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Conclusion

A cell is equipped with different protein quality control mechanisms that continuously work to maintain proteostasis or protein homeostasis state. The importance of protein quality control mechanisms can be understood from the fact that disturbance in these pathways may prove to be detrimental for the cells. The involvement of these pathways in cell survival mechanisms has made them an interesting target to modulate and utilize for therapeutic purposes including diseases like neurodegenerative disorders and cancer. Improved understanding of the working of these mechanisms and identifying the downstream consequences of its modulation may prove to be quite useful in predicting and determining potential advantages and complications if used for possible therapeutic applications in near future.

5.1 Summary

The current study was conducted to identify proteasome inhibitors and understand the downstream consequences of proteasome inhibition. Two nonsteroidal anti-inflammatory drugs diclofenac and indomethacin were identified as inhibitors of proteasome. The inhibitory effects of diclofenac or indomethacin on proteasome were concluded on the basis of various experiments conducted that involved both cell based and cell free systems. With the help of substrates specific for chymotrypsin and caspase like active sites of proteasome it was observed that both diclofenac and indomethacin have the capability to interfere with the protease activity of the proteasome. Through various immunoblot experiments increased ubiquitylation was observed in the presence of these two NSAIDs which further confirmed the interference of these drugs with proteasome activity, as increased presence of higher molecular weight derivatives of ubiquitylated proteins is one of the characteristic features of proteasomal dysfunction. In addition, increased stability of proteasome targeted model substrate, d1EGFP was observed in the presence of diclofenac or indomethacin, when analyzed in the presence of protein synthesis inhibitor cycloheximide. Subsequently, using immunocytochemistry analysis, increased accumulations of proteasome targeted proteins were also observed. Additionally, as proteasome inhibition was previously observed to induce apoptosis, it was important to identify the role of NSAIDs in induction of apoptosis pathway. It was noticed that both of these drugs caused enhanced accumulation of pro-apoptotic and cell cycle regulatory proteins Bax, p53, p27 and p21. Further, treatment with diclofenac or indomethacin resulted in mitochondrial membrane depolarization and cytochrome *c* release in cytosol, an important step in mitochondrial mediated apoptosis pathway.

5.2 Concluding Remarks

Together, with help of the work done, proteasomes were identified as a target of diclofenac and indomethacin which could be possibly involved in generation of apoptotic outcomes of these drugs. The study might be useful in gaining understanding of NSAIDs mediated anti-proliferative effects and may also help to unravel new pathways associated with them. Further, it also confirms the importance of key proteostasis pathway, the ubiquitin proteasome system in the cells; disruption of which may prove to be deleterious for cells.

5.3 Closing Comments

Identification of novel targets of existing approved drugs is quite useful in understanding their hidden possible applications and even gaining knowledge about their side effects. The present work found proteasomes as the target for diclofenac and indomethacin showing the ability of these NSAIDs to effect the functioning of proteasome. The study helps in increasing our existing knowledge of the mechanisms underlying the NSAIDs mediated apoptotsis. This capability of diclofenac and indomethacin can be utilized for therapeutic purposes, however, for that, more research is needed to identify their exact potential and underlying complexities.

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