J Endocrinol Reprod 17 (2013) 1 :31-40 **Review Article**

Glucocorticoids in health and disease with special reference to glucocorticoid receptor signalling pathways and molecular networking

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Summary

Glucocorticoids have a diverse role in physiological and pathological conditions, making them a class of potent drugs in clinical use ranging from organ transplantation, arthritis, asthma, and chronic obstructive pulmonary diseases (COPD) to oncological problems. The use of glucocorticoids in clinical practices, however, is associated with many side effects and steroid resistance in certain diseases. Understanding the underlying mechanism of glucocorticoid action is an ultimate concern in overcoming these complications. Several studies have pointed the role of glucocorticoid receptor interaction with many other signalling pathways in these situations. Designing of drugs that could modulate these interactions, while maintaining glucocorticoid receptor molecular network in a homeostatic balance, is a challenge in glucocorticoids pharmacology. Some prospective molecules like theophylline and compound A, are reported to have such effect.

Key words: Glucocorticoids associated problems, Glucocorticoid receptors, Glucocorticoid receptor signalling and molecular network.

Introduction

In 1929, Philip Hench found that the arthritis of a patient began to improve after he became jaundiced. He postulated that an endogenous substance, called substance x, from the adrenals is responsible for the remission. By 1940, some steroids were isolated from the adrenal gland by various laboratories. Compound E, known to Reichstein as compound Fa, was suspected by Kendall and Hench as the substance x. This compound came to be known as dehydrocorticosterone (Lloyd, 2002). Later, the Nobel Prize in Physiology or Medicine 1950 was awarded jointly to Edward Calvin Kendall, Taedeus Reichstein and Philip Showalter Hench for their discoveries relating to "the hormones of the adrenal cortex, their structure and biological effects".

Since this discovery, the glucocorticoids (GCs) were found to have a great potential as a therapeutic drug and were considered as magic drugs. The action of GCs is executed by the glucocorticoid receptor (GR) signalling pathway and a complex molecular network (Kharwanlang and Sharma, 2011). GCs application in clinical scenario ranges from arthritis, asthma, chronic obstructive pulmonary diseases (COPD), inflammatory bowel and autoimmune diseases to oncological disorder and organ transplantation (Barnes, 2006; Walsh and Avashia, 1992). However, the clinical uses of GCs have adverse side effects like diabetes, Cushing's syndrome, adrenal suppression and insufficiency, osteoporosis and dyslipidemia. Apart from the side effects, the clinical use of GCs is hindered by resistance for GCs in some cases (Lansang and Hustak, 2011). The complexity of the GR signalling and molecular network needs to be accurately studied, so that the problems in GC therapy can be clearly defined as well as tackled. This review unveils the role of GCs in relation to GR signalling and molecular networking in health and disease.

Physiological and clinical role of glucocorticoids

Glucocorticoids, named by Hans Selye, also known as glucocorticosteroids or corticosteroids, have the capability of life supporting functions (Szabo et al, 2012). Their role in health and disease is overwhelming. They influence wide range of biological activities like the intermediary metabolism, immune/inflammatory reactions, stress, the central nervous system and the cardiovascular system. GCs, being catabolic in nature, upregulate the influx of substrates such as glucose, amino acids and fatty acids into mitochondria for oxidation. They also influence blood pressure, and salt and water balance. GCs play an important role in development and aging (Sharma, 1988; Kharwanlang and Sharma, 2011; Szabo et al., 2012). The ability of GCs to alter energy allocation, physiology and behaviour influences key life-history traits like age-specific transitions, reproduction and survival. GCs also mediate the trade-off between life-history traits (Crespi et al., 2013). Failure in GCs secretion leads to Addison's disease

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which is characterised by lethargy, weight loss and postural hypotension. Chronic GCs excess causes Cushing's syndrome leading to morbidity and mortality, due to obesity, osteoporosis, hypertension and hyperglycaemia (Macfarlane et al., 2008).

