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STEROIDS AND THEIR MECHANISMS OF ACTION*

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When in 1949 Hench and colleagues first used corticosteroids in the treatment of rheumatoid arthritis,[1] they opened the door to a new era in the management of inflammatory diseases, not only of the joints but also of the airways and skin. The first successful use of topical corticosteroids in inflammatory skin disease was reported by Sulzberger and Witten in 1952.[2] Their paper is often quoted as showing that the agent (Compound F, hydrocortisone acetate, in a 2.5 per cent ointment base) was highly successful. In fact, of the 19 treated patients, only 2 were described as being 'much better' and 6 as 'slightly better'. The patients were however, a difficult group, all refractory to previous treatment and 8 suffering from skin diseases that we now know would be very unlikely to respond to 2.5 per cent hydrocortisone.

This review considers primarily the topical use of corticosteroids in the management of inflammatory skin disease. Nonetheless, much of what is written also applies to their mechanism of action in the management of malignant and auto immune disease. There can be no doubt that their introduction into therapeutics was one of the most dramatic therapeutic advances of the post-war years, and for which Philip Hench rightly won the Nobel prize for medicine.

Early studies provided a clue to one of the principal factors which determine the topical activity of corticosteroids. One investigation of the effect of topical application of Compound E (cortisone) in inflammatory skin disease had found it to be inactive;[3] this was a clue to the relevance of the pharmacology of a glucocorticoid to its potency when applied topically.

The introduction and subsequent use of steroids in therapeutics was almost entirely empirical. If it worked, then it was used. The decade which followed the first descriptions of the therapeutic value of topical hydrocortisone was marked by the large number of synthetic corticosteroids which were synthesised. The development of steroids according to a rational understanding of various aspects of their activity soon followed. The first major breakthrough was the development of a reliable assay for their potency. The introduction of the vasoconstrictor assay (VCA) by Mackenzie and Stoughton allowed for steroids to be ranked according to their ability to induce blanching when applied to human skin.[4] Importantly, it became apparent that the results of this assay correlated well with the potency of steroids in clinical use.[5] The VCA remains the best screening method we have for assessing the likely anti-inflammatory potency of topical steroids. While the VCA has its critics,[6] nonetheless, it has provided a very useful and reproducible basis on which to make some of our clinical decisions.

In a recent review, Stoughton has emphasised how the correlation between the vasoconstrictor potency of a topical steroid and its clinical activity allows us to answer many of the questions which are of most relevance to its use in clinical practice.[7] For example, does the vehicle affect the potency? Does it matter whether the patient applies a thick coating of cream or ointment or a thin one? Does dilution of a topical steroid necessarily result in dilution of its potency? Does the vasoconstrictor assay correlate with the results of other methods of assessing the anti-inflammatory potency of a topical steroid?

Secondly, an understanding of structure activity relationships of topically active steroids emerged, to the extent that a new steroid molecule could be designed with reasonable certainty that it would have high or low potency.[8]

But neither of these advances allowed us to separate the desirable anti-inflammatory effects of steroids from their undesirable local and systemic side-effects.[9] In general, the more potent a steroid, the more likely it was to induce cutaneous atrophy and to inhibit the production of endogenous cortisol by suppressing the hypothalamic-pituitary-adrenal (HPA) axis. The apparent inevitability of this association between desirable and undesirable effects is one of the main reasons for our reluctance to use potent topical steroids in children with atopic eczema (although paradoxically, we are generally happy to use them long-term in treatment of atopic airways disease in children), and in medium-term or long-term treatment of adults with inflammatory skin disease. Until now, the avoidance of the unwanted side-effects of topical steroid therapy has involved manipulation of the methods of administration or the vehicle in which they are applied. The most recent advances in understanding how steroids work promise to change this.

Thirdly, and most importantly, the steroid receptor was described.[10] With a gradually improving understanding of the molecular mechanism of action of steroids comes the opportunity to design steroids that do what we want them to do, and not do what we do not want them to do; to separate the topical anti-inflammatory effects from systemic and topical unwanted effects. The purpose of this review is to describe what we now understand about how steroids work, and discuss how this allows us the possibility of improving those for topical application.

