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INHIBITION OF γ-REPAIR BY QUINACRINE IN *TETRAHYMENA PYRIFORMIS*

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ABSTRACT

Cultures of *Tetrahymena pyriformis* were exposed to γ-rays and incubated with quinacrine afterwards. Although the concentration used scarcely affects either growth rate or morphology, it enhances the damage caused by radiation, as measured by a delay in growth. It is proposed that the latter effect is due to an inhibition of some DNA repair mechanism.

RESUMEN

Diferentes cultivos de *Tetrahymena pyriformis* se expusieron a rayos γ y posteriormente se incubaron con quinacrina. A pesar de que la concentración utilizada casi no afecta ni la velocidad de crecimiento, ni la morfología, sí incrementa el daño causado por radiación, en función del retardo en el crecimiento. Se propone que dicho efecto se debe a la inhibición de alguno de los mecanismos de reparación del ADN.

INTRODUCTION

Quinacrine (Atebrin) is a drug of the aminoacridine group, which links easily to the DNA molecule. Since long ago, it has been used against different diseases like malaria, giardiasis, taeniasis, and so forth.

This drug forms a complex with DNA (Van Dyke *et al.*, 1970), with no base specifity. The complex quinacrine DNA is stable to urea and unstable to ionic cosolutes, which suggests that the drug binds to DNA by ionic attraction (O'Brien *et al.*, 1966). The result is a stabilization of the DNA molecule, preventing the separation of both strands.

Studies with synchronized cells of *T. pyriformis* demonstrated that a concentration of 14 μg/ml quinacrine ,inhibits cell division, especially the second mitotic division (Chou *et al.*, 1968) and up to 16 μg/ml have a lethal effect (Clancy, 1968).

According to some authors, quinacrine also inhibits the *in vitro* activity of both RNA and DNA polymerases but there is greater damage on the latter (Ciak and Hahn, 1967; O'Brien *et al.*, 1966; Van

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Dyke *et al.*, 1970). Due probably to the effect mentioned above, it was noted that, in some bacteria as well as in the protozoa *Tetrahymena pyriformis* (Chou and Ramanathan, 1968; Conklin and Chou, 1970, 1972, Conklin *et al.*, 1969) and *Plasmodium berghei* (Van Dyke *et al.*, 1970), large quantities of quinacrine inhibit DNA, RNA and protein synthesis.

Fucks and Smith (1971) working with *Escherichia coli* showed that quinacrine increases the damage caused by X-rays and that the effect is irreversible. To achieve this, the drug must be added immediately after irradiation. Otherwise, only 10 minutes later, there is a decrease of about 95% of the effect. Using rec^- and rec^+ mutants, the authors found that quinacrine does not affect rec^-. Since this strain is repair deficient, they suggest that quinacrine inhibits in some way the DNA repair mechanisms.

The results reported herein describe the synergistic action of quinacrine on the damage caused by γ-rays to cultures of *T. pyriformis*.

MATERIALS AND METHODS

An axenic amicronucleate strain of *Tetrahymena pyriformis* G.L. was used. Stock cultures were kept at 22°C in PP_3Y medium, containing 2% proteose peptone No. 3 (Difco) and 1% yeast extract (Difco).

Samples of stock cultures, diluted 1/100, were incubated at 28°C in a water bath shaker at 77 r.p.m., during approximately six days, or until stationary phase was reached. Samples of 2 ml each were exposed to a ^{60}Co source (Gammacell 200 A.E.C.L. at a dose rate of 51.45 rads/sec, diluted afterwards 100 times and incubated during six days with and without quinacrine. Growth rate was measured indirectly by optical density in a spectrophotometer (Spectronic 70 Bausch & Lomb) at 546 nm.

RESULTS

Exposure of *Tethrahymena pyriformis* to gamma radiation (^{60}Co) results in an increase of the lag phase. The effect is enhanced when the cells are incubated after irradiation to certain chemical substances such as quinacrine.

Quinacrine inhibits growth but unlike radiation there is no lag phase and the effect seems to be irreversible (Fig. 1).

Concentrations of 6×10^{-6}M, 1.2×10^{-5}M and 2.4×10^{-5}M had inhibitory effects of 28%, 52% and 57% respectively.

As may be seen in figure 2, three different concentrations of the drug were tested and it may be observed that all of them enhanced greatly the damage caused by radiation. The highest dose wich usually has an inhibitory effect of about 57%, together with radiation results in 100% inhibition.