GCs are regularly used in the treatment of various ailments associated with inflammation such as asthma, COPD, inflammatory bowel disease, autoimmune disorder and arthritis (Barnes, 2006). They are used as immunosuppressive agent in organ transplantation. GCs also have profound benefits in clinical oncology. They were first used in treating oncological disorders showing lympholytic activity in mice and regression of lymphoid tumours in human. Later, GCs were routinely used in treating various oncological problems like multiple myeloma, leukaemia and others (Walsh and Avashia, 1992).

The clinical use of GCs as drugs, however, causes adverse side effects on the endocrine system, including diabetes and worsening of hyperglycemia in known diabetics, Cushing's syndrome, adrenal suppression and insufficiency, osteoporosis and dyslipidemia (Lansang and Hustak, 2011). The hyperglycemic inducing activity of GCs could be correlated to impaired peripheral glucose uptake, insulin resistance and stimulation of gluconeogenesis (Macfarlane et al., 2008). The therapeutic potential of GCs in clinical scenario is devaluated not only by the associated side effects, but is also often accompanied with GCs resistance in few regimen. Tackling of these problems requires a comprehensive understanding of the glucocorticoid action mechanism at the level of the glucocorticoid receptor structure, signalling and molecular network, though ligand availability is a crucial factor. The dynamic milieu of glucocorticoid receptor molecular network at various physiological and pathological conditions is a hurdle in GCs therapy.

Glucocorticoids mechanism of action

HPA axis regulation on GCs secretion

The hypothalamus-pituitary-adrenal (HPA) axis is a major part of the neuroendocrine system, managing homeostasis via the regulation of GCs in a feedforward and feedback loops (Sriram et al., 2012). Under stressful conditions, the hypothalamus releases the corticotrophin releasing hormone (CRH) which then binds to the CRF_1 receptor in the anterior pituitary. The binding stimulates the release of adrenocorticotrophic hormone (ACTH), which activates the ACTH receptor on the zona fasciculata of the adrenal cortex, triggering the synthesis and release of GCs from the adrenal glands. The feedforward action of the HPA axis is stabilized by the negative feedback action of the GCs on the axis itself. The negative feedback inhibition on the HPA axis can be elucidated in part by the binding of activated GR on the negative glucocorticoid response element (nGRE) of the CRH gene, thereby downregulating the transcription of CRH gene (Kageyama and Suda, 2009).

CBG and HSD enzymes

GCs, released from the adrenal glands, are transported in the plasma to various sites by a carrier protein called corticosteroid binding globulin (CBG). CBG regulates the bioavailability of GCs to various tissues and thereby regulate the GCs metabolism and action (Petersen et al., 2006). Upon release, GCs are again acted upon by a set of enzymes called the 11β-hydroxysteroid dehydrogenases (11β-HSDs). Hence, these enzymes also regulate the cellular availability of GCs. 11β-HSDs are a set of isoenzymes that catalyse the interconversion of active GCs and their inert 11-keto forms. 11β-HSD type 1 is a reductase converting inert GCs into active ones, whereas 11β-HSD type 2 is a dehydrogenase that inactivates GCs (Sandeep and Walker, 2001).

Glucocorticoid receptor structure and its isoforms

GCs exert their action through intracellular receptors called the glucocorticoid receptor (GR). The GR comprises an N-terminal domain carrying the transactivation region, a central Zn^{2+}-finger DNA binding domain (DBD) and a C-terminal ligand binding domain having ligand-dependent transactivational function (AF-2) (Ford et al., 1997; Black et al., 2001). The DBD consists of two zinc fingers which are accountable for binding to the glucocorticoid response elements (GREs). One of the zinc fingers is involved in receptor dimerization while the other interacts with NF-kB and AP-1 (Reichardt et al., 1998; Tao et al., 2001). The transactivation regions of GR are involved in the regulation of gene transcription by interacting with chromatin remodelling factors and general transcriptional apparatus (GTF). Two such regions have been described. AF-1 positioned at the N-terminus is ligand-independent and constitutive, whereas AF-2 in the C-terminus is GCs-dependent (Godowski et al., 1987; Hollenberg et al., 1988).

