ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 4, 2016

Online - 2455-3891
Print - 0974-2441

Review Article

ACNE VULGARIS: A REVIEW ON PATHOPHYSIOLOGY AND TREATMENT

SHYAM BABOO PRASAD*

Department of Pharmacognosy, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab - 144 411, India.
Email: b.shyam2@gmail.com

Received: 15 May 2016, Revised and Accepted: 24 May 2016

ABSTRACT

Acne vulgaris is a disease associated with sebaceous follicle. It starts appearing after the onset of puberty and can extend up to 40-50 years of age. As far as the pathogenesis of acne is concerned, it is not fully understood up till now. Treatment of acne is very frustrating and involves an understanding of etiopathological factors. This review focuses on various factors accountable, pathogenesis, and therapy of acne.

Keywords: Acne, Skin diseases, Acne therapy, *Propionibacterium acnes*, *Acne vulgaris*.

INTRODUCTION

Acne is considered as one of the most widespread skin diseases [1]. When extreme disfiguration occurs, it results in the development of severe consequences among the young people and may result in depression and suicide. *Acne vulgaris* is the second uppermost reason of suicide among skin diseases. When a person suffering from acne is compared with an individual who is not suffering from acne than it is found that the former has higher level of anxiety, more socio inhibition and has more aggressiveness [2].

Acne is an exclusive disease associated with skin occurs when sebaceous glands (SGs) attain special conditions. This disease occurs in both male and female; there is no preference among them, but the course is more severe in males [3].

ETIOLOGY OF ACNE

Factors that can lead to acne are as follow: SG produces more sebum, hypercornification of the sebaceous ducts, colonization of *Propionibacterium acnes* in the pilosebaceous ducts and inflammation.

Severity of acne is connected with seborrhea which is associated with follicular infundibulum. In case of milder acne, there occur hypercornification, hyperkeratinization, and hypodesquamation of keratinocytes of the infundibulum which lead to the production of comedones. In severe acne, infundibulum breaks and releases sebum into the dermis which produces inflammatory responses.

INCREASED ANDROGENS AND SEBUM PRODUCTION

As far production of sebum in the body is concerned, it is produced by the sebocytes dissolution in sebaceous lobules than they are passed to the follicle via sebaceous ducts, and finally, reach to skin surface by means of infundibulum. SG is mainly found in face and truck, i.e., the regions where acne generally forms [4-6].

After the onset of puberty androgen, production increases within the body and SG are the main target organ for the same because they have higher androgen receptor in skin [7,8]. Testosterone is the main androgen responsible for acne. One of its derivative dihydrotestosterone formed in the body by the action of enzyme 5α-reductase (type-1). Type-1 of 5α- reductase is the foremost isotype found in human skin, specifically in SG rich area [9-12]. As sebum content rises in acne patients due to rise androgen level and also regulated by them [13].

METABOLISM OF ANDROGENS IN SEBOCYTES

Sebum production is mainly regulated by dehydroepiandrosterone sulfate (DHEA-S). This androgen has highest sebum concentration and is present in an equal amount in both male and female. DHEA-S is a considered as weak androgen, but the sebocytes have required enzymes to convert it into strong androgens (androstenedione, testosterone, and dihydrotestosterone). Despite there formation within the sebocytes, these androgens can also be re-up taken by the sebocytes. After the reuptake, testosterone and dihydrotestosterone binds to the receptor of cytoplasmic androgen to form complex of androgen and receptor which enters the nucleus via nucleopore and bind to specific gene sequences in the nucleus (Fig. 1) [14].

In most of the patients of seborrhea, despite normal levels of androgens sebum production is high because of increased sensitivity of sebocytes toward androgen (it may be the cause) [15]. Hence, seborrhea and acne can occur both at normal as well as higher levels of androgens [16,17].

Fatty acids

It was hypothesized by Weeks *et al.* that free fatty acid formed by action of lipase enzyme of *P. acnes* on the triglycerides of sebocytes is highly inflammatory and chemotactic. They also showed that inhibition of lipase cause the reduction in the free fatty acid amount in the skin, but it does not suppress the acne because of the formation of pro-inflammatory fraction of lipid by the other mechanisms than the bacterial lipase and are responsible for inflammation in acne [18].

