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Letter to the Editor

Synergic Effect of $NADP^+$ on the Antitumor Properties of Low Doses of 9 OH-Ellipticine towards Walker Cells*

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ELLIPTICINES are DNA-intercalating, antitumor agents. The mode of action of ellipticines and their derivatives remains unknown. Two observations led us to think that NADPH might be able to modulate the activity of some of the ellipticine derivatives. First, when incubated with microsomes and with NADPH as an electron donor, ellipticines produce reactive intermediates (probably free radicals). On the other hand, peroxidase and hydrogen peroxide are able to generate free radicals in ellipticines [1, 2]. NADPH, as well as $NADP^+$, can reduce such radicals.

Cell cultures

Walker cells were derived from Walker carcinosarcoma 256. The cytotoxicity test of ellipticines on Walker cells was performed according to the method of Philipps [3]. We found that ellipticines have a cytotoxic effect on Walker cells, resulting in an ID_{50} of 1 μmol/l after a 1-hr exposure at 37°C [4]. The toxicity of 9 OH-ellipticine and of 9 NH_2-ellipticine for Walker cells appears to be increased by a NADPH generating system (the final concentrations were: 1.7×10^{-3} mol/l G6P; 1.3×10^{-3} mol/l $NADP^+$; 0.4 units/ml G6PDH) (Fig. 1). The NADPH generating system is in itself not toxic to Walker cells in this system. This ellipticine activation effect was confirmed by an *in vivo* count of the surviving cells. Cells were incubated with the drug according to the same protocol, with or

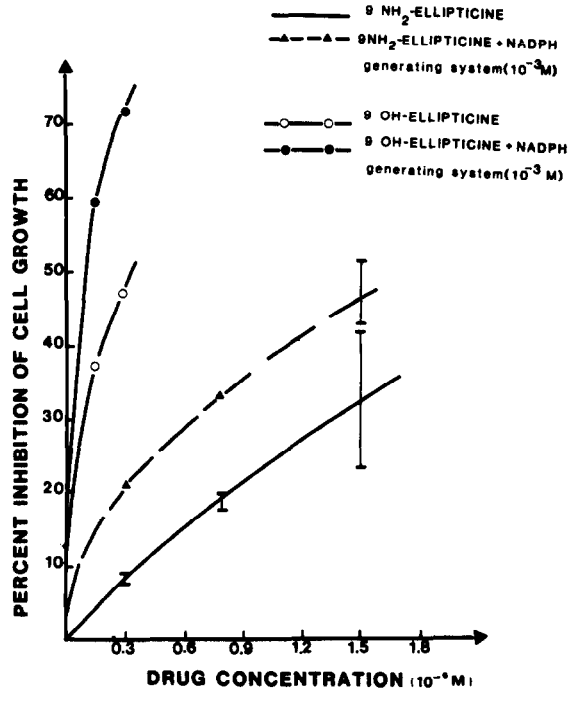

Fig. 1. An NADPH generating system (10^{-3}M NADPH) increases the cytotoxic potency of 9 OH- *and* 9 NH_2-*ellipticine towards cultured Walker cells. The NADPH generating system alone does not inhibit the cell growth.*

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Abbreviations: G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate deshydrogenase; $NADP^+$, nicotinamide adenine dinucleotide phosphate, oxidised form; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; LD_0, lethal dose 0; ID_{50}, inhibitory dose 50.

without cofactors, before being injected into rats, with control animals receiving cofactor-treated cells. The relative increase in survival time of injected animals started at 26.1% for rats injected with drug-treated cells, increasing to 61% when a NADPH generating system was added to the drug.

Antitumor effect on the rat

Experiments were carried out using inbred Furth Wistar rats, 7–8 weeks old. The rats to be treated were injected with 10^5 tumor cells; drugs were injected i.p. 24 hr later.

Intraperitoneal injections of 10^2–10^5 Walker cells cause death in all experimental rats. The survival times correspond to a dose–effect curve. Increasing doses of 9 OH-ellipticine cause an increased survival for some rats in the experimental population while preventing others from dying.

First we established the LD_0 of 9 OH-ellipticine on Wistar rats to be about 50 mg/kg. In order to measure the increase in experimental animal life span it was necessary to use the drug at doses sufficiently low (0.1 mg/kg) to initiate only a