GR's transcriptional regulation on gene expression, which depends on the cell milieu, nature of response element binding and interacting factors during

transcription, is further augmented by various GR isoforms produced by alternative splicing and alternative translation of the *NR3C1 gene* (Starr et al., 1996; Lu and Cidlowski, 2004). The human GR gene consists of 9 exons which generate two isoforms on alternative splicing, the GRα which is functionally active and GRβ, a transcriptionally inactive isoform. Both the isoforms have similar amino acid sequence from the N-terminal up to amino acid 727. Beyond this position GRα has 50 more amino acids as compared to the 15 non-homologous amino acids in GRβ. GRα and β mRNA each produces 8 additional daughter isoforms from different initiation sites, resulting in 18 isoforms including the parent isoforms (Cidlowski and Lu, 2006).

Glucocorticoid receptor signalling and molecular network

The unliganded GR is retained in the cytoplasm with attached complex of proteins, that include two sub-units of heat shock proteins 90 (hsp90) acting as molecular chaperones, immunophilins and various other modulatory proteins. This complex is involved in ligand binding and maintaining GR in a state of high affinity for GCs, nucleo-cytoplasmic trafficking and proper folding of the GR. Upon ligand binding, hsp90 dimers dissociate and the hormone-receptor complex (HR-complex) translocates into the nucleus. Nuclear localization of HR-complex is followed by the interaction of the GR with the DNA at specific response elements (Picard et al., 1990; Tai et al., 1992; Truss et al., 1993). Genes responsive to GCs have GREs located at upstream or downstream from the promoter (Winter et al., 1990; Hao et al., 2003). Binding of GRs to GREs regulate the transcription of these genes resulting in induction or repression of such genes (Jantzen et al., 1987; Meyer et al., 1997). The induction is due to interaction of GR with the usual GRE, having sequence of 15 bps of the order GGTACAnnnTGTTCT, while for repression a negative GRE (nGRE) of a variable sequence ATYACnnnTnATCn is required (Truss et al., 1993). The transcriptional regulation of gene expression by GR binding to its response element is generally termed as genomic action or classical GR signalling pathway. GC-mediated action on gene expression and metabolism is further accounted by the crosstalk between GR with the other pathways like MAPK signalling pathway. This describes the non-genomic action or molecular network of GR (Fig. 1). Protein-protein interaction between GR and members of these pathways mutually modulate their genomic actions. GC anti-inflammatory property in a portion could be explained by the interaction of GR with AP-1, NF-kB, JNK, Raf and MSK1, apart from the classical GCs repression on inflammatory genes (Kharwanlang and Sharma, 2011). The interaction of GR with these players could vary at various physiological and disease states as well as under changing external factors.

Glucocorticoid receptor signalling and molecular network role in health and disease

GCs role in physiology and disease is regulated by various parameters ranging from ligand availability to GCs action mechanism. Ligand availability depends upon the activation of the HPA axis, CBG and HSD enzymes, whereas GCs action mechanism depends upon the glucocorticoid receptor signalling and its interaction with other players in the molecular network (Fig. 2). GR interaction with other prominent players of various diseases and physiological conditions is diverse and complicated. Compilation of these players in a single platform is central to further understand the role, therapeutic application and problems of GCs in health and disease.

GR in aging

In senescence, the capacity to adjust to internal as well as external variations declines and results in unstable homeostatic balance. GCs are key regulators in various physiological and biochemical activities. Alterations in GR level with age in various tissues in experimental animals have been reported extensively (Sharma and Timiras, 1987; Ranhotra and Sharma, 2000). A higher level of GR in young mice may be an important reason for the role of GCs in development but a reduction in the level of GR with age may impair metabolic and intermediary functions resulting in unstable homeostatic balance. In addition, the *in vitro* activation of GR was more pronounced in young mice as compared to old ones which may be another factor in down-modulating functional changes in GCs action in senescent animals (Ranhotra and Sharma, 2001). The role of dietary restriction (DR) without malnutrition has been known to influence various physiological processes, delaying aging and extending life span. The mechanism controlling the adaptive response to reduced caloric intake may involve dynamic interplay between signalling pathways that control energy balance, cell proliferation, apoptosis and inflammation (Leaky et al, 1994). It has been earlier reported that DR regulates the level of GR in mice. DR in both young and old mice causes increased level of GR

