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Effect of tilorone and quinacrine on translation in reticulocyte lysates

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Tilorone [2-7-bis-(diethylamino)-ethoxy-fluoren-9-one dihydrochloride] is an antiviral agent which increases circulating interferon levels [1] and shows a variety of host-mediated biological activities [2]. Considering its antitumoral and antiviral spectra, tilorone resembles dsRNA (double stranded RNA), another well-known inducer of interferon. Tilorone may be also compared to dsRNA in that it is a potent immunological adjuvant [2] and depresses cytochrome P-450 mono-oxygenases [3]. Otherwise, the antimalarial agent, quinacrine, [6-chloro-9-[4-(diethylamino)-1 methylbutyl] amino-2-methoxy-acridine dihydrochloride] also resembles dsRNA and tilorone by its ability to induce interferon [4] and to depress cytochrome P-450-dependent mono-oxygenases [3]. The mechanism of action of tilorone and quinacrine at the molecular level has not been established. As protein synthesis in reticulocytes lysates is strongly inhibited by dsRNA [5], we have decided to examine whether tilorone and quinacrine are also active in inhibiting protein synthesis.

Phenylhydrazine chloride was furnished by Merck Laboratories, quinacrine was obtained from Sigma Laboratories, tilorone was furnished by Merrel Laboratories and [^{14}C]leucine (297 mCi/mmole) was obtained from CEA.

White New Zealand (2–2.5 kg) rabbits were made anemic by five daily injections of phenylhydrazine chloride. On the seventh day, blood was obtained by heart puncture and lysates were prepared as described [6].

The optimal hemin concentration was 25 μM in the lysate used. Protein synthesis was assayed by the incorporation of [^{14}C]leucine. The reaction mixture (100 μl) containing 50 μl of reticulocytes lysate and other components required for protein synthesis [6] was incubated at 30°. After the incubation times indicated in the figures, 10 μl aliquots were removed, added to 0.5 ml of 1 mM leucine in 0.1 N NaOH, incubated for 15 min at 35°, and then combined with 1.5 ml of 10% TCA. After one night at 4°, the precipitates were washed three times with 10% TCA, collected and counted.

Figure 1(a) shows the effect of different concentrations of tilorone on protein synthesis by reticulocytes lysates in the absence of added hemin; Fig. 1(b) shows the synthesis in the presence of 25 μM hemin. In the absence of hemin, tilorone decreased the rate of protein synthesis, and this effect increased with increasing drug concentrations (10–700 μM). In the presence of hemin, an inhibitory effect of tilorone was also observed. Hemin-enhanced leucine incorporation was completely blocked by 200 μM tilorone.

Figure 1(c) shows the incorporation of leucine in the absence of hemin at different concentrations of quinacrine (10–200 μM). Figure 1(d) shows the same experiment except that 25 μM hemin was added. Quinacrine was more efficient than tilorone in preventing the hemin-promoted stimulation. At 50 μM, quinacrine almost completely blocked the effect of hemin. Unlike tilorone, concentrations of quinacrine which prevented the effect of hemin did not strongly alter the rate of protein synthesis during the first minutes of incubation.

Tilorone- and quinacrine-induced inhibition of translation may be compared with inhibition by dsRNA. After addition of dsRNA, protein synthesis in the presence of hemin proceed for a few minutes before an abrupt drop in the rate of synthesis and disappearance of the met-tRNA$_F$ complexes [6]. Twenty-five to fifty micromolar quinacrine also prevented the effect of hemin without strongly affecting the rate of leucine incorporation in the first minutes of incubation. (Higher concentrations of quinacrine, however, slowed the rate of leucine incorporation in the first minutes.) However, all concentrations of tilorone which prevented the effect of hemin, immediately altered the rate of incorporation, indicating that its sites of action may be different from that of quinacrine. Tilorone and quinacrine may affect translation at many sites. By example, these drugs can intercalate into an RNA of the reticulocyte system (as is the case with ethidium bromide, another intercalating agent which acts upon transfer RNA [7]). On the other hand, the kinetics observed in the presence of quinacrine also suggest that this drug can antagonize the action of hemin. Since, as we have shown previously [8], tilorone and quinacrine prevent the inactivation by hemin of a casein kinase present in various mammalian cells, one can imagine that this kinase mediates the action of both drugs on protein synthesis. At present, there is no direct evidence bearing on such a possibility. There are, however, some examples for the control of protein synthesis via protein kinases. Thus, dsRNA is known to activate a kinase which phosphorylates eIF2 (eukaryotic initiation factor 2) [9–12] and hemin prevents the activation of a specific protein kinase which also acts on eIF2 [13–16].