The synergistic effect may be conveniently described in terms of the growth reduction factor (GRF), which is the absorbance obtained as a result of treating the irradiated cells with the drug. For each drug concentration this value was computed as:

$$GRF = \frac{AD/A}{ID/I}$$

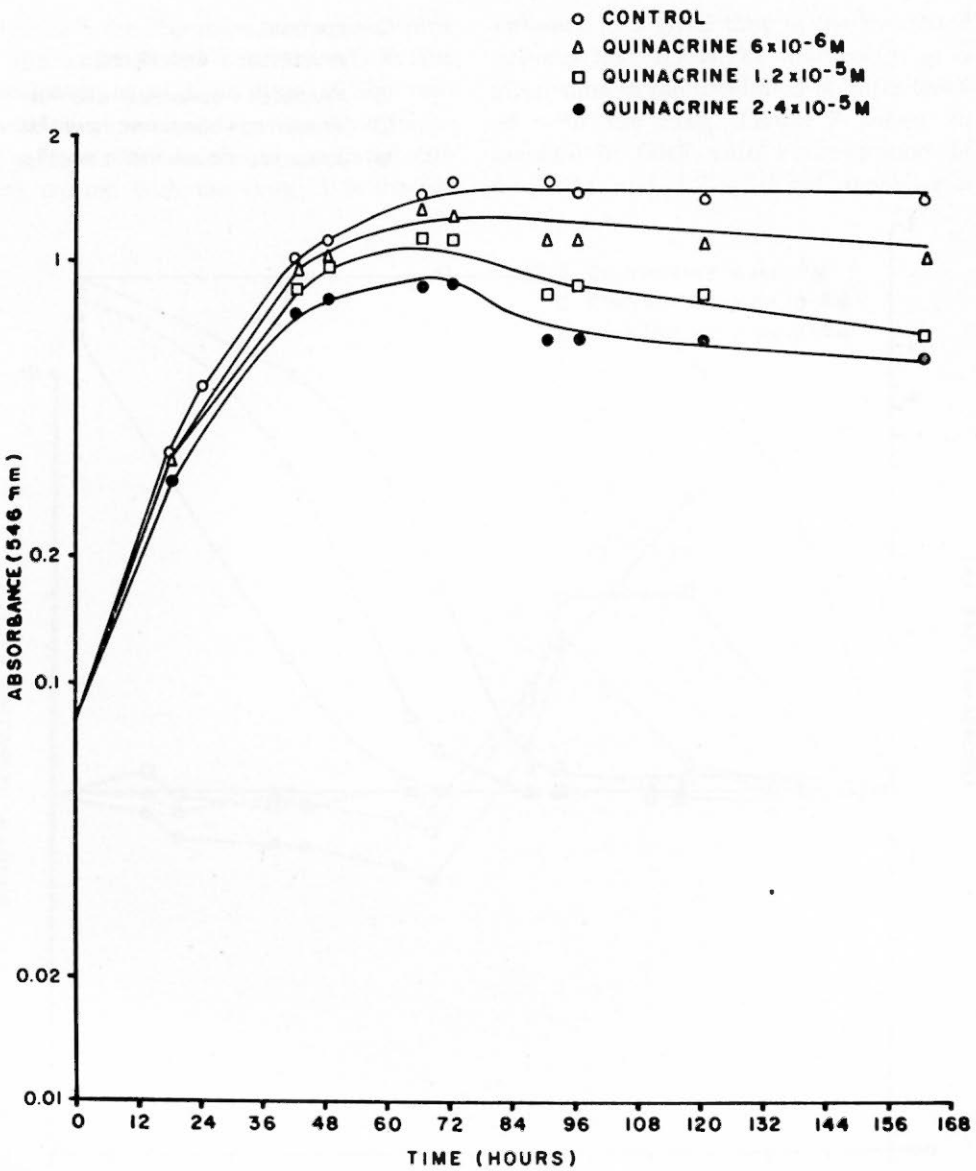

Fig. 1. Cultures of *Tetrahymena pyriformis* incubated with and without quinacrine. In the beginning all of them follow the same growth pattern but at a certain time according to the drug concentration, growth stops with no further recovery.

