ON THE MODE OF ACTION OF KUWAITIMYCIN

IBRAHIM R. SHIMI

Biochemistry Department, Faculty of Science,
Ain Shams University,
Cairo, Egypt

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Kuwaitimycin influences the translocation of alkali metal cations in liver mitochondria. In presence of valinomycin, which stimulates uptake of potassium ions by the mitochondria, the addition of kuwaitimycin could augment the loss of these ions when the source of energy was mainly an oxidizable substrate. However, kuwaitimycin failed to cause loss of K^+ from mitochondria in presence of appropriate amounts of ATP. A relation between the ionic size of the alkali metal cations and the inhibiting effect of the antibiotic on glutamate oxidation could be demonstrated. The ability of kuwaitimycin to restrain ATP supported oscillatory swelling associated with alkali metal cations transport induced by monazomycin was also demonstrated. The more voluminous the cation the more pronounced the effect on cation transport. The effect of kuwaitimycin on ATP hydrolysis in presence of different alkali metal cations was found to be weak and independent of the cationic size.

In light of the results obtained it is suggested that the antibiotic is exerting its effect on ion transport by inducing pores in the mitochondrial membrane and that the addition of appropriate amounts of ATP might alter the conformation of the mitochondrial membrane in such a way as to nullify the effect of the antibiotic.

The transport of alkali metal cations by mitochondria and the requirement for K^+ to generate adenosine triphosphate (ATP) has been thoroughly studied through the use of specific reagents including antibiotics[1~6]. Kuwaitimycin, an antibiotic which was recently isolated by SHIMI *et al*[7]. was found to influence the alkali metal ion transport in rat liver mitochondria. The present work was conducted to explore the mode of action of kuwaitimycin.

Materials and Methods

Liver mitochondria were prepared according to the method of JOHNSON and LARDY[8] using male rats of 250 g average body weight. Continuous recording of light-scattering changes, variation in extramitochondrial concentration of alkali metal cations were conducted as described by PRESSMAN[9,10] and GRAVEN *et al*[11]. Adenosine triphosphatase activity was assessed after ESTRADA *et al*[12]. and LARDY *et al*[13]. Reaction mixtures and experimental conditions are given in the figure legends.

Results

Fig. 1 demonstrates the effects of kuwaitimycin on mitochondrial K^+ transport stimulated by valinomycin in presence of succinate and two different levels of ATP. The initial addition of valinomycin was immediately followed by a rapid increase in the accumulation of K^+ into mitochondria accompanied by a simultaneous decrease in the light-scattering and a pronounced increase in the succinate oxidation. When the reaction mixture reached the anaerobic stage a short transitory period followed as indicated by deflection of the K^+ and light-scattering trace. Immediately after the addition of kuwaitimycin loss of the K^+, accompanied by marked

Fig. 1. Effect of kuwaitimycin on mitochondrial K^+ transport stimulated by valinomycin.

Basic reaction mixture: 7.3 mM KCl; 10 mM acetate (triethanolamine) pH 7.4; 3 mM $MgCl_2$; 10 mM (triethanolamine) (Cl^-), pH 7.4; 160 mM sucrose; 3 mM ATP tris (hydroxymethyl) aminomethane, pH 7.4; 10 mM succinate; mitochondria equivalent to 2.0~2.3 mg N in a 5.0 ml volume at 28°C.

Other additions were as follows: valinomycin; 1×10^{-7} M; kuwaitimycin, 2 μg/ml, kuwaitimycin 2 μg/ml+5 mM ATP tris (hydroxymethyl) aminomethane. Each trace represents a separate individual experiment.

Swelling (of mitochondria) is indicated by a decrease in light scatter.

Increase [K^+]: refers to the extramitochondrial concentration of potassium ion.

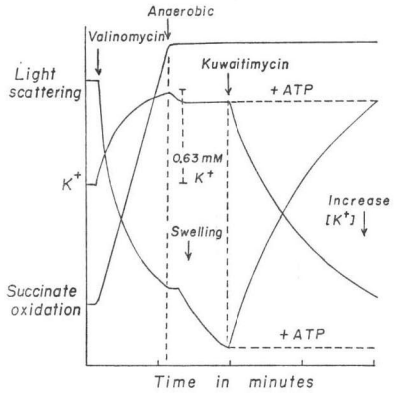


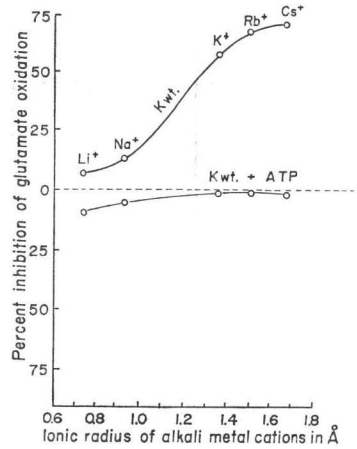
Fig. 2. Effect of kuwaitimycin on glutamate oxidation stimulated by valinomycin in presence of different alkali metal cations.