with no change in the affinity of GCs for GRs (Dutta and Sharma, 2004). Downstream in GR signalling, a higher *in vitro* GR activation is also seen in the liver of older mice. GR level and activation alteration in older mice during DR may modulate GCs action during aging for better adaption to metabolic changes (Dutta and Sharma, 2003; 2004; Sharma and Dutta, 2006). Regulation of GR activation may also play an important role to modulate GCs action. *In vitro* studies have shown that polyunsaturated fatty acids (PUFA) modulate heat activation of both hepatic and renal GR. Both linoleic and arachidonic acids inhibit activation of GR in both young and old mice; however, the degree of inhibition is greater in young as compared to old mice. Modulators like PUFA showing inhibitory effect on GR activation and DNA binding could be used to downregulate GR signalling pathway in unwanted physiological and pathological conditions (Ranhotra and Sharma, 2001).

GR in Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder associated with old age, characterised by memory loss and cognitive weakening. Pathologically, AD is characterised with amyloid β (Aβ) plaques and neurofibrillary tangles leading to synaptic loss and neuronal cell death (Ball et al., 1985). In relation with the GCs signalling pathway, HPA axis dysfunction and high circulating level of GCs are associated with AD (Popp et al., 2009). GCs may mediate a number of events in the genesis of AD. In mouse model of AD, GCs were found to increase the Aβ formation by increasing the level of amyloid precursor protein (APP) and β-APP cleaving enzyme. The underlying mechanism of GCs-induced AD pathological condition points to a plausible role of GR in the pathophysiology of AD. In the same study, it was found that mifiprestone, a GR antagonist, blocked the GCs-mediated enhancement of Aβ, whereas spironolactone, a mineralocorticoid receptor antagonist, was found to have a frail inhibition. Hence, the modulation of GCs on Aβ is mediated by specific binding to GR. The presence of GRE within the promoter of APP gene and GCs-mediated enhancement on APP mRNA, demonstrates a mechanism in which GCs increase Aβ level by GR transactivation of APP (Green et al., 2006).

GR in diabetes

GCs, as the name implies, are associated with glucose metabolism. GCs up-regulate gluconeogenesis by activating expression of key gluconeogenic enzymes like PEPCK, TAT and glucose-6-phosphatase. Prolonged usage of GCs as therapeutic agents leads to diabetes or adverse hyperglycemia in known diabetes. Glucocorticoid receptor plays an important role in the induction of hyperglycemia by GR binding to GRE of these gluconeogenic enzymes (Clark and Lasa, 2003; Beck et al., 2009). Further, in cre-transgenic mice, deletion of GR in hepatocytes confirms the requirement of GR for GCs-induced hyperglycemic activity. Mutant mice showed reduced glucose level on starvation as compared to normal mice due to inactive gluconeogenic activity. In addition, streptozotocin-treated mice showed hyperglycemic activity whereas deletion of GR ameliorates streptozotocin induction of diabetes (Opherk et al., 2004). Earlier, a reduction in activation of hepatic GR has been reported in streptozotocin-induced diabetic mice (Ranhotra and Sharma, 2000).

GR in depression

Depressive disorder is often associated with hypercortisolemia and dysfunctional HPA axis. Negative feedback on the HPA axis is impaired resulting in hypersecretion of GCs. The decrease in the level of GR in the lymphocytes of depressed patients may be one of the reasons for hypercortisolemia (Whalley et al., 1986). Decreased GR may down-regulate GCs feedback inhibition on HPA axis by decreased GR binding to nGRE of CRH (Kageyama and Suda, 2009). In suicide victims, reduced level of GR and FKBP5 (co-chaperone of hsp90) is seen in the amygdala. The alteration of GR and FKBP5 may have resulted in abnormal GR signalling, HPA axis dysfunction and emotional response (Pérez-Ortiz et al., 2012). Interestingly, antidepressant drug enhances GCs-feedback inhibition on the HPA axis. The molecular mechanism underlying this effect is due to GR translocation into the nucleus and GR-mediated gene modulation, probably by binding to nGRE of CRF. A drug possessing such ability is desipramine and its pharmacological property could pave way for a new generation of drugs (Pariante et al., 1997).