Linoleic acid deficiency

Linoleic acid plays an important role in the creation of intracellular lipid lamellae after incorporation with sphingolipids in follicular epithelium. Linoleic acid deficiency is very important in the etiology of acne as such condition causes impairment in the follicular epithelium barrier which allows the other free fatty acids produced by bacterial lipase activity or/ and by the metabolism of sebocytes to enter the epithelium and cause localized deficiency of essential lipids. Zouboulis showed that linoleic acid can regulate the interleukin (IL)-8 secretions and thus regulate the inflammatory responses [19,20].

Ductal hypercornification

It is known that during the formation of acne infundibulum changes occurs but the mechanism behind the changes yet clearly not known. One of the hypotheses said that local follicular insufficiency of linoleic acid effects IL-1α and androgens which cause apparent early cornification of keratinocytes [21-23].

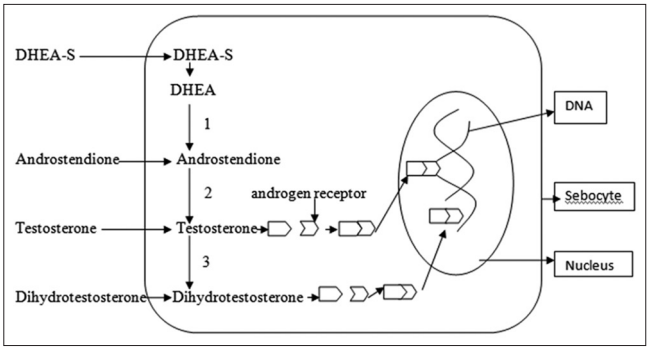

Fig. 1: Metabolism of androgens in sebocyte. DHEA-S: Dehydroepiandrosterone sulfate, DHEA: Dehydroepiandrosterone, 1: 3β-hydroxysteroid dehydrogenase, 2: 17β-hydroxyester dehydrogenase, 3: 5α- reductase. These androgens are formed within as well as reuptake by the sebocyte. They bind to the cytoplasmic androgen receptors to form androgen-receptor complex which enters the nucleus via nucleopore and bind to specific gene sequences in the nucleus. Binding effect is that the reading rate of the particular gene gets altered

Follicular hyperkeratosis

Further prerequisite condition for the development of acne is keratinization of follicular cells which further lead to hyperkeratosis. In normal skin, hair follicle has loosely layered keratinocytes. There regular desquamation occurs, and they are carried to the skin surface by sebum flow. There exist a balance between the newly formed keratinocytes and the desquamated ones. Whereas in acne affected skin, there is increased proliferation of keratinocytes.

Keratinocytes of the hair follicle get linoleic acid from the sebum. Increased flow of sebum means more supply of linoleic acid to the keratinocytes and decline of linoleic acid concentration in sebum and thus results in local follicular linoleic acid deficiency. Further analysis showed that there are also other comedogenic sebum components that contribute toward acne development. These include fatty acid peroxides and squalene peroxides, which are created by the action of ultraviolet radiations on sebum lipid squalene. Androgens are also thought to contribute toward it because antiandrogens are found to reduce comedo formation irrespective of increased sebum production in some patients. Body's inflammatory mediators are also contributing toward it. IL-1a increases the keratinocyte proliferation [24].

Oxygen stress and free radicals

One of the hypotheses also said that in response to the invading microorganism the phagocytes of the body such as neutrophils release reactive oxygen species (ROS) to cause lysis of the invading cell. These ROS are involved in inflammation [25].

Microorganism

Acne is neither a contagious disease nor infectious. Those bacterial species that exist in human skin as residential flora can colonize in the follicular ducts of the SG. Only three form of microorganism thus contributes toward the development of acne lesions. These are: Bacteria (*P. acnes* and *Staphylococcus epidermidus*) and yeasts (*Malassezia furfur*).

However, it is found that acne does not improve after treatment with antifungal drugs. Therefore, yeast cannot be involved in pathogenesis of acne. Staphylococci can also be excluded because during first weeks of treatment in most of the patients it develops resistance [26]. Hence, scientific data mainly concentrated toward *P. acnes*. *P. acnes* is a Gram-positive having characters such as non-motility and pleomorphism. They are rod-shaped cells which cause the fermentation of sugars so as to yield propionic acid as one of their metabolic end products. This bacteria predominantly found in SG rich area of the skin in adults [27]. In the human skin, it exists from the birth till the death of the person [28-34].

