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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are class of drugs that are commonly used for its analgesic, antipyretic, and anti-inflammatory effects. Interestingly, there are abundant findings that uncover the anti-proliferative and pro-apoptotic outcomes of NSAIDs [Bock *et al.*, 2007; Chan, 2002]. Further, various NSAIDs have been found to have anti-cancerous properties and regular use of NSAID like aspirin has been found to reduce risks of several cancers [Algra and Rothwell, 2012; Brunelli *et al.*, 2012; Pantziarka *et al.*, 2016; Rüegg *et al.*, 2003]. However, still much work is required to identify the underlying mechanisms different NSAIDs induce, that result in their anti-proliferative property. One of the central mechanisms involved in maintenance and survival of cellular system is the protein quality control mechanism. Cellular proteome is composed of large number of proteins that interact with each other forming a well regulated interaction network to achieve diverse functions occurring inside the cells. However, cells regularly face different kinds of stresses like formation of misfolded or aberrant proteins. In order to cope up against such stress situations, cells maintain their state of homeostasis by well regulated protein quality control (PQC) processes. The ubiquitin proteasome system (UPS) is one of the key PQC mechanisms that is involved in maintaining protein homeostasis state in the eukaryotic cellular system. The UPS system involves identification of unwanted or misfolded proteins which are tagged with chains of ubiquitin molecules as a signal to target them towards proteasome for degradation. As the proteasomes are the end points of the UPS proteolytic system, any disturbance in this machinery may lead to huge consequences inside the cell due to accumulation of the ubiquitylated proteins targeted towards proteasome for degradation. Thus being a crucial process and important for cellular survival; disturbance in UPS system may result in stress condition due to disruption in the state of proteostasis, which may ultimately lead to induction of anti-proliferative activity.

1.1 PURPOSE OF THE STUDY

Developing new drugs is costly and time consuming process due to limitations in existing procedures of drug development [Dickson and Gagnon, 2004]. The cost may reach up to 3 billion dollars with approximately 15 years of time period for a drug to reach in market [Nosengo, 2016]. Using natural molecules as lead compounds may add to cost and labor depending upon chemical complexity and available knowledge of the same. Scarcity of sources and concerns with intellectual property rights adds difficulty in using natural products at commercial level [Cragg and Newman, 2013; Harvey, 2008]. Drug repurposing or repositioning can prove to be an effective alternative both in terms of time and cost. In drug repositioning strategy, known or existing drugs are studied for their effectiveness to treat new indications or diseases, as can be understood by examples of HIV drug plerixafor and antidepressant milnacipran, which were later approved for multiple myeloma and fibromyalgia, respectively [Sleigh and Barton, 2010]. Similarly, various epidemiological and experimental studies on drugs used in treatment in cancer, cardiovascular disorders, anti-inflammatory diseases have also identified novel applications. Anti-inflammatory drugs like celecoxib upregulate E3 ubiquitin ligase Casitas in B-lineage lymphoma B cells (Cbl-b), which results in inhibition of Akt activation through rapamycin thereby attenuating gastric cancer cell resistance to rapamycin [Cao *et al.*, 2015]. In addition, derivative of salicylic acid, diflunisal has been shown to act as pharmacological chaperone, in transthyretin (TTR) amyloidosis, as it stabilizes the tertiary

configuration of this protein [Sekijima *et al.*, 2006]. Thus these few studies provide evidence that anti-inflammatory drugs have role in regulating and modulating the proteostasis pathways. Inhibition of proteasome, a component of UPS system of proteostasis pathway, leads to disturbance of cellular proteostasis and ultimately may direct cells towards apoptosis as shown in Figure 1.1. This strategy has been used in developing therapeutic approaches for cancer, as it has been thought that cancer cells being highly proliferative, have higher requirement of proteins, making them sensitive towards proteasome inhibition [Crawford *et al.*, 2011].