GR in cancer

GCs are clinically used in the treatment of oncological problems. The molecular mechanism underlying the anti-oncogenic property of GCs may be related to the molecular interaction between GR and NF-kB. NF-kB is a transcription factor that plays a significant role in regulating genes involve in development and progression of cancer. Abnormal or constitutive expression

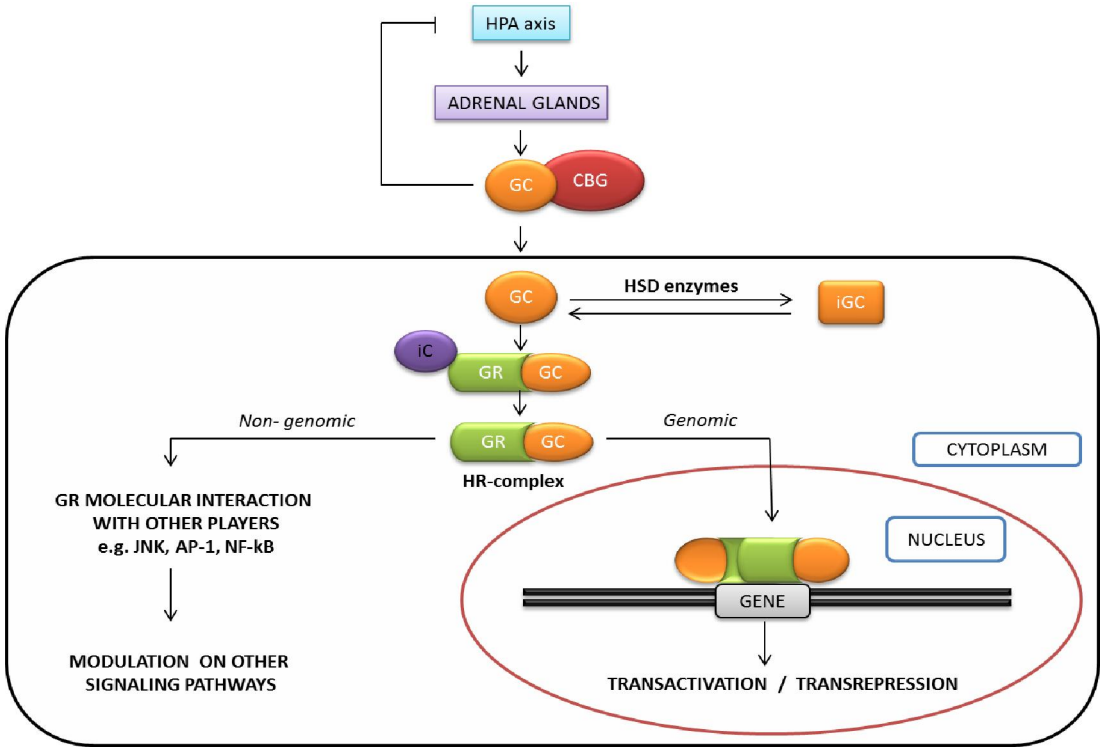

Fig. 1. Schematic diagram showing the regulation of glucocorticoids (GCs) secretion by HPA axis, GCs bioavailability by HSD enzymes, glucocorticoid receptor (GR) signalling pathway (genomic) and molecular interaction (non-genomic) with other signalling pathways. iGC= inert GCs; iC= inhibitory complex, HSD=11β-hydroxysteroid dehydrogenases.