P. acnes and its metabolite possibly interact with sebocytes or keratinocytes via cell-mediated immunity and then produce cytokines. These than non-specifically attract the lymphocytes. According to the primary data, both T-helper 1 and T-helper 2 play a very important role in the inflammation process. *P. acnes* is also found to have mitogenic activity. Hence, there are two mechanisms via which *P. acnes* cause lymphocytic activation, i.e., antigen-driven and mitogen-driven [35].

Genetic factors

Predisposition of acne can also occur genetically. Very slight knowledge is obtained about the hereditary mechanisms. Numerous genes are responsible for it. Among them, the main are cyt-P450-1A1 and steroid 21-hydrolase which controls adrenal glands androgen production. People having XYY karyotype shows severe type of acne usually [36].

Immunological factors and inflammation

The development and course of acne are influenced by immunological and inflammatory factors in various ways. Earlier it was thought that inflammation is produced as the result of other factors responsible for acne, especially bacterial metabolites but from the new data, it was found that the patients of acne have a leaning of follicular inflammation from the outset [37]. It is accepted that follicles are surrounded by leukocytes, especially T-lymphocytes. They initiate the comedone formation by the production of IL-1, which is the way to the development of acne. Inflammation triggers sebum production. Leukotriene B4 binds to peroxisome proliferator –activated receptor-α on the sebocyte and thus regulates the lipid metabolism. Further support for the above observation is that there occurs a decline in sebum lipid production when leukotriene antagonist zileuton was used [38].

Pathogenesis of acne

The various factors mentioned in the etiology contribute to the pathogenesis of acne in the following way (Fig. 2).

Seborrhea increase androgen concentration due to genetic factors as well as because of attainment of puberty all ultimately leads to the increased sebum production. On puberty, body's androgen production increases. In the sebocyte, androgens are synthesized as well as reuptake. These androgens than form androgen-receptor complex within the cytoplasm. These than enter nucleus via nucleopore and alter the specific gene sequence and thus affect the reading rate in the result of which sebum production by the sebocyte increases. The sebum thus produced flow through the pilosebaceous ducts and reaches the skin surface. During the flow, this sebum supplies its linoleic acid to the keratinocytes of the hair follicle. Due to this, there occurs local linoleic acid deficiency which leads to the impairment in the follicular barrier. This allows the free fatty acid formed by *P. acnes* by action of its enzyme lipase or by other mechanisms on triglycerides, to enter the follicle. The impairment in the follicular wall can also occur because of oxygen stress or by generation of free radicals by phagocytes in response to invading micro organism. The entered free fatty acids are highly chemotactic and lead to the production of various cytokines such as IL-8 and IL-1α which lead to inflammation and upward regulation of keratinocyte proliferation. This leads to ductal hypercornification and formation of dense horny lamellae. Retention-proliferation hyperkeratosis results because of it. Retention-proliferation hyperkeratosis first form microcomedone, which further grow and convert into comedone and this comedone further develop and form acne [6-36,39].

Treatment of acne

Aim of acne treatment

The main goal of treatment of acne is to stop scarring and minimize the duration of disease. It also focused to decrease the psychological stress that affects at least half of sufferers [40-43]. So, clinical trial evidence of

