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Comparative *in vitro* investigations of the interaction between some macrolides and Cu(II), Zn(II) and Fe(II)

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Received July 9, 2002, accepted October 28, 2002

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Pharmazie 58: 223–224 (2003)

Azithromycin (Az), clarithromycin (Clar) and roxithromycin (Rox) are relatively new macrolide antibiotics with improved pharmacological and pharmacokinetic properties [1]. Literature reports indicated that they might be significantly different in antimicrobial activities and pharmacokinetic properties [2]. In a detailed study using polarographic, absorption and NMR spectroscopic techniques, Az was shown to form 1 : 1 or 4 : 1 (drug : metal) complexes with copper in methanol and aqueous buffer [3]. However there has been no systematic study – to best of our knowledge – which compares the complexation behavior (possible complex species and formation constants) of the three compounds to biologically important metal ions. Potential differences in the possible complex species as well as the strength of the formation constants of the drugs with the various metal ions could explain some of the differences in their biological and pharmacological properties. The purpose of this study was to investigate complexation reactions between the macrolides and metal ions: Cu(II), Fe(II) and Zn(II).

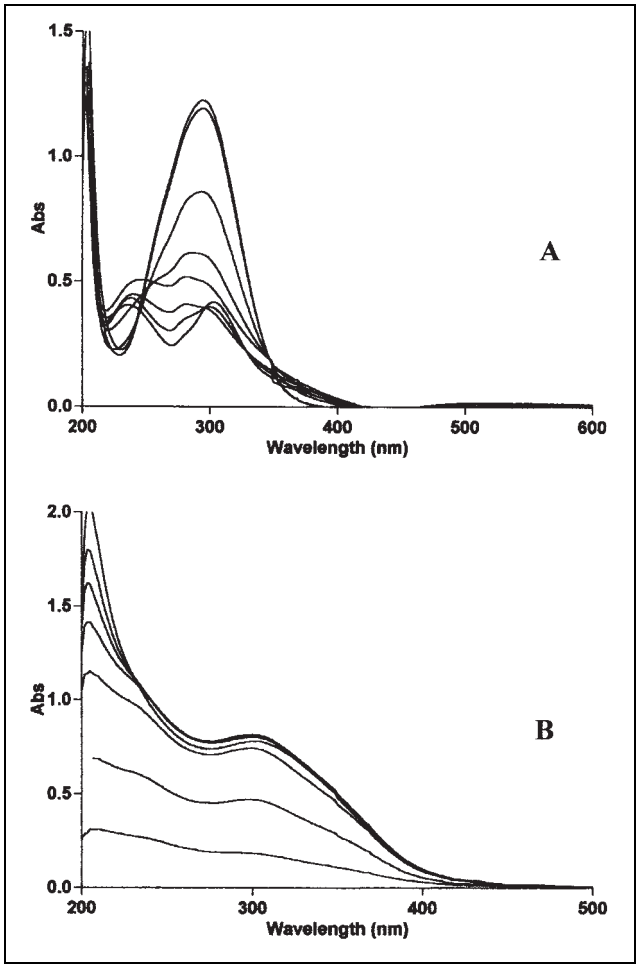

Fig. 1: In (A), overlaid UV spectra of $CuBr_2$ solutions (2×10^{-4} M) titrated with Az. In (B), overlaid UV spectra of $FeSO_4$ solutions (1.46×10^{-4} M) titrated with Az. Similar spectral changes (in general) were obtained for Rox and Clar. Generally the absorbance increased as the drug to metal ratio was increased

Scanning of titration solutions (see Experimental) for copper and iron, in the range 200–800 nm, showed a progressive hyperchromic shift at about 300 nm (Fig. 1). Since non of the three macrolides has any absorption at wavelengths higher than 230 nm, the UV spectra obtained at r = zero were essentially that of the metal. Thus the observed hyperchromicity was explained – as previously reported for azithromycin [3] – in terms of complex formation between the copper and the macrolide compounds. Isosbestic points characterestic of the presence of chemical species under equilibrium (free and bound) were obtained for the reaction of copper with Az and Clar at 347 nm, 328 nm, 295 nm, 252 nm and 244 nm (Fig. 1). For the reaction of Rox with copper, three isosbestic points were detectable: 347 nm, 327 nm and 250 nm. The hyperchromic shift was associated with slight shift in λ_{max}. Plots of λ_{max} against molar ratio of drug to metal (r) were obtained for each of the macrolides (when titrated with copper). It could be concluded from the obtained plots that both Az and Clar can form 1 : 1, 3 : 1 and 4 : 1 complex species, where Rox can form 1 : 1, 2 : 1 and 4 : 1 complex species. However, other complex species cannot be excluded based on these plots only, because they might have formed but without causing significant changes in λ_{max}. Therefore absorption molar ratio plots were obtained for the complexation reaction of each of the macrolides with copper (Fig. 2). Similar to a previous report [3], Az was shown to form 1 : 1 or 4 : 1 complexes with copper. Also a 3 : 1 complex form was evident. Molar ratio plots for Clar and Rox (monitored at 295 nm and 294 nm respectively) indicated the formation of the overall complex species 4 : 1. The presence of 1 : 1 complex species was also evident for both Clar and Rox. However, only Rox was shown to form 2 : 1 complex species. No evidence of 3 : 1 Rox : Cu(II) complex form was obtained. On the other hand no evidence for the presence of 2 : 1 Clar : Cu(II) complex species was obtained. It is interesting to note the similarity of the stepwise complexes of Clar and Az which were different from Rox. Various reports have compared the antimicrobial activity of the three macrolides and shown Clar and Az to be close in their antimcrobial activity but significantly more potent than Rox [2]. Therefore it might be possible that the complexation behaviour of the macrolide compounds, to metal ions, has some role in their mechanism of action.

