CHAPTER V

CONCLUSIONS

Structure-activity relationship (SAR) of curcumin analogues on PDEs: Cell free experiments showed that all curcumin analogues were moderately active PDE5 inhibitors (Table 4). When the meta-methoxy group was missing from 1, the activities of 2 and 3 were reduced suggesting that this group is important for PDE5 inhibitory activity. Demethylation of 1 gave the more polar analogue 4 resulting in a 2-fold lower inhibitory activity while removing both methoxyl groups yielded compound 3 which produced a 4-fold reduction in activity. It might be possible that these meta-positions need these bulky substituents for binding to the active site.[114]

Replacement of the ketomethylene group in 1 giving 5 slightly increased the inhibitory activity whereas a similar structural modification of 2 to 6 increased the activity. Taking compound 6 and transposing the two hydroxyl groups from positions 4′ to 2′ and 4′′ to 3′′ and removing the aromatic methoxy group yielded a highly potent compound 7.

Water solubility was a major challenge for curcumin analogues (1-7) and solubility was improved by adding a triazole carboxylic group to the 2′ hydroxyl group in 7 to give 8 but this compromised PDE5 potency. In contrast, a similar substitution on the opposite 3′′-hydroxyl group of 7 yielding 9 preserved the inhibitory activity as well as offering superior water-solubility.

The inhibitory activity on PDE6 is another concern because it disrupts cGMP signaling pathway used in retinal transduction and this is avoided in the highly selective PDE5 inhibitor, tadalafil.[113] The inhibition by these compounds on PDE6 suggests that 1, 3 and 4 had weak actions compared to the corresponding actions on PDE5 and accords with the 10-fold selectivity of sildenafil. [114] These results suggest that further modification of curcuminoid analogues could achieve the required specificity and high activity needed to realize clinical usefulness.

Vasorelaxant effects of curcuminoid analogues: In these experiments, sildenafil potency on intact pulmonary artery was 0.04 μM (0.074±0.016

μMcalculated as EC_{50}) which accords with previous work in the rat pulmonary artery[2] and aorta.[114] Furthermore, the potency of sildenafil here was similar to the cell-free action on PDE5 protein. Endothelial removal caused a dramatic decrease in sildenafil potency (200-fold less) both here and previous work on aorta.[115] This confirms that the vascular smooth muscle relaxation was largely mediated through the endothelium but notwithstanding this, sildenafil acts on the vascular smooth muscle. [116] It is note the vasorelaxation effects were expressed as EC_{40} as curcumin and analogs had limit solubility and low potency which preventing us from determining the supramaximal effect.

All the curcuminoids showed vasorelaxation effect on endothelium intact pulmonary artery with the same range of potencies (Table 2). Compounds 2, 5, 8, 9 and possibly 4 had actions which indicate that the endothelium was necessary for a large proportion of their action and thus essential for their effect. But, these four compounds each had similar potencies which did not reflect those variations seen in the cell free assays on either PDE5 of PDE6. Thus for compounds 2, 5, 8 and 9, there was clearly endothelium-dependent, but the poor potency correlation with the cell-free studies does not clearly indicate that they are acting on PDE5. Compounds 1 and 7 were unaffected by endothelial denudation suggesting that they act directly on vascular smooth muscle through a mechanism probably unrelated to PDE5. These might include a direct action on soluble guanylyl cyclase[117] on β-receptors, and cytosolic Ca^{2+} handling.[118]

There are two important differences between the cell-free and vascular relaxation studies which might affect potency of our compounds (i) the compounds have to gain access to the cell interior and numerous bioavailability studies have shown that the membrane permeability of at least curcumin itself is very poor [119], (ii) a vast number of cellular effects for curcuminoids have been described.[120, 121] However, the very high concentrations needed to have any effect on the aorta do suggest that these compounds have some selectivity for the pulmonary artery. This alone suggests that the compounds may form the basis for the development of drugs that selectively target the pulmonary circulation. Finally, the multiple cellular actions of curcuminoids may be an asset in the treatment of a disease such as PAH where

there are multiple pathologies including inflammation, PDE5 upregulation, ionchannelopathies, vasoconstriction, endothelial dysfunction and vascular hyperplasia.

Future perspectives

As our studies showed the effects of curcumin analogs on PDE5 and isolated pulmonary artery, further investigations on many aspects of this group of compounds are needed to be conducted. For examples:

1. Structural design for the new curcumin analogs based on the information we have reported here. The in silico approach using the publish models for the catalytic site using the present PDE5 inhibitors and the curcuminoids we already have are one of the possibilities.

2. Further studies on the most active curcuminoid. The curcuminoids appear to be good candidates for PAH because of their multi-target effects including PDE5 inhibition, Rho-kinase inhibition, sGC inhibition, anti-inflammation (NF-kB), antioxidant (NRF-2) (Figure 13). Already in two studies, curcumin itself has been shown vascular relaxation effect in low nanomolar concentrations [82];[83]. Although the animal models for PAH currently used do not represent the human condition very well [122], the *in vivo* study is still nessessary to prove the effect of the curcuminoids on pulmonary artery.

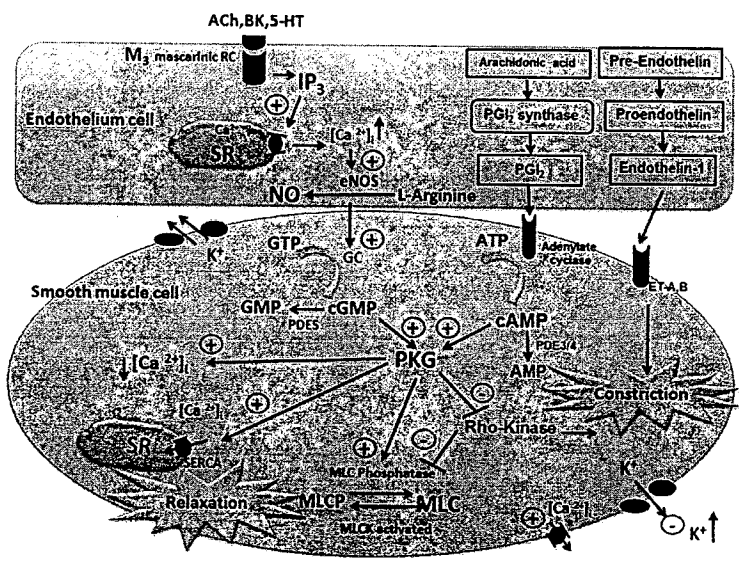

Figure 13 Signaling pathway on smooth muscle relaxation and constriction

Conclusions

Curcumin analogues showed PDE5 inhibitory activity with varying potencies and some showed selectivity for PDE5 over PDE6. There were clear endothelium-dependent vasorelaxation effects to which the pulmonary artery was more sensitive compared to the aorta. These results suggest that these curcuminoids could underpin the further development of highly selective and potent compounds which could discriminate the pulmonary arterial circulation as PDE5 inhibitors or on a presently unknown different pulmonary selective target.