CHAPTER IV

DISCUSSION AND CONCLUSION

The results presented in chapter III demonstrate that ketoconazole has inhibitory effect on important bioenergetic function of isolated rat liver mitochondria namely, oxidative phosphorylation. This drug inhibits states 3 and 3u respiration by mitochondria respiring with NAD^+-linked substrates but has little effect when succinate is substrate. The mitochondrial ATPase activity is unaffected whereas MAO activity is strongly inhibited by this antifungal agent. The discussion below concerns with the mechanism of ketoconazole action on mitochondrial bioenergetics and whether these effects might participate in the pharmacological and/or toxicological action of ketoconazole

Effect of ketoconazole on mitochondrial oxidative phosphorylation .

The ability of ketoconazole to inhibit states 3 and 3u respiration when mitochondria are respiring with glutamate plus malate or other NAD^+-linked substrates indicates that ketoconazole may inhibit oxidative phosphorylation through effect on the respiratory chain since the inhibition pattern, the blockade of both states 3 and 3u respiration, resembles that of the respiratory chain inhibitors. However, when

succinate is substrate, the state 3 and 3u respiratory rate are only slightly altered by ketoconazole. In this respect the effect of ketoconazole to inhibit both states 3 and 3u respiration with NAD^+-linked substrates but not succinate is similar to rotenone, a well known and potent respiratory chain inhibitor acting at complex I [31,44]. Thus ketoconazole appears to exert inhibitory effect at complex I which mediates the transfer of electron from NADH to coenzyme Q. The inhibition by ketoconazole of NADH oxidation with osmotic-shocked mitochondria also supports this conclusion since NADH directly feeds electron to complex I. If this drug inhibits oxidative phosphorylation through depletion of mitochondrial NADH by blocking substrates transport across mitochondrial inner membrane or by inhibiting substrate dehydrogenases in the matrix, ketoconazole should have no effect on NADH oxidation. Further experiments with isolated complex I are needed to prove that ketoconazole act directly on this complex to inhibit electron flow from NADH to ubiquinone.

The inhibition by ketoconazole on states 3 and 3u respiration suggests the respiratory chain but does not exclude the ATP synthase complex as the site of ketoconazole action. The possibility that ketoconazole might also inhibit oxidative phosphorylation by interfering with the ATP synthase complex can be assessed by studying the effect of ketoconazole on the uncoupler - activated

mitochondrial ATPase activity. The ATPase reaction induced by the DNP-type uncouplers is generally believed to represent the reversed process of the respiratory chain linked ADP phosphorylation. Since ketoconazole has been found inactive on the DNP-activated ATPase activity, it therefore appears unlikely that ketoconazole inhibits mitochondria oxidative phosphorylation through inhibition of ATP synthase [45,46].

Factors modifying ketoconazole action on oxidative phosphorylation.

As ketoconazole is a weak base [4], raising the medium pH will increase the concentration of the unionized form. This form presumably can penetrate the mitochondrial inner membrane more readily than the ionized form due to its higher lipophillic property and acts on the respiratory chain enzyme located in the inner membrane. If this is the case then ketoconazole should be more active when medium pH is increased. Although there is a tendency for ketoconazole to be more active when medium increased from 6.8 to 7.6, the change in inhibitory activity is very small. The explanations for this observation may be that the ionized and unionized forms of ketoconazole can penetrate mitochondrial inner membrane and/or can inhibit complex I about equally, with the latter being slightly more active.

The sulfhydryl group (-SH) in the mitochondrial inner membrane is known to play an important role in several inner membrane functions (permeability of inner membrane, the coupling of oxidative phosphorylation, the function of ATP synthase and several ions transport) [48,49,50]. The observation that dithiothreitol (DTT), a sulfhydryl group-protecting substance [51], can not attenuate the effect of ketoconazole makes it unlikely for ketoconazole to inhibit mitochondrial function by combining with the mitochondrial-SH group.

Bovine serum albumin (BSA) is the only factor which has been found to clearly influence ketoconazole 's action on oxidative phosphorylation. The presence of BSA in reaction mixture reduces, in a dose-related fashion, the inhibition produced by ketoconazole on state 3 respiration. Since BSA is not expected to pass through mitochondrial membrane, the antagonizing effect of BSA presumably exerted outside mitochondria. By complexing with extramitochondrial ketoconazole, BSA can reduce the free drug concentration around mitochondrial vicinity and thereby facilitate the dissociation of ketoconazole from mitochondrial inner membrane. Ketoconazole presumably binds BSA with relatively low affinity since 20 mg BSA causes only partial reversal of the ketoconazole's effect on oxidative phosphorylation.

Effect of ketoconazole on mitochondrial calcium transport.

Mitochondria possess elaborate systems for transporting calcium ions across their inner membrane. Mitochondria could uptake large amounts of calcium ions by uniporter, a mechanism that facilitates the diffusion of an ion down its electrochemical gradient and does not couple the transport to that of any other ion or molecule [52]. Calcium ions penetrate across the inner membrane in response to the negative membrane potential established inside the inner membrane by the activity of the respiratory chain or by ATP hydrolysis [53]. Thus inhibitors of respiratory chain, e.g., rotenone and inhibitors of ATP synthase, e.g., oligomycin can inhibit mitochondrial calcium uptake by preventing the generation of membrane potential across mitochondrial inner membrane. The observed inhibitory effect of ketoconazole on calcium-stimulated respiration, Therefore, Can be explained by its action on the respiratory chain, i.e., complex I inhibition by ketoconazole leads to decreased membrane potential and consequently reduces calcium uptake and calcium-stimulated respiration by the mitochondria.

Whether the mitochondrial effects of ketoconazole described here are involved in the pharmacological and/or toxicological actions of this drug is at present a matter of speculation. After oral doses of 400 and 800 mg, peak

plasma concentrations of ketoconazole are approximately 8 and 20 μg/ml. In blood, 84-99% of ketoconazole is bound to plasma proteins, largely albumin; 15% is bound to erythrocytes, and only 1% is in free form [54]. Evidently, the concentration of free ketoconazole in blood is much less than concentration of ketoconazole employed in this study (10-100 μg/ml). Therefore, the mitochondrial effects of ketoconazole reported here are unlikely to contribute to the antifungal activity of this compound. On the other hand, it is tempting to speculate that these effects, particularly the inhibition of oxidative phosphorylation, may, at least partly, play a role in the toxicity of ketoconazole-especially hepatitis and hepatic necrosis. Thus it is proposed that of ketoconazole may accumulate in hepatic tissue during prolonged used with high doses, resulting in impairment of mitochondrial ATP synthesis and, eventually hepatic dysfunction. Moreover, the inhibition of MAO by ketoconazole raises the possibility that this drug may potentiate the actions of certain sympathomimetic amines leading to undesirable effects on the cardiovascular system.

In conclusion, the major effect of ketoconazole on mitochondrial bioenergetics is to inhibit states 3 and 3u respiration probably by blocking electron transport at complex I of the respiratory chain. This action may partly responsible for the hepatotoxicity of this drug. The possible drug interaction between ketoconazole, as well as other

related antifungal agents, and sympathomimatic amines deserves further investigation.