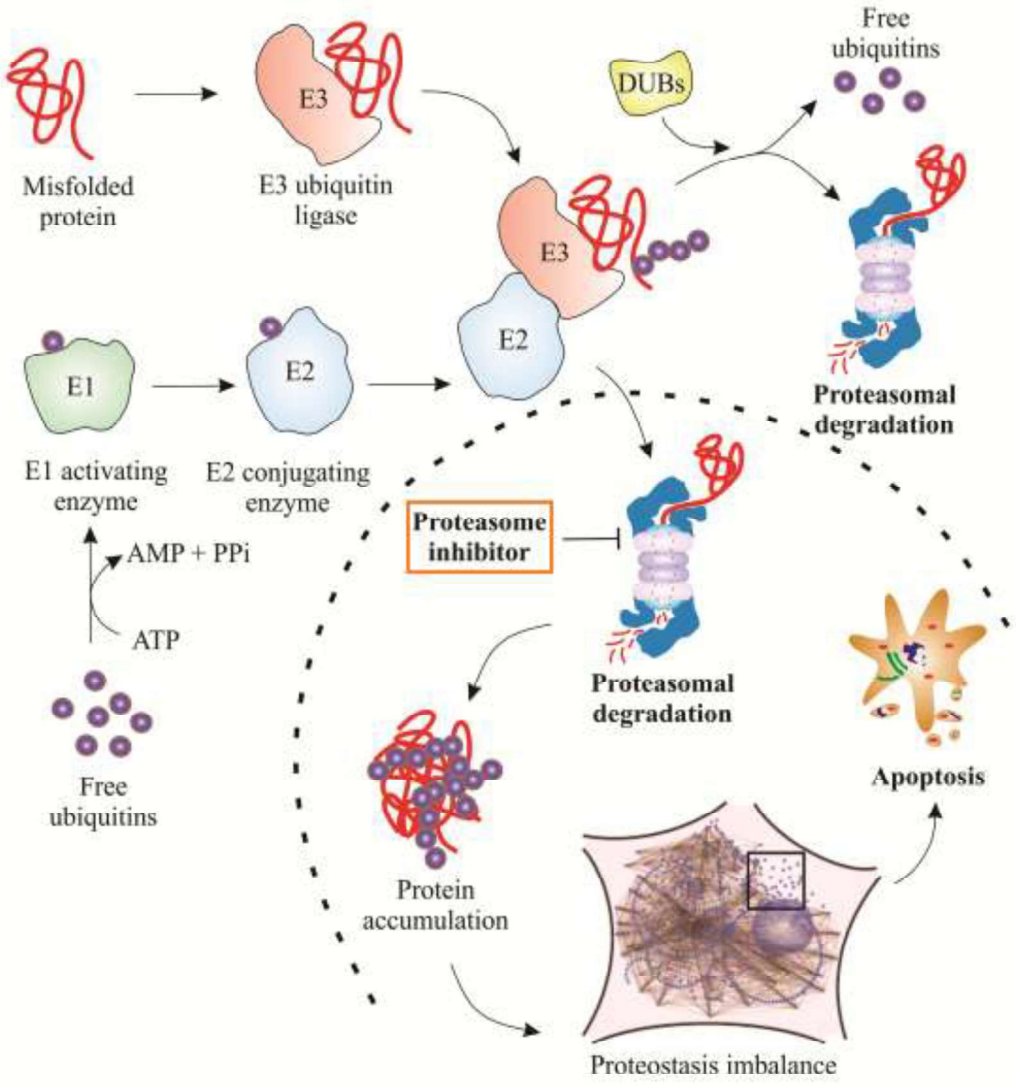

Figure 1.1 : *Ubiquitin proteasome system and cellular proteostasis:* Ubiquitin proteasome system (UPS) is a central protein degradation mechanism playing an important role in maintaining protein homeostasis state along with other protein quality control mechanisms. Disruption in the proper functioning of UPS system due to conditions such as proteasome inhibition, may in turn affect the overall cellular protein homeostasis state causing stress which may lead to induction of apoptosis.