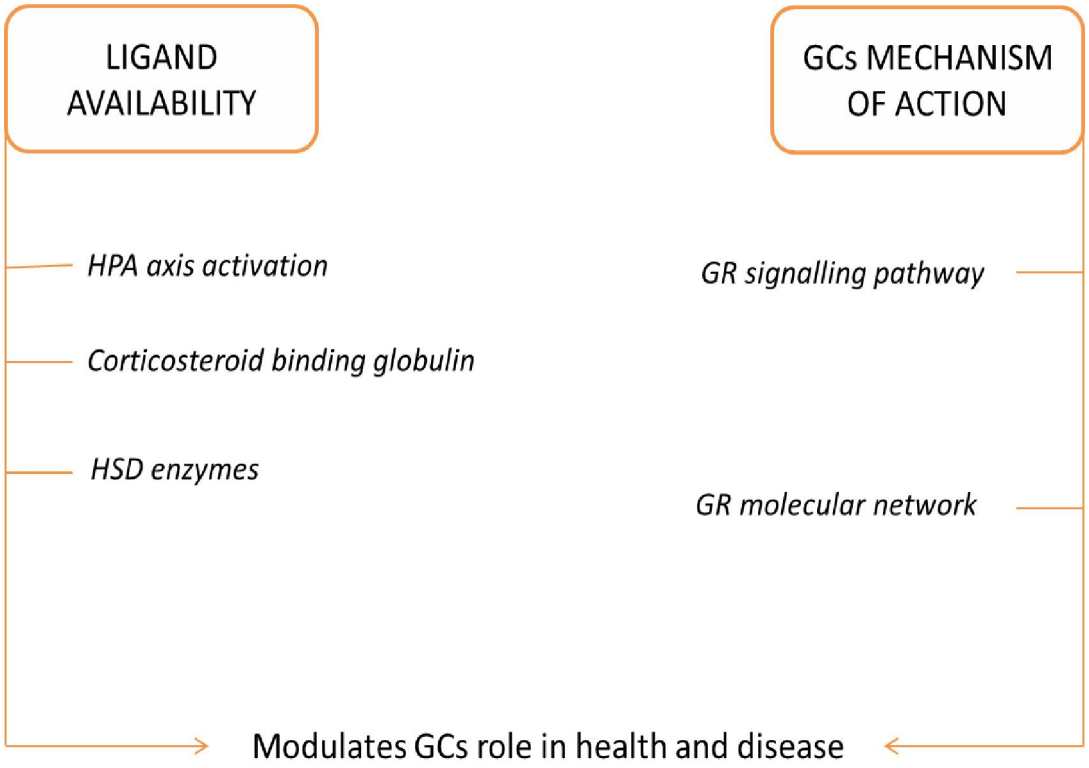

Fig. 2. GCs role in health and disease as therapeutic agents is regulated by ligand availability and GCs mechanism of action.

GCs Associated Problems	Underlying Causes	Possible Theraputic Design
Unwanted GCs effects in other tissues/cells	Delimited ligand availability	Regulating HSD enzymes and using tissue specific agonist/antagonist
Side effects	GR transactivation and transrepression	Modulators having dissociative property, e.g, cpd A
GCs resistance	Altered gene expression of factors interacting with GR	Modulators regulating expression of these factors, e.g, theophylline on HDAC.
Decreased homeostasis during Aging	Decreased GR level	Dietary restriction without malnutrition

Fig. 3. GCs-associated therapeutic problems, their underlying causes and possible therapeutic designs.

of NF-kB is detected in many human malignancies (Dolcet et al., 2005). However, in a few cases the anticancer property of GCs is lost, which may be due to resistance against GCs. This resistance may be due to GR loss of function or GR decreased expression, thereby decreasing the protein-protein interaction and down-regulation of GR on NF-kB (Schlossmacher et al., 2011). In addition, it is reported that GR repression on NF-kB mediated gene expression involve a third partner which is a tumour suppressor molecule named p53. Downregulation of p53 could result in the loss of GCs anti-oncogenic activity. p53 does not alter NF-kB or GR upstream signalling; however, it modulates GR-mediated transcription. The possible mechanism put forward was that p53, NF-kB and GR interact collectively and block the interaction of NF-kB with its response element (Murphy et al., 2011).