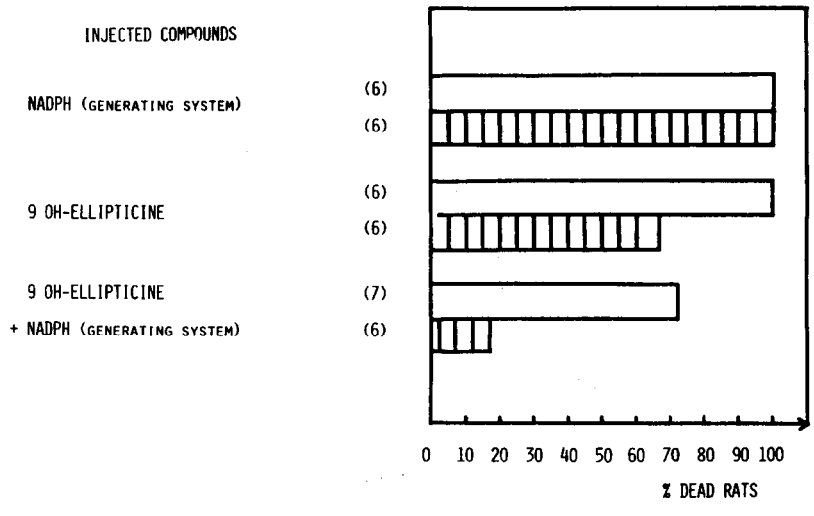

Fig. 2. Synergic effect of an NADPH-generating system on the antitumor properties of 9 OH-*ellipticine at low doses. Empty bars: experiment No.* 1, *NADPH generating system: NADPH,* 6.6×10^{-2} mol/l; 9 OH-*ellipticine,* 0.5 mg/kg. *Shaded bars: experiment No.* 2, *NADPH generating system: NADPH,* 1.5×10^{-2} mol/l; 9 OH-*ellipticine,* 0.7 mg/kg. 9 OH-*ellipticine was dissolved in acetate buffer* (0.1 mol/l), *pH* 5, *before injection as indicated in the text. The total number of animals used in each experiment is indicated in brackets.*

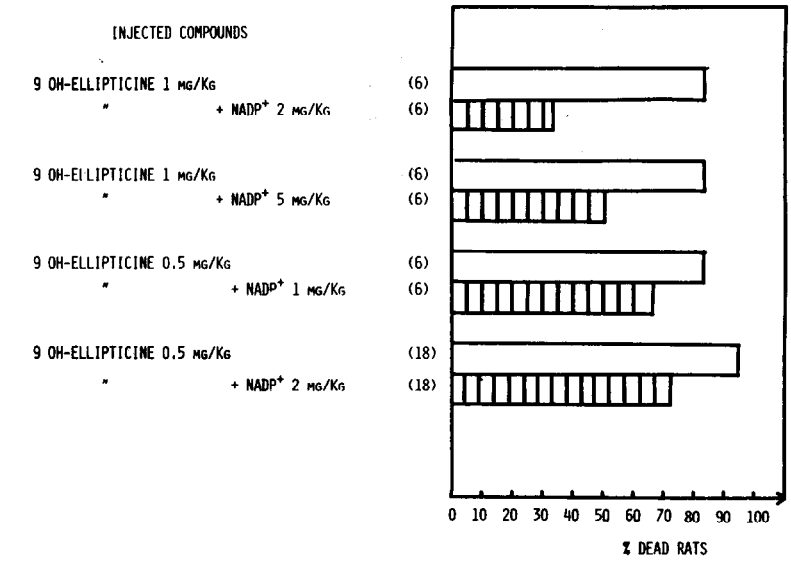

Fig. 3. Synergic effect of low doses of $NADP^+$ *on the antitumor properties of* 9 OH-*ellipticine at low doses. Treatment of rats was performed as indicated in the text. The variation of the doses is due to the use of different production lots of* 9 OH-*ellipticine which display slightly different antitumor effects at the same dose. We added the four independent experiments in order to test the efficiency of* $NADP^+$ *by the paired series method* [6]. *Statistical calculations were made for all the possible pairings in each experiment. In the* 5 *possible configurations* $|t| < 1.96$ *and* $P > 0.02$. *Empty bars:* 9 OH-*ellipticine; shaded bars:* 9 OH-*ellipticine* + $NADP^+$.

delayed death of the rats. We chose to evaluate the chemotherapeutic effect by counting the number of surviving rats. On one hand Fig. 2 shows that a low dose of 9 OH-ellipticine has an antitumor effect in the presence of an NADPH regenerating system. This is in agreement with the results observed in cell culture. On the other hand, $NADP^+$ given at the time of ellipticine treatment significantly increases the number of rats surviving 9 OH-ellipticine (Fig. 3). $NADP^+$ when injected alone and even at much larger doses has no effect on control animals and does not change the established LD_0 of 9 OH-ellipticine for rats to any appreciable extent.

Our method was not able to show any stimulation at a dose of 5 mg/kg 9 OH-ellipticine because all the tumor-bearing animals survived. The conclusion of experiments performed with different doses of drug is that the cofactors show a significant increase only at drug doses which alone have very little effect. Non-published results show that NADPH, G6P and phosphogluconic acid (which is the oxidation product of G6P) injected alone do not produce any stimulation of the antitumor potency of 9 OH-ellipticine. The comparison of the effect of an NADPH generating system and of $NADP^+$ in *in vivo* experiments shows that a low dose of $NADP^+$ is able to produce a quantitatively more dramatic effect on the antitumor potency of a low dose of 9 OH-ellipticine.

Altogether these data suggest that residual $NADP^+$ is responsible for the action of the NADPH generating system on 9 OH-ellipticine. Indeed, at concentrations of 1.7×10^{-3} mol/l G6P and 1.3×10^{-3} mol/l $NADP^+$, the $NADP^+$ reduction is not 100%; the explanation lies in the values of the K_i for NADPH (2.7×10^{-5} mol/l) and the K_m for G6P (6.9×10^{-5} mol/l) [5].

The very low solubility of a mixture of $NADP^+$ and 9 OH-ellipticine suggests that these two compounds may form a complex which is internalised by Walker cells in a manner which could be different from the uptake of the drug alone.

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