Reaction mixture: 10^{-7} M valinomycin; 3 mM $MgCl_2$; 12 mM tris (hydroxymethyl) aminomethane PO_4, pH 7.4; 10 mM glutamate (triethanolamine); 30 mM alkali metal cation (Cl^-); 10 mM triethanolamine (Cl^-), pH 7.4; 150 mM sucrose and 0.5 ml of a suspension of rat liver mitochondria in 0.25 M sucrose containing 1.3~1.8 mg of N in a volume 3 ml.

Valinomycin and kuwaitimycin (2 μg/ml) were incubated with mitochondria and 30 mM metal cation 8 minutes prior to addition of glutamate (upper trace).

Valinomycin, kuwaitimycin (2 μg/ml) and 8 mM ATP tris (hydroxymethyl) aminomethane pH 7.4 were incubated with mitochondria and 30 mM metal cation 8 minutes prior to addition of glutamate (lower trace).

Data were calculated from QO_2N values obtained from the experiments conducted at 30°C for 10 minutes.



increase of the light-scattering, could be observed. If an appropriate amount of ATP was added with the antibiotic the loss of K^+ was abolished and hence the light scatter remained rather unchanged. Similar results were obtained with other metal cations though their magnitudes were different.

In Fig. 2 the glutamate oxidation stimulated by valinomycin was restrained to different extents by kuwaitimycin depending on the alkali metal cation existing in the reaction mixture. Inhibition of the glutamate oxidation was the lowest in magnitude in the medium containing Li^+ followed by Na^+, K^+, Rb^+ and Cs^+. When kuwaitimycin and ATP were supplemented together to the reaction fluids the inhibitory influence of kuwaitimycin was not only antagonized but was also reversed into a feeble stimulatory effect in lithium and sodium containing media.

The antibiotic monazomycin stimulated the oscillatory transport of alkali cations in presence of an oxidizable substrate or ATP[14] As shown in Fig. 3 addition of kuwaitimycin to the reaction fluids resulted in a detectable increase in the extramitochondrial alkali cation contents accompanied by a pronounced increase in the light-scatter. It is worth pointing out that the more voluminous the alkali cations the more pronounced were the changes in the light-scatter.

The effects of different alkali metal cations in relation to the inhibition exerted by kuwaitimycin on ATP hydrolysis stimulated by monazomycin are demonstrated in Fig. 4. The most

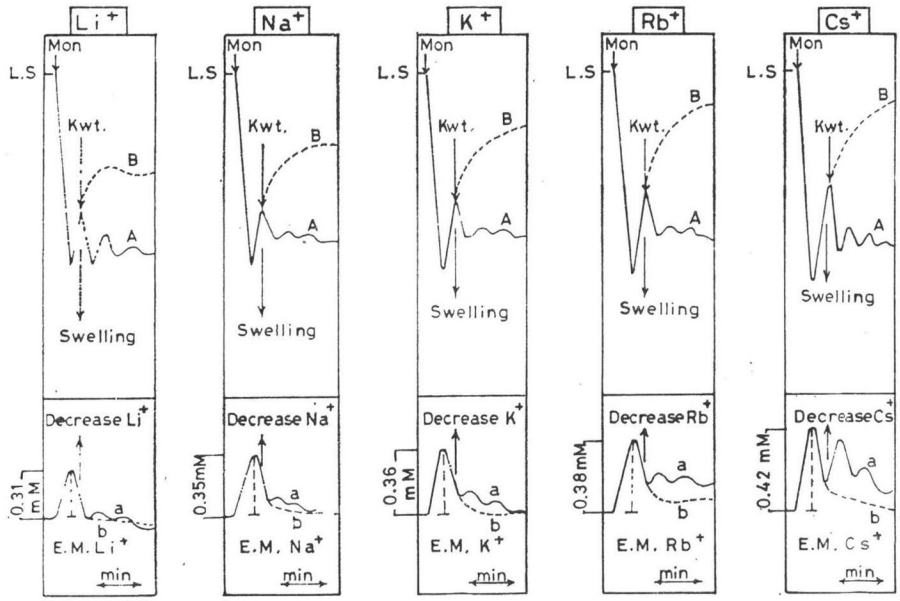
Fig. 3. Effect of kuwaitimycin on the movements of alkali metal cations and light-scattering changes of liver mitochondria induced by monazomycin·

Reaction mixture contained: 6 mM Tris-ATP, 10 mM acetate (triethanolamine) 180 mM sucrose, 3 mM $MgCl_2$; 7.5 mM of the indicated alkali metal cations, 2.3×10^{-7} M monazomycim and mitochondria equivalent to 1.0~1.2 mg of N in 5 ml volume; Final pH 7.4 at 28°C. Kuwaitimycin was added to attain 2 μg/ml of the reaction mixture.