In a similar manner to the UV absorption studies, conductimetric molar ratio plot was obtained for each drug by plotting conductivity against the molar ratio (r). The conductivity of the control solutions (see Experimental) showed only little and steady increase in response to increasing concentrations. Conductimetric titration curves indicated an overall formation ratio of 4 : 1 for the three macrolides, which was in good agreement with the UV experiments. Consistent with the UV data and with a previous report (for Az) the two forms 1 : 1 and 4 : 1

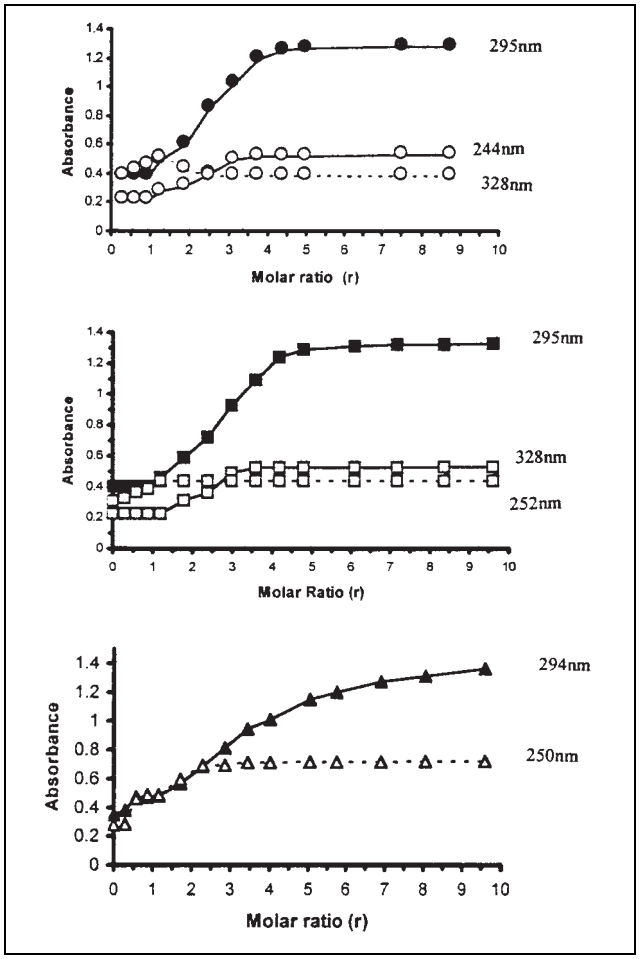

Fig. 2: Molar ratio plots (UV) for the titration of $CuBr_2$ solution (2×10^{-4} M) with Az (circle), Clar (square) and Rox (triangle). The wavelength at which the reaction was monitored is indicated

Az : Cu(II) or Clar : Cu(II) complexes were detectable [3]. In additional support to the UV data 2 : 1 complexes of Rox were evident.

In the case of iron, molar ratio plots (UV) suggested complex formation with an overall stoichiometry of 2 : 1 for the three macrolides. Presence of 1 : 1 complex species was also evident for the three macrolides. Conductimetric titration plots were consistent with an overall stoichiometry of 2 : 1 for the three macrolides.

Because neither Zn nor the macrolides have a significant absorption in the UV region it was not practical to study complexation reactions using UV absorption method. Conductimetric molar ratio plots for the complexation of Zn to the different macrolides indicated an overall stoichiometric ratio of 4 : 1 for the three macrolids. There was no obvious difference in the behaviour of the three macrolides when bound to Zn(II).

The apparent formation constants for the 4 : 1 (copper) and 2 : 1 (iron) complex species were estimated from the UV molar ratio plots. When complexed to copper, Clar exhibited the highest formation constant (2.5×10^{15}) followed by Az (7.7×10^{14}) then Rox (9.2×10^{13}). A similar trend was also observed in the case of iron: Clar (6.1×10^{10}), Az (5.4×10^{10}) and Rox (1.5×10^{10}). These differences in the strength of the complexes could have implications on the differential antimicrobial activity of the macrolides. Although the relative antimicrobial activity for these compounds is variable depending on the type of the bacteria being tested, it is generally accepted that Clar is the most powerful followed by Az then Rox [4]. This trend in antimicrobial activity could be roughly correlated to the formation constants where the generally more active macrolides, Clar and Az, had higher formation constants than Rox.

Experimental

All metal salts were of analytical grade and obtained from Sigma (Saint Louis, USA). The macrolides were of pharmaceutical grade and obtained from Al hikma Pharmaceutical Company (Amman, Jordan). HPLC grade methanol was obtained from Labscan (Dublin). All experiments were carried out in HPLC grade methanol because macrolide complexes were insoluble in water. The design of the experiments was based on the mole ratio method [5] where a series of test tubes containing a fixed concentration of the metal (app. 1.5×10^{-3}) and increasing concentrations of the drug was prepared for each experiment. At least 15 solutions were prepared for each experiment so that the molar ratio (r) of the drug to metal was in the range of 0.2–10. After standing for 15 min the absorbance or the conductivity of each test tube was measured using methanol as a blank. All experiments were repeated at least three times and average values were obtained for obtaining the various plots. In all cases the relative standard deviation of measurements was less than 2.5%. Apparent formation constants were calculated manually as described in [5].

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