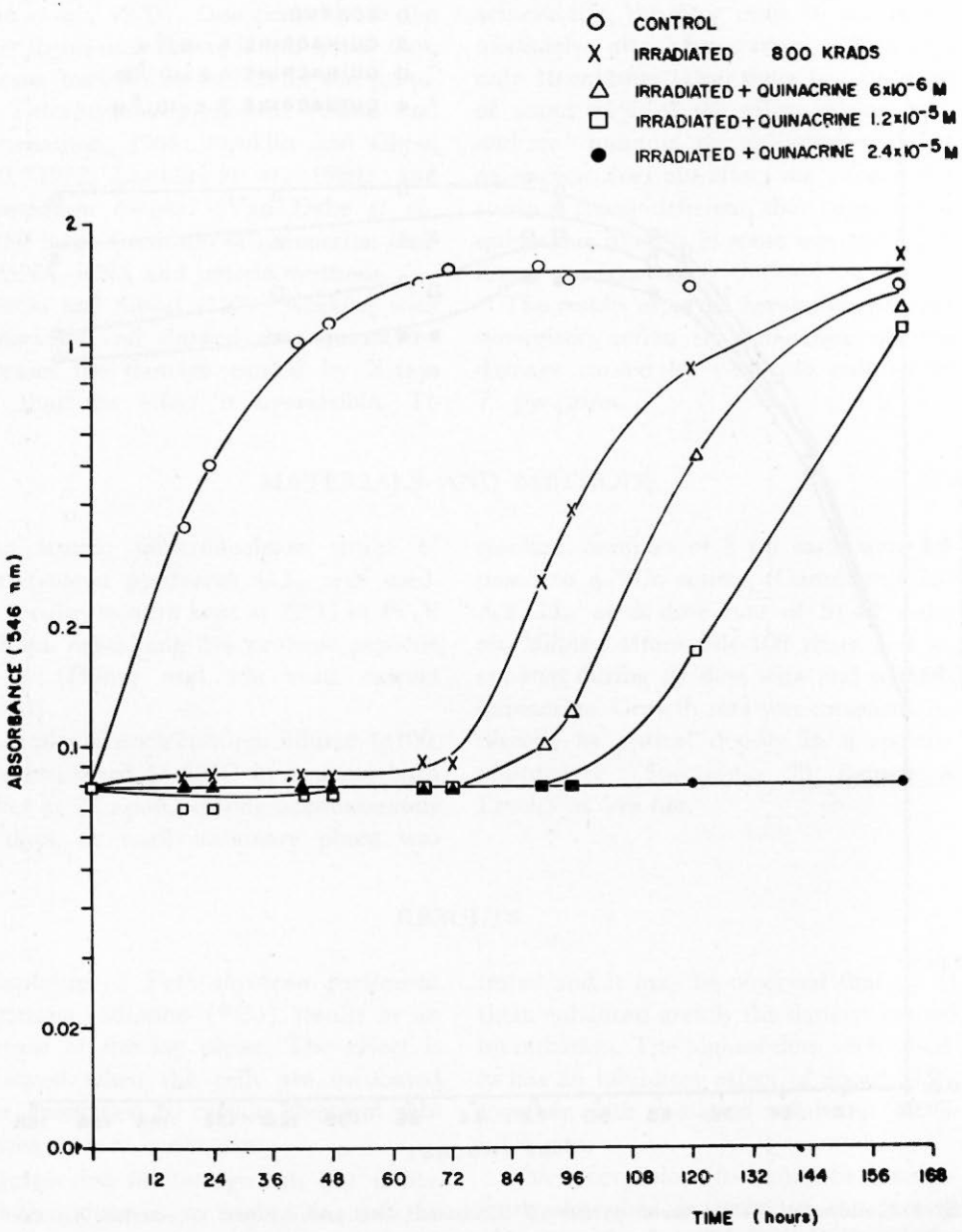

Fig. 2. Growth curves of *Tetrahymena pyriformis* γ-irradiated (800 Krads) and incubated with different concentrations of quinacrine. The effect of the drug, as compared with the untreated culture, is seen very clearly, espcially at th highest concentration where 100% of inhibition is achieved.

where A is the absorbance at a given time in the unirradiated control; AD is the absorbance at a given time in the unirradiated control; AD is the absorbance at a given time in the unirradiated cultures treated with the drug; I is the absorbance at a given time in the irradiated cultures and ID is the absorbance at a given time in the irradiated cultures treated with the drug. Figure 3 shows an increase in GRF with concentration of $6 \times {}^{-6}$M and 1.2×10^{-5}M reaching a