A: without kuwaitimycin, B: with kuwaitimycin.

↑ Decrease in extramitochondrial (EM) concentrations of alkali metal cations.

↓ Decrease in light scattering due to swelling of mitochondria.



marked inhibition to adenosin triphosphatase, though of low magnitude, was recorded in Na^+ and K^+ containing reaction mixtures while Rb^+ had no detectable effect. Cs^+ on the other had induced a very weak stimulatory infleunce.

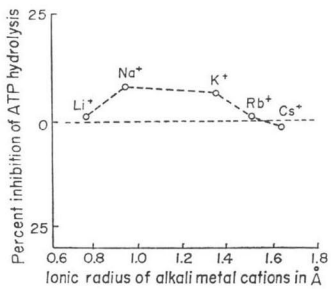
Discussion

Kuwaitimycin is a polypeptide antibiotic that contains isohexadeca-3,6-dienoic acid attached to the rest of the molecule through its carboxylic group. Effect of kuwaitimycin on loss of accumulated alkali metal cations from mitochondria is closely similar to that of monensin which is a non-nitrogenous cyclic antibiotic with a free carboxylic group[15]. Monensins are very effective in causing loss of alkali metal cations from mitochondria when an oxidizable substrate is the main source of energy However, when ATP is the source of energy the specificity of the monensins is greatly increased so as to cause loss of potassium but not of other ions such as Li^+, Rb^+

Fig. 4. Effect of different alkali metal cations on the inhibition by kuwaitimycin of ATP hydrolysis stimulated by monazomycin.

Basic reaction medium; 6 mM ATP (tris) (hydroxymethyl) aminomethane, pH 7.4; 10 mM triethanolamine (Cl^-); 15 mM acetate (triethanolamine) 30 mM of the designated metal cation (Cl-salts); 100 mM sucrose and mitochondria obtained from 0.1 g of the liver; monazomycin, 8×10^{-7} M and kuwaitimycin was added at a final concentration of 2 μg/ml.

Final volume 1 ml. Reaction temperature 28°C.



and Cs^+.

Kuwaitimycin is completely effective in causing loss of alkali metal cations with no discrimination when an oxidizable substrate provides the main source of energy. Nonetheless, the addition of appropriate amounts of ATP abolishes the loss of all cations including K^+. Furthermore, monensins could strongly inhibit ATP hydrolysis in media containing K^+ whereas kuwaitimycin exerted a considerably weaker effect. Thus the mode of action of kuwaitimycin on alkali metal cation transport is quantitatively different from that of the monensins. Furthermore, kuwaitimycin possessed relatively weak inhibitory effect on ATP hydrolysis and a cationic size dependent inhibitory influence on glutamate oxidation. Moreover the effect of kuwaitimycin on cation transport induced by monazomycin was more pronounced the larger the cationic size of the alkali metal used.

From these various lines of evidence it seems likely that kuwaitimycin exerts its effect by inducing pores in the membrane as suggested by CHAPPELL and CROFTS[16] CHAPPELL and HEARTHOFF[17] and CHAPPELL *et al*[18], to interpret their results with gramicidin, valinonycin, nigericin and dianemycin. If this assumption is correct it would be predicted that smaller cations could pass more freely through the mitochondrial membrane than larger ones, depending on the pore dimensions in relation to the ionic radius of alkali metal cations. This prediction is in harmony with the results of Figs. 2 and 3 where the effect of kuwaitimycin became more pronounced the larger the ionic radius of the cation. This may possibly indicate that the dimensions of the pores presumably induced by kuwaitimycin in the mitochondrial membrane were large enough to allow a rather free passage of Li^+ and Na^+ because of their relatively small ionic radii. The data recorded in Fig. 4 demonstrate that there is no regular relation between the cationic size and the percent inhibition of the ATP ase activity which was rather too weak to account for the potent effect of kuwaitimycin recorded in the preceding figures.

Thus addition of appropriate amounts of ATP may alter the conformation of the mitochondrial membrane in such a way as to nullify the effect induced by kuwaitimycin.

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