STEROID RECEPTORS

Discovery. Glucocorticoid receptors were first described in rat thymocytes in the late 1960s.[11] It was soon shown that they shared several properties with receptors for other steroid hormones.[12] In particular, that once a steroid bound to its receptor, the steroid-receptor complexes thus formed were rapidly transported from the cytoplasm where it seemed the receptors normally existed, to the nucleus of the cell.[13] The effects of these steroid-receptor complexes on the production of protein by the cell can be blocked by inhibitors of RNA synthesis, and we have known since the 1950s that steroids induce the synthesis of hepatic enzymes.[14] Even at this early stage therefore, it seemed likely that steroids were involved in regulating the transcription of specific genes.

Briefly, the steroid-receptor complex binds to a specific 'glucocorticoid responsive element (GRE)' on DNA and is then able to stimulate or inhibit the production of mRNA for one of a variety of gene products, depending on the gene adjacent to the GRE.[15,16] Although steroids may on occasion act through other mechanisms, the steroid-receptor/DNA interaction described here can apply to all actions that steroids have, and no other convincing alternative explanation of their actions exists.

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Structure. Early experiments were dogged by artefacts of processing, and there were conflicting reports of the size of the receptor. The current view is that the receptor comprises a 100-kDalton receptor protein that contains a steroid binding domain at the carboxy terminus. An adjacent domain binds to DNA (the DNA-binding domain). The development of this model has been greatly facilitated by studies of the products of enzymatic cleavage of the receptor, but also by the cloning of cDNA for the steroid binding proteins of the receptor which consists of about 795 amino acids. The steroid-binding domain resides in amino acids 541–795, while the DNA-binding domain occupies amino acids 440–505. The 440 amino acids adjacent to the amino terminus of the receptor are described as the 'immunogenic domain'.

In vivo however, the receptor comprises a 300-kD structure, and it has become apparent that two 90kD heat-shock proteins (Hsp90), and a 59kD protein (p59) are integrated.[17] When a steroid enters the cell and binds to a receptor, it causes the loss of these two heat shock proteins and the p59 protein, with the consequence that the DNA-binding domain is then exposed. This allows the steroid-receptor complex to enter the cell nucleus, bind to DNA, and then inhibit or stimulate the production of specific gene products at the transcriptional level.

Effects of glucocorticoids on nuclear DNA. The phenomena which immediately follow the binding of a glucocorticoid to the receptor are known as 'activation' of the receptor,[18] of which one of the principal features appears to be the loss of the two heat-shock proteins as well as the p59 protein. Consequent conformational change exposes the DNA-binding domain of the receptor, and the drug-receptor complex is now capable of binding to DNA, to specific regions known as 'glucocorticoid-responsive elements (GREs)'.[19] These are found close to the transcription initiation sites for the relevant genes. Once bound, the steroid-receptor complex has the capacity either to enhance the transcription of mRNA from the adjacent gene or to inhibit transcription. The precise mechanism by which glucocorticoid-receptor complexes accomplish this is a matter of intense research. Identification of this would then make possible the design of glucocorticoids which act to enhance or inhibit transcription of a limited number of genes.

The family of steroid receptors. This article addresses the question of the mechanism of the anti-inflammatory action of the glucocorticoids. However, glucocorticoids are members of a much wider steroid family, which includes oestrogen, androgen, progesterone and mineralocorticoid hormones, and the sterol 1,25 dihydroxyvitamin D_3. The activity of each of these members of the steroid family is mediated by their effect at their own specific receptor. For example, glucocorticoids are unable to exert an anti-inflammatory effect except in the presence of glucocorticoid receptors.

Steroids used for their anti-inflammatory effect may also have unwanted effects mediated by their activity at other steroid receptors. For example the fluid-retaining and hypertensive effects of systemic steroids such as prednisolone are mediated through their effect at mineralocorticoid receptors. The acnegenic affects of glucocorticoids, and their ability to cause hirsutism at the site of application seem likely to be related to their effect at the androgen receptor. It seems likely that many of the unwanted cutaneous effects of topical steroids are mediated through their effects at receptors other than the glucocorticoid receptor.

The ability to manipulate the coding sequences for steroid receptors have produced some startling revelations concerning its specificity. Perhaps the most picturesque of these is the work of Green and Chambon.[17] They produced a receptor which had the DNA binding domain of a glucocorticoid receptor, but the steroid binding domain of an oestrogen receptor. When stimulated by an oestrogen, this chimeric receptor had the effects of a glucocorticoid!