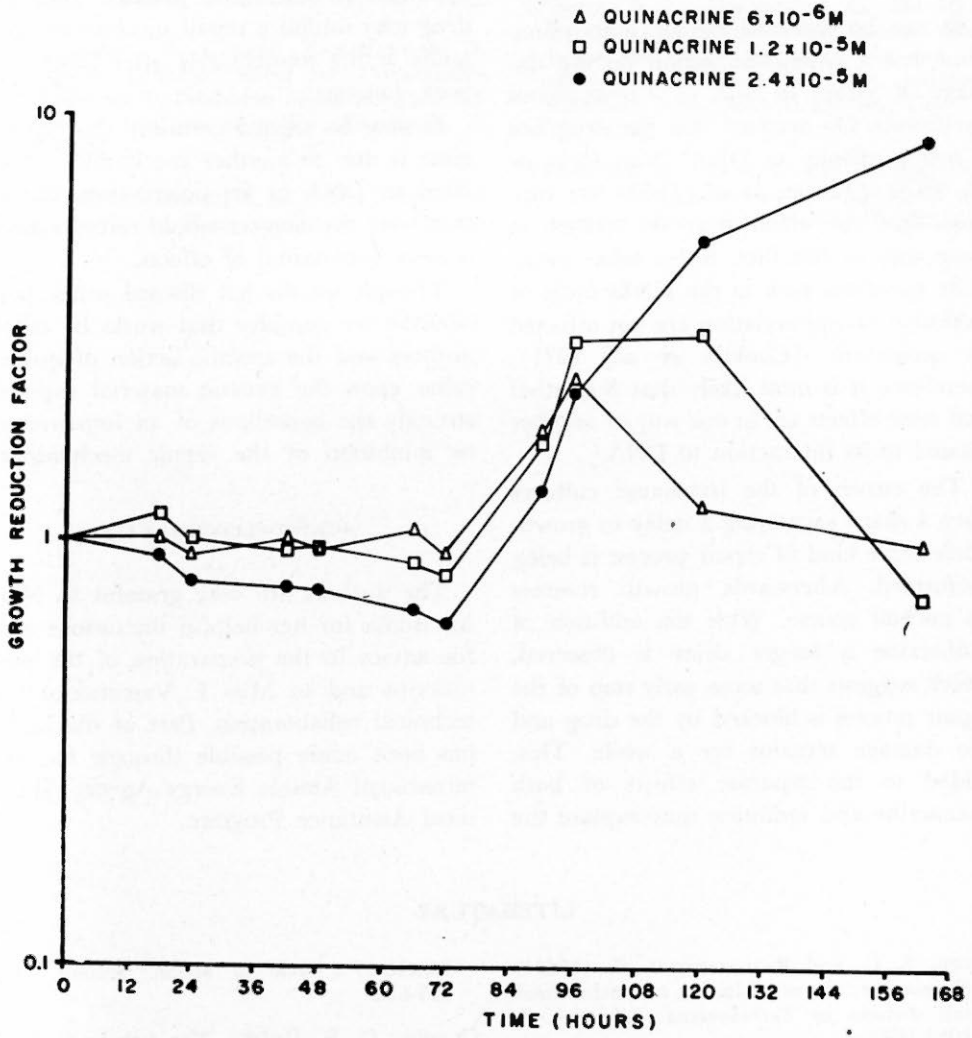

Fig. 3. Inhibition factor of quinacrine (GRF) as measured by the differences in growth between controls and irradiated cultures treated with the drug, at different times of incubation (see text). At concentrations of 6×10^{-6}M and 1.2×10^{-5}M, this value reaches a maximum around 96 hours declining afterwards when cells aproach the stationary phase. With 2.4×10^{-5}M of quinacrine cells never recover from the damage, hence the continuous increase in GRF.

peak at about 96 hours after irradiation. Thereafter, when cells start to grow, this factor decreases altogether. At the highest concentration (2.4×10^{-5}M) there is a continuous increase in GRF which eventually remains at a maximum value of 8.5, since cells never recover their growth capacity.

It can be observed clearly that quinacrine has a synergistic action on the damage of γ-rays to cells of *Tetrahymena pyriformis*. On account that the drug has a strong affinity to DNA (Van Dyke *et al.*, 1970; O'Brien *et al.*, 1966) we suppose that the effect may be related in some way to this fact. Some other metabolic functions such as the Krebs cycle or oxidative phosphorylation are not affected by quinacrine (Conklin *et al.*, 1971), thereferoe it is most likely that the lethal and toxic effects are in one way or another related to its interaction to DNA.

The curves of the irradiated cultures have a shape suggesting a delay in growth while some kind of repair process is being performed. Afterwards, growth resumes its normal course. With the addition of quinacrine a longer delay is observed, which suggests that some early step of the repair process is blocked by the drug and the damage remains for a while. This, added to the separate effects of both quinacrine and radiation may explain the synergism, effect that becomes particularly evident at the highest drug concentration. The results reported by Fuks and Smith (1971) in normal and repair deficient strains in *E. coli*, especially their finding that quinacrine acts within a limited range of time after irradiation, confirm this hypothesis. Hence it is possible that the drug may inhibit a repair mechanism normally acting immediately after DNA has been damaged.

It may be argued certainly that synergism is due to another mechanism unrelated to DNA or its polymerases but if that were the case we would rather expect a mere summation of effects.

Though we do not discard other possibilities we consider that works by other authors and the specific action of quinacrine upon the genetic material support strongly the hypothesis of an impairment or inhibition of the repair mechanisms.

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