order to obtain a final concentration of 10,000,000 cells per ml. Each animal was inoculated with 0.1 ml **intra-****musculary** (1,000,000 **tumoral** cells) in their right thigh.

Experiment I

There were three groups. Each group had 5 male AJ mice inoculated with TA3 tumor cells. The first group was the control, receiving tap drinking water. The second group received water with quinacrine in a concentration of 100 **mg/L**. The third group received water with quinacrine 100 **mg/L** plus 100 **mg/L** of CuSO₄.

Experiment II

There were four groups. Each group had 5 male **AJ** mice. All the animal: were inoculated with 1,000,000 TA3 **MTXR tumoral** cells in the **right** thigh. The first group was the control group and received regular diet **and** tap drinking water. The second group received drinking water **ad libitum** with quinacrine in a concentration of 100 **mg/L** from the **day** of the

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inoculation of the tumor cells. The third group received drinking water with a solution of **100 mg/L** quinacrine and **100 mg/L CuSO₄**. The fourth group received drinking water with a solution of **100 mg/L** of quinacrine and **100 mg/L ZnSO₄**.

Experiment III

In this experiment, four groups of mice received drinking water with different solutions for two weeks prior to inoculation with **1,000,000** TA3 tumor cells in their right thigh, and then were treated with quinacrine. The first group received drinking water with a solution of **25 ml/L CuSO₄**, prior to inoculation and **25 mg/L** of quinacrine after inoculation. The second group received drinking water with a solution of **50 mg/L CuSO₄** prior to inoculation and drinking water with **100 mg/L** quinacrine after inoculation. The third group received drinking water with **25 mg/L** quinacrine before and after inoculation, and the fourth group received quinacrine **25 mg/L** plus **CuSO₄ 25 mg/L** before inoculation and quinacrine **25 mg/L** after inoculation.

In each experiment, the evolution of the tumors was registered by measuring the maximum and minimum diameter every 3-4 days. The final results of the average of **tumoral** diameter were analyzed using Student's t-test. Water consumption was measured weekly for each group of mice. **All the chemicals were acquired from Sigma Laboratories.**

Results

Experiment I

A graphical view of the average tumor growth pattern in each **group** of experiment I appears in Figure 1.

Control group: The TA3 tumor presents a Gompertzian pattern of tumor growth, increasing from an average diameter of 7 mm at day 3, **up** to an average of 30, the day at which animals began to die.

The group of animals treated with quinacrine had a slower rate of tumor growth and one animal had complete remission. **At day 30**, the average diameter was 15.4 mm.

The third group of animals which received quinacrine plus **CuSO₄** in drinking water presented three animals with regressions (60%) and reached an average of 11.7 mm (Table 1).

Experiment II

A graphical view of the average tumor growth pattern in each group of experiment II appears in Figure 2.

Control group: The TA3 MTXR tumor showed a Gompertzian pattern

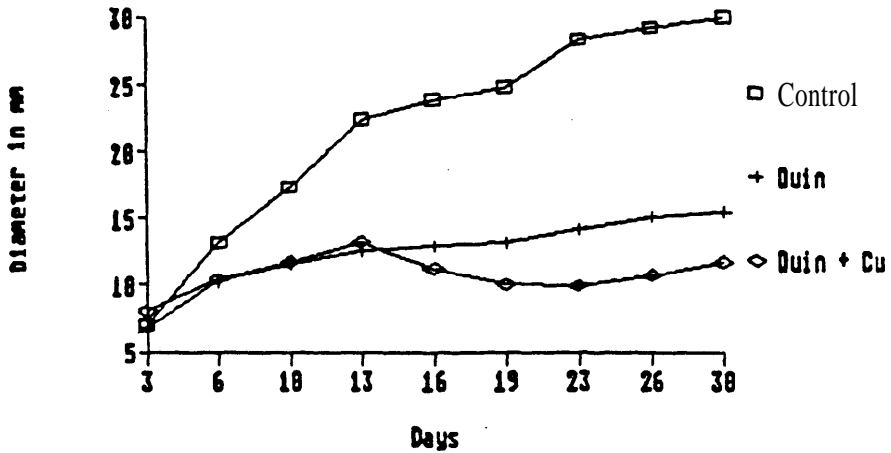


FIGURE 1. Growth curves of TA3 tumors.

TABLE 1. Effect of quinacrine plus copper over TA3 tumor

Treatment Group	Average Diameter	Number of Regressions
Control	30.0	0/5
Quinacrine	15.4	1/5
Quin + Cu	11.7	3/5

of growth, increasing from an average diameter of 12 mm at day 4, up to an average diameter of 34 mm at day 30, when five animals died.

The transplanted tumor in animals that received water with quinacrine had a minor rate of tumor growth, increasing from an average of 11.3 mm at 4 days up to an average diameter of 26.3 mm at day 30. The average diameter of tumor in this group was 22.6%, less than the control group. The animals that drank water with quinacrine and CuSO_4 had a less developed tumoral mass. One animal died at day 28 and another presented regression, following two weeks of increasing tumor volume. The average diameter of tumor of this third group was 39.7% less than the control group ($p < 0.001$). Those animals that received water with quinacrine and ZnSO_4 showed a tumoral development similar to the control group ($p > 0.01$), and two animals died on day 28. The average tumor diameter of each group on day 30 is shown in Table 2.