It is possible that a glucocorticoid, specific for the glucocorticoid receptor with little or no activity at other steroid receptors, will have a reduced propensity for local side-effects. Research on new anti-inflammatory steroids should aim at glucocorticoids which are free of affinity for other members of the steroid receptor family.

Affinity. There are a number of other implications that this understanding of steroid receptors has for future developments in topical steroids. For example, with the ability to synthesise steroid receptors, we can now study the pharmacology of the association between a therapeutic steroid and its receptor. This may seem at first sight to have little consequence for clinical practice. But glucocorticoids with a prolonged receptor occupation time seem likely to have a prolonged action on transcription. This should allow for increased dosing intervals, a particularly desirable quality in topical steroids, where the inconvenience of frequent application of ointment or cream is a major disincentive to good compliance.

Steroids with a high affinity for the glucocorticoid receptor are, in general those with a greater potency.[20] One important property which predicts high affinity is the degree of lipid solubility (lipophilicity) of the steroid molecule. Highly lipophilic steroid molecules, in general, have a high affinity for the steroid receptor. This is likely to be because of their ability to enter cells more readily, since the movement of steroids across cell membranes seems largely to be by passive diffusion. However, the steroid receptor is also associated in the cell with a modulating phosphoglyceride,[21] and it may be that the more lipophilic steroids interact differently with this steroid receptor modulator than less lipophilic ones. Furthermore, our ability to study the action of a steroid at its receptor also means that we can study the effects of its metabolites. For example, many glucocorticoids have metabolites which also have some activity at various steroid receptors. This presents a genuine problem. When we study the vasoconstrictor potency of a topical steroid, it is the parent compound whose activity we are examining. This may not give an accurate view of its actual potency if it happens to have active metabolites. While the VCA is, as stated, generally a reliable guide to the clinical activity of a steroid, there are occasional exceptions to this.[6] An interesting approach to this problem has been to explore the concept of the 'soft drug' or ante-drug.[22] These are compounds which show high activity at the site of application, but which are rapidly metabolised to inactive metabolites and therefore have reduced systemic activity.[23] This concept of reducing the propensity of a steroid to have effects at sites other than the site of application may be thought of as the 'hit and run' concept, and can be applied to the anti-inflammatory effects of steroids in many areas of therapeutics.

In this context, sulphur-containing steroids hold particular promise, and thio-containing steroids have been investigated. Several of these show potent anti-inflammatory activity coupled with low systemic activity. The sulphur group,

provided it is readily available on the molecule, can be easily converted to sulphoxides or sulphones and excreted as such. The presence of a sulphur group in the molecule may also imply an affinity for the skin; for example, some sulphur-containing antibiotics preferentially localise to the skin after topical application.[24] Equally, there are a number of steroids whose metabolites have more activity than the parent compound. Cortisone is converted to hydrocortisone before exerting its anti-inflammatory effects. The lack of anti-inflammatory activity of topical cortisone, quoted earlier, would have been predictable had the investigators known this.

CONCLUSIONS

New understanding of the molecular mechanisms of steroid activity has made it possible to account for all their anti-inflammatory actions with a unified theory. It has now become possible to investigate candidate molecules for the desirable property of glucocorticoid receptor affinity, and eliminate as far as possible molecules with affinity for other steroid receptors. Similarly, the metabolism of potential new steroids can easily be investigated with a view to producing a 'hit and run' topical steroid. The desire to separate the anti-inflammatory potency of a steroid from its local and systemic side effects has become more than the pious hope it has sometimes been. An example of a topical steroid already used in treatment of inflammatory skin and airways disease which embodies many of these concepts is fluticasone propionate, a thio-steroid which appears to have very little propensity for systemic absorption.[25] It is metabolised completely and rapidly to a metabolite with negligible activity at the steroid receptor.[26] It has particularly high affinity for the glucocorticoid receptor with very little if any activity at other steroid receptors. The development of topical steroids should begin to take advantage of our increasing knowledge of how steroids actually affect gene transcription. Perhaps in the near future we can expect to see steroids with the favourable pharmacological profile of fluticasone propionate with the ability selectively to enhance or inhibit transcription of specific genes. Certainly our new knowledge concerning how steroids work allows real hope that these may be medicines of the future at least as much as they have been medicines of the past.

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