The mechanism of action of tilorone and quinacrine at the molecular level is not well understood. These drugs have an intercalative mode of binding to DNA [17, 18] and inhibit the template activity of DNA in DNA- and RNA-polymerase reactions *in vitro* [18]. Our observations show that they also act on the protein synthetizing machinery. Then, it is tempting to think they promote some of their biological effects by acting at the translational level.

It also seems possible that other antiviral drugs which are not structurally related, such as dsRNA [5] and ARaA (9-β-D arabinofuranosyl adenine) [19], promote some of their biological effects by interacting with the protein synthetizing machinery of the mammalian cells.

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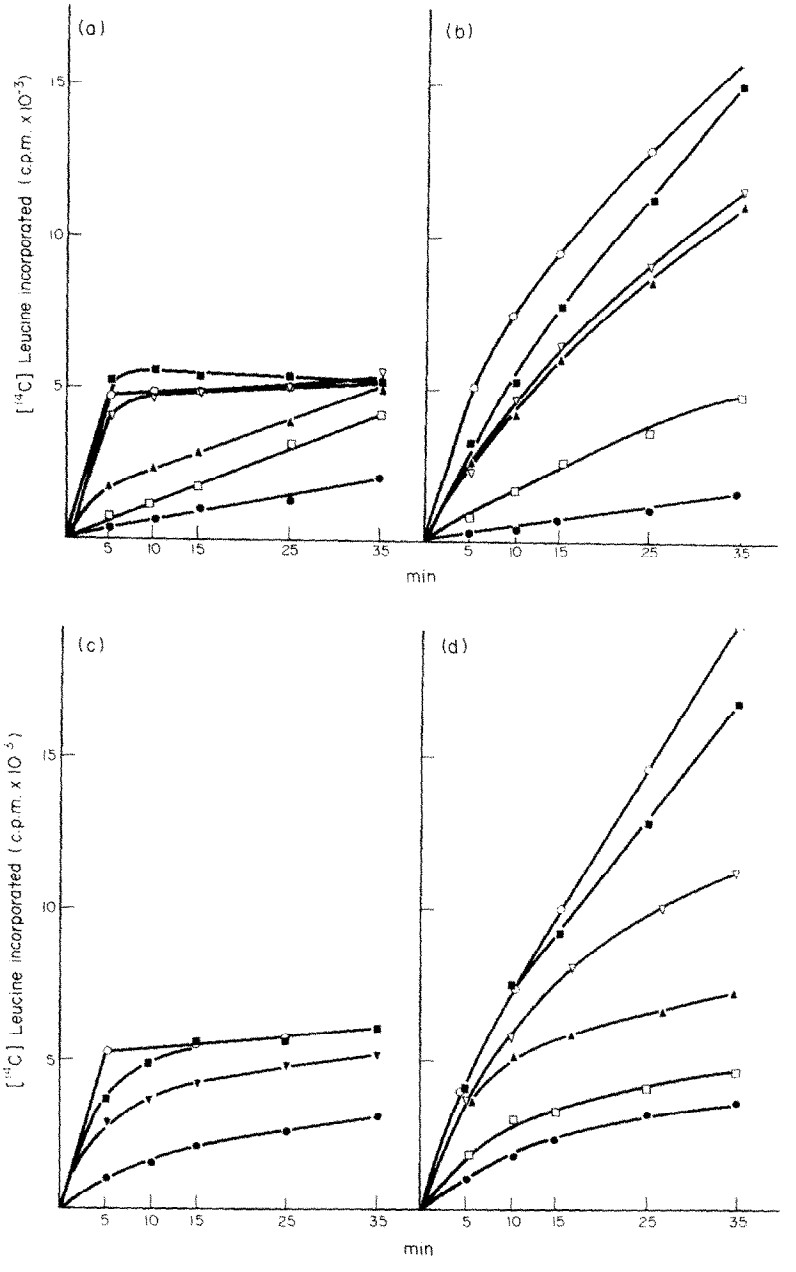

Fig. 1. Protein synthesis inhibition by tilorone and quinacrine. ^{14}C-Leucine incorporation was assayed as described in the text. ○— control (without drug) tilorone concentrations [Fig. (a) and (b)] were 50 μM (-■-); 100 μM (-▽-); 200 μM (-▲-); 350 μM (-□-); 700 μM (-●-). Quinacrine concentrations [Fig. (c) and (d)] were 10 μM (-■-); 25 μM (-▽-); 50 μM (-▲-); 100 μM (-□-); 200 μM (-●-). Incubation was performed in the absence [Fig. (a) and (c)] and in the presence of 25 μM added hemin [Fig. (b) and (d)].

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