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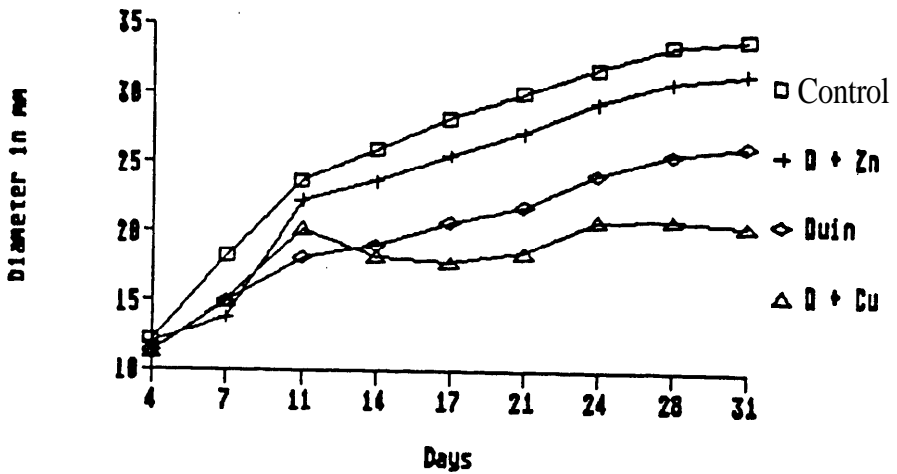


FIGURE 2. Growth curves of TA3 MTXR tumors.

TABLE 2. Average diameter of TA3 MTXR tumors at 30 days post-transplantation

Group	Number	Diameter	SE	Student's t
Control	5	34	0.32	
Quinacrine	5	26.3	0.37	15.67'
Quin + Cu	4	20	2.9	5.45' 2.45†
Quin + Zn	3	31.5	0.73	3.69† 7.09' 3.31†

SE = Standard Error.

. = $p < 0.001$.

† = $p > 0.01$.

Experiment III

A graphical view of tumor growth pattern in experiment III is shown in Figure 3.

In this experiment, a suboptimal dose of quinacrine (25 mg/L) was used after inoculation. In the group pretreated with quinacrine, all animals developed tumor and there were no regressions. In groups pretreated with CuSO₄, quinacrine treatment potentiated antitumor growth, obtaining 2 and 3 out of 5 regressions (4040%). In the group pretreated with a higher dose of CuSO₄ (50 mg/L) and a higher dose of quinacrine (100 mg/L), the results were not as favorable as treatment at the lower doses (Table 3).

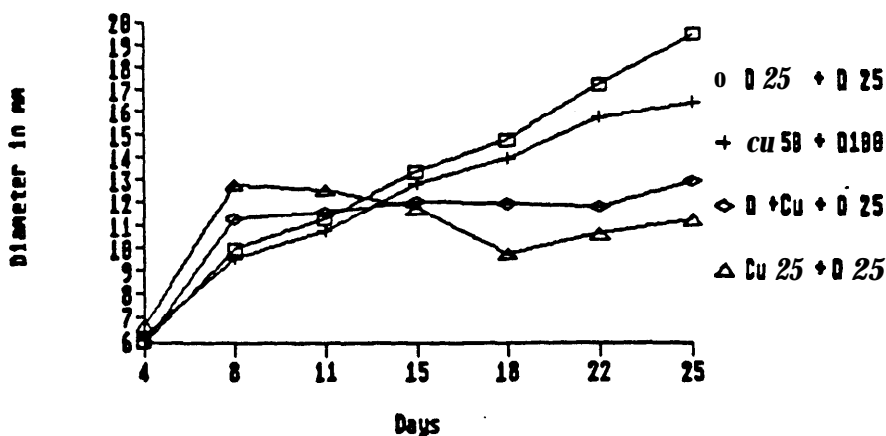


FIGURE 3. Growth rate of TA3 tumors with different treatments.

TABLE 3. Effect of pretreatment on TA3 tumor growth

Treatment Group		Average Diameter	Number of Regressions
Pretreatment	Treatment		
Quin 25 mg/L	Quin 25 mg/L	19.5	0/5
Cu 50 mg/L	Quin 100 mg/L	16.5	1/5
Quin 25 + Cu 25	Quin 25 mg/L	13	2/5
Cu 25 mg/L	Quin 25 mg/L	11.3	3/5

Discussion

For both tumor types, TA3 and TA3 MTXR, copper salts improve the antitumoral action of quinacrine. As the consumption of water was uniform, this effect cannot be attributed to a higher dose of quinacrine.

Considering that each animal drinks an average of 5 ml/day of water, quinacrine in a concentration of 100 mg/L would be 0.5 mg daily. Since each mouse weighs 20-25 g, it could be extrapolated that for a person who weighs 50 Kg, this quantity would be equal to 500 mg/day of quinacrine, five times higher than the amount used as preventive in malarial regions. No toxic manifestations or rejection of water by the animals was observed. Quinacrine significantly retarded the growth of the neoplastic nodules in the TA3 tumor resistant to methotrexate, and this is consistent with the observation of Alade in which quinacrine inhibited the expression of glycoprotein P, one of the main mechanisms of multi-resistance

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to antineoplastic drugs.¹² The addition of **CuSO₄** in drinking water reinforced the antineoplastic **property** of quinacrine, while the addition of **ZnSO₄** decreased its effect. This observation must be used in considering the biochemical mechanism of action of quinacrine which probably is targeted against a B-containing protein.

Data in experiment **III** established two main points. A small amount of copper given prior to tumor inoculation, strongly potentiates the effect of a suboptimal dose of quinacrine, but a larger dose had an adverse effect. This is in accord with epidemiological studies in women which demonstrated a U-shaped relation between plasma copper levels and the risk for developing breast **cancer**.¹⁴

This antitumoral synergism of quinacrine and copper is significant because of the large number of users of copper intrauterine devices. These patients could occasionally receive quinacrine as treatment. Our experiments showed that there is no pharmacological incompatibility between these two substances and that there is, in fact, an anticarcinogenic synergism between them.

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