Inhibitors of proteasome have been found to cause endoplasmic reticulum and oxidative stress [Du *et al.*, 2009; Fribley *et al.*, 2004]. Further, proteasome inhibition has been found to be another mechanism involved in generation of anti-inflammatory activity [Chen *et al.*, 2012; Elliott *et al.*, 2003]. Different nonsteroidal anti-inflammatory drugs have also been found to

generate oxidative and ER stress responses in the cell [Basivireddy *et al.*, 2002; Franceschelli *et al.*, 2011; Hickey *et al.*, 2001; Nagappan *et al.*, 2015]. Previously, NSAIDs such as aspirin and meclofenamate sodium have been shown to interfere with proteasome activity [Dikshit *et al.*, 2006; Ghosh *et al.*, 2016]. However, more studies are required to identify the mechanisms of NSAIDs induced proteasomal dysfunction and their underlying consequences. Thus, the present study was aimed to identify novel members of this class of drugs for their potential to inhibit proteasome activity and if yes, what could be the possible mechanisms underlying the inhibitory activity of these drugs on proteasomes.

1.2 BRIEF OUTCOMES AND FUTURE POSSIBILITY OF THE STUDY

The aim of the present study was addressed with the support of extensive literature survey that was used in designing and conducting appropriate experiments. Following section provides a brief outcome of the study executed.

1.2.1 Nonsteroidal Anti-inflammatory Drugs; Diclofenac or Indomethacin treatment, Elicits Proteasomal Disturbances and Develops Apoptosis Through Mitochondrial Abnormalities (Published as Amanullah et al., in Journal of Cellular Biochemistry and Journal of Cellular Physiology)

In the current study, it has been observed that diclofenac and indomethacin, two commonly prescribed nonsteroidal anti-inflammatory drugs (NSAIDs) for pain, fever and inflammation treatment individually induce proteasome malfunction and promote accumulation of different critical proteasome targeted substrates, including few pro-apoptotic proteins in cells. Exposure of diclofenac or indomethacin consequently elevates aggregation of various ubiquitylated misfolded proteins. Further, it has been shown that treatment with diclofenac or indomethacin promotes apoptosis and reduced cell viability in cells, which could be due to mitochondrial membrane depolarization and cytochrome *c* release into the cytosol. It has been found that both diclofenac and indomethacin have inhibitory effects on the post-glutamyl peptide hydrolase-like protease (β1) like and chymotrypsin (β5) like active sites of the proteasomes. The inhibitory activity was identified both in cell and cell free systems i.e. in the purified proteasomes using substrates for specific enzymatic sites (β1and β5) of the proteasomes. Further, through Saturation Transfer Difference Nuclear Magnetic Resonance (STD-NMR) studies it was found that indomethacin interacts with proteasome through its aromatic groups. Interestingly, treatment of indomethacin induced accumulation of ubiquitylated proteins in crude mitochondrial lysates.

The use of proteasome inhibition as a therapeutic strategy requires further studies despite being approved for diseases like multiple myeloma as various limitations of the existing proteasome inhibitors have been reported, such as drug resistance and toxic side effects [Chen *et al.*, 2011]. As diclofenac and indomethacin are well studied drugs, identification of proteasomes as their target can prove to be quite useful in the field of development of proteasome inhibitors as a therapeutic option in conjuction with other anti-cancer agents. These findings further improves the current understanding of how NSAIDs can exhibit crucial anti-proliferative outcomes in cells in addition to identification of target, the proteasome, of these commonly used drugs. This study also sheds light on the importance of UPS as the protein quality control mechanisms in the maintenance of cellular integrity and proper functionality. However, being a key player in maintaining cellular homeostasis and linked with large number of different crucial cellular mechanisms, careful inspection unraveling hidden aspects of modulation of this system are needed for better outcomes in terms of application in disease therapeutics.