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Artemisinin RNA Interaction

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Abstract: The bioactivity of Artemisinin is assessed by its damaging effect on RNA fragment. Incubation of artemisinin with RNA at different reaction conditions, including irradiation with light, heat and mild acidic media revealed no RNA damage when examined by agrose electrophoresis. However artemisinin/Fe (II) caused RNA damage in pH-dependent manner. In contrast hemin didn't show the same effect when used instead of Iron (II).

Key words: Artemisinin · RNA · Electrophoresis

INTRODUCTION

Cytological studies has shown that artemisinin causes ultra structural changes to plasmodium parasites membrane [1] and ^{3}H-dihydroartemisinin and ^{14}C-artemisinin covalently bind heme serum albumin during *in vitro* incubation with human serum [2]. The drug serum interaction product is resistant to mercaptoethanol and urea indicating covalent bonding as supported by mass spectrometry. This covalently bound protein increases in presence of iron (II) by 40% and diminish in presence of chelators e.g. desferrioxamine [2].

DNA is known to be damaged by Fenton oxidant [3], this inspired Wu group [4] experimenting with Fe (II)/artemisinin analogue. PUC18 super coiled DNA, calf thymus DNA and Salmon DNA in slightly acidic condition exhibited exhaustive cleavage of DNA [4].

Experimental: Lyophilized Ribonucleic acid sodium salt was obtained from BDH Chemicals Ltd. England. All electrophoresis were purchased from Sigma Chemical Co., United Kingdom.

Preparation of electrophoresis running solutions, gel loading, reaction buffers and agarose gel followed literature methods [5]. Reaction mixtures were mixed with gel loading buffer and loaded into slots by micropipette using sterile disposable tips. Electrophoresis experiments were run at 80-250 V for a period of 0.75-2.14 h on a horizontal electrophoresis system. The gel was examined in ultraviolet trans-illuminator and photographed using low land camera.

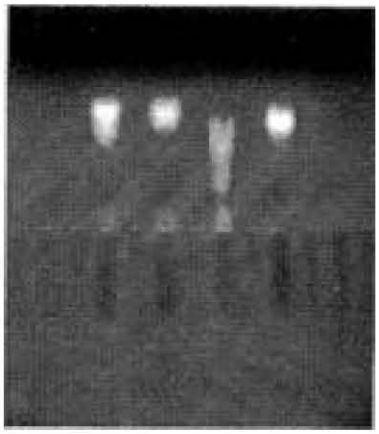

Fig. 1: Ribonucleic acid sodium salt (RNA) (2.0 mg ml^{-1}) was incubated for 24 h at 37°C with artemisinin and Fe (II) ion in phosphate buffer (pH 5.5) and analyzed by gel electrophoresis eats (0.6% agarose). Lane 1, RNA control; Lane 2; RNA+Artemisinin (10.0 mM); Lane 3., RNA+ Artemisinin (10.0 mM) + Fe (II) ion (10.0 mM); Lane 4, RNA + Fe (II) ion (l0 mM).

RESULTS AND DISCUSSION

Mixtures of artemisinin and RNA in the presence of Fe (II) ion at pH 7.5 when subjected to agarose gel electrophoresis revealed RNA damage (Fig. 1). Hemin in place of Fe (II) didn't show any damage (Fig. 2). Similar observation for DNA with γ-radiation has been reported [6]. The apparent acid dependence is due to

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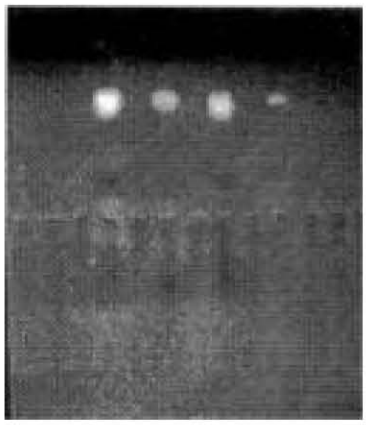

Fig. 2: Ribonucleic acid sodium salt (RNA) (2.0 mg ml^{-1}) was incubated for 24 h at 37°C with artemisinin and Fe (II) ion in phosphate buffer (pH 6.5) and analyzed by gel electrophoresis (0.6% agarose). Lane 1, RNA control; Lane 2; RNA + Artemisinin (15.0 mM); Lane 3., RNA + Artemisinin (15.0 mM) + hemin (15.0 mM); Lane 4, RNA+ hemin (15.0 mM)

reactive radical intermediate on pH since increase in hydrogen ion concentration doesn't change products or their ratio.

No kinetic work have been done to probe the mechanism of RNA/DNA damage and so many species have been suggested as responsible for the attack on nucleic bases. Hydroxyl and ferryl radical and secondary carbon radicals have been suggested, the latter alkylated DNA [6].

In contrast to alkylation of protein which is very efficient in basic media DNA/RNA interaction with artemisinin are more efficient in acidic media. This may be acceptable if carbon centered radicals cleave DNA through hydrogen abstraction [7]. The carbon centered radical has been spin trapped [8-10].

Hemin causes no RNA damage when used instead of Fe (II) ion in the same reaction condition in agreement with early epr work and spin trapping [11], suggesting heme to be responsible for the degradation of artemisinin.

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