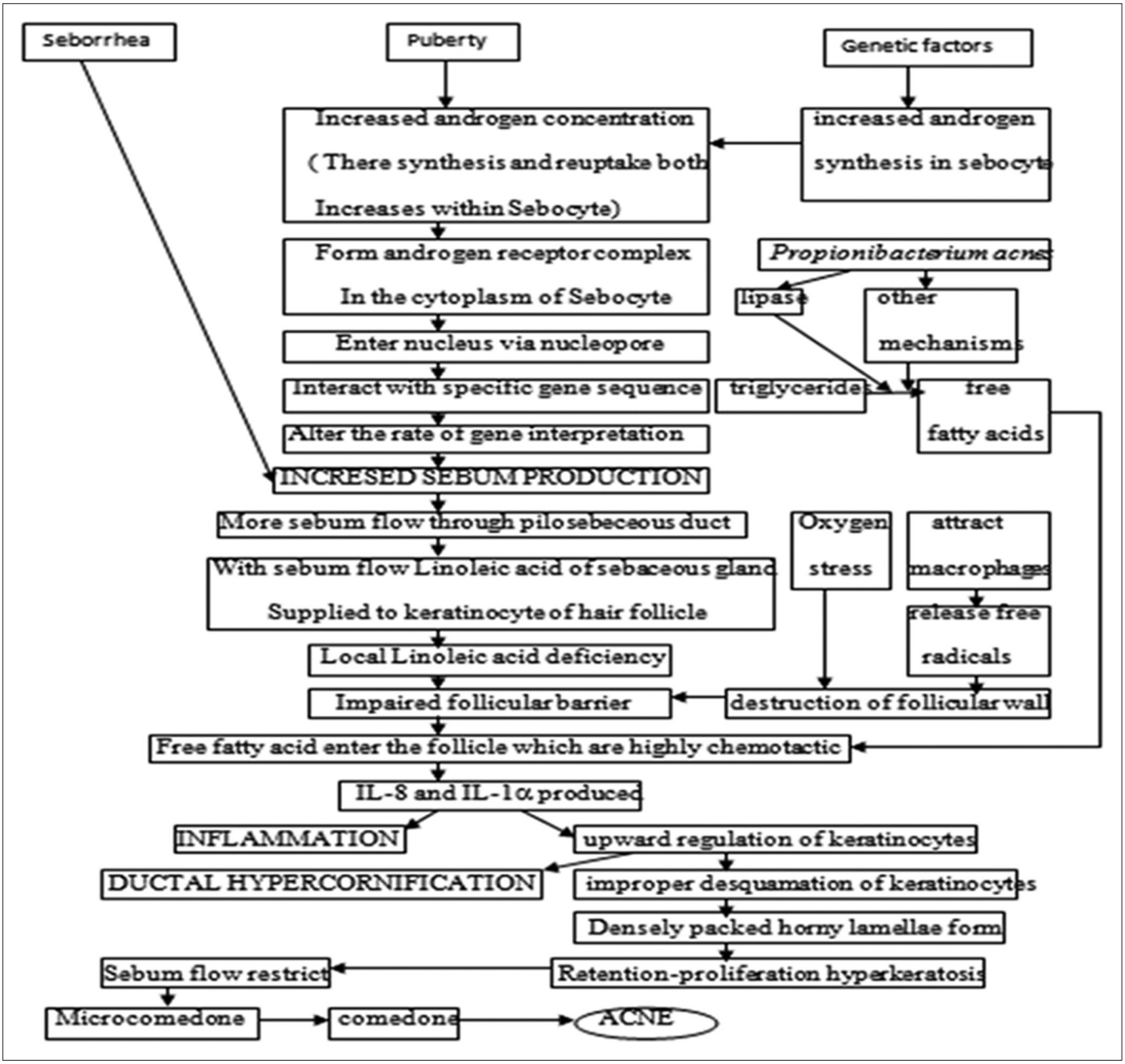

Fig. 2: Pathogenesis of acne

effectiveness of medicament plays important role in the management of acne.

Treatment of different types of acne

Treatment of mild acne

The topical preparation usage is the main way to treat it, and preparations that can be used for the treatment of mild acne are shown in Fig. 3.

The above-mentioned treatments are most popular among the patients, but they require good explanation regarding the use for their good compliance among the patients. Moreover, they are slow beneficial drugs [43].

Benzoyl peroxide

It is a powerful oxidizing agent with keratolytic and antibacterial properties. Benzoyl peroxide does not lead to the induction of any type of alter in the resistance pattern of aerobic bacteria toward antibiotics, but benzoyl peroxide can check such resistance when used along with topical formulation of erythromycin.

It is available in the form of lotions and creams in the market in a concentration of 2.5-10%. They can be used once in a day. There is no available dose-response profile which can show the increase in efficacy with higher doses. The main adverse effects associated with benzoyl peroxide are transient irritation of skin, occasional allergic contact dermatitis and bleaching of clothes. In long-term or in conjunction with oral antibiotics they can be used in the cure of moderate type of *A. vulgaris* [44].

Azelaic acid

It is keratolytic in nature and may lead to change in the composition of free fatty acid of skin surface lipids, and it can significantly reduce the follicular bacterial density, but it can cause local irritation and photosensitization. Its treatment generally limits to 6 months. It is used in a concentration of 20% and can a used twice a day [45].

Topical retinoid formulation

They are valuable for the treatment of mild as well as moderate acne. It can be applied once or twice in a day. Tretinoin is available as cream and lotion. Isotretinoin is used in as gel preparation. Both formulations have comedolytic activity. They have untoward effects such as desquamation, occasional hyperpigmentation or hypopigmentation, erythema, and sensitization of the skin to sunlight. It shows malformation in infants born to women who have used topical retinoids during early pregnancy [46,47].