GR in osteoporosis

Long term use of GCs leads to many side effects including osteoporosis. GCs-induced osteoporosis (GIO) may be due to GCs' apoptotic activity in osteoblasts, osteocytes and possibly suppression of their differentiation (O'Brien et al., 2004). GCs' downregulation of genes involved in bone formation such as *Col1a1* and *Runx2,* mediates suppression of bone formation (Canalis et al., 2007). Rauch et al. (2010) reported that GIO requires a monomeric GR in attenuating osteoblast differentiation. Using cre-transgenic mice, these authors found that at pharmacologic dose GCs-mediated osteoporosis requires a functional monomeric GR whereas GR dimerization is dispensable. Further, in GIO downregulation of IL-11, a cytokine associated with osteoblastogenesis is seen in GR^{dim} mice. Chromatin immunoprecipitation of GR resulted in amplification of a DNA element having Il-11 gene in which its promoter lies adjacent to two AP-1 binding sites. Hence, protein-protein interaction of monomeric GR with AP-1 rather than GR transrepressive activity could be the plausible underlying molecular mechanism of GCs-mediated osteoporosis and inhibition of IL-11 (Rauch et al., 2010).

Future perspective of GCs therapy in relation to GR

GCs therapeutic application is limited by various side effects and resistance in some regimen. However, GCs appear to be indispensable in current clinical scenario and its usage in future will probably escalate owing to its diverse role in physiology and medicine. Thus, searching for the mechanism underlying the GCs-associated problems requires utmost attention. Since GCs effect is regulated by ligand availability, GR signalling and molecular

network, a technology that could fine-tune these parameters but keeping the physiological conditions in homeostasis will be of a huge advantage to clinical science. Currently, antagonists with tissue selective pharmacology are being engineered, e.g., hepatic-specific antagonist conjugated-bile acid could have reduced systemic effect. In addition, tissue-specific inhibitors of HSD enzymes possibly will be useful in regulating cell-specific bioavailability of GCs. Such a technology will further augment the tissue-specific agonist /antagonist property (von Geldern et al., 2004). However, achieving tissue- or cell-specific GCs-mediated action would solve only a portion of the problems. In the cell, GCs have diverse roles mediated by GR signalling and molecular network. In GR signalling, molecules that could dissociate GR transactivation from transrepression or reversibly could potentiate GCs therapeutic action from undesirable effects (Fig. 3). Compound A (cpd A), a non-steroid plant-derived phenyl-aziridine precursor, mediates gene inhibitory effect by activating GR but does not heighten genes containing GRE (De Bosscher et al., 2005). Similarly, modulators that could separate classical GR signalling from GR molecular network could be another good prospective.

GR molecular network mediated by GR interaction with players like JNK, AP-1, NF-kB, MSK-1, Raf, and p53 in various physiological and pathological states has a great potential to identify GCs' therapeutic problems. For example, anti-oncogenic property of GCs is mediated by GR downregulation of NF-kB in the presence of p53. Resistance to GCs in some oncological problems could be due to reduced p53 expression though GR functions normally. Thus, a modulator that could increase p53 could overcome GCs' resistance. For instance, GCs therapy in COPD accompanied with smoking reduces GCs effect due to reduced histone deacetylase (HDAC) expression and occupancy on pro-inflammatory genes, though GR is bounded perfectly. However, treatment with theophylline increases HDAC level and reverse GCs resistance in COPD (Cosio et al., 2004). In the opposite way, a modulator that could reduce GR interaction with AP-1 by reduction in AP-1 expression could lessen osteoporosis during GCs therapy. Balancing the molecular network of GR at homeostatic level in designing such modulators is important because destabilizing the network could deviate it in any direction and result in undesired effects.

Conclusion

In conclusion, developing new smart technologies in understanding GR's role in other pathological models, understanding the relationship of GR with more signalling pathways, and designing drugs that specifically modulate this interaction but keeps the network intact requires serious consideration. GCs' therapeutic application in future will require holistic approach, by keeping into consideration the factors regulating ligand availability and molecular mechanism of action of GR in various physiological and pathological conditions.

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