Topical antibiotics

They are particularly used in the treatment of mild to moderate acne and in acne which shows resistance to benzoyl peroxide. These antibiotics affect the metabolic pathways of *P. acnes*. Topical preparations of clindamycin and erythromycin are similar in terms of efficiency. These topical preparations are suitable for greasy skins because of their alcoholic base. Clindamycin in a lotion base is less irritating to dry or scaly skin and is preferred by women. Topical tetracycline is less effective and leaves a residue that may fluoresce under ultraviolet light. The development of antibiotic resistance in *P. acnes* limits the use of these topical antibiotics now [48].

Treatment for moderate acne

Treatment of moderate type of acne can be done in following ways [48] as shown in Fig. 4.

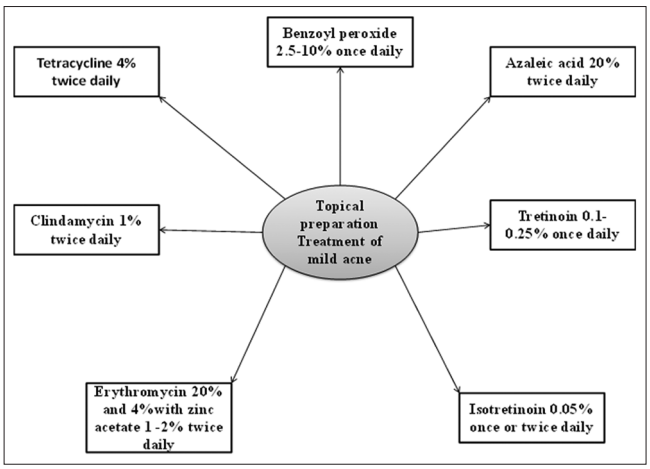

Fig. 3: Treatment of mild acne

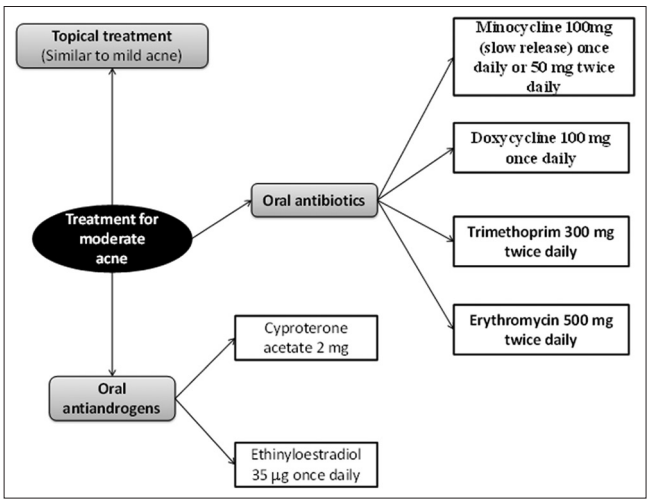

Fig. 4: Treatment of moderate acne

Oral antibiotics

Systemic antibiotics administration remains the main way to treat the disease, and tetracycline remains the first choice for treatment. To make sure satisfactory absorption, oral antibiotics should be ingested half an hour before meal and patients must avoid simultaneous use of iron supplements or milk. Development of resistance by *P. acnes* and *Staphylococcus epidermidis* for erythromycin is the limiting factor for its use in treatment. Minocycline is the main suggested systemic antibiotic for the treatment of acne. There is slight differentiation between minocycline and tetracycline clinically as there is less dietary restriction in case of minocycline. Doxycycline or trimethoprim can be used further as an alternative. Adverse drug reaction associated with oral antibiotics involve gastrointestinal tract disturbance upset, vaginal candidiasis, and hyperpigmentation when elevated dose of minocycline are used [49].

Hormonal therapy

The SGs inflammation results in the formation of acne. There is a few evidence which shows support to any hormonal disturbances in girls with acne, but 46% of women population with acne between the ages of 18 and 32 has little increase in the level of testosterone and also there is inhibition of sex hormone binding globulin [50]. Cure can be done with antiandrogens like cyproterone acetate 2 mg along with ethinyl estradiol 35 mg which is similarly effective as oral tetracycline but duration of treatment is 3-6 months when used alone [51]. Cyproterone acetate (50 or 100 mg) from days 5-15 of the menstrual cycle beside 35 mg of ethinyl estradiol from days 5-26 had reported to show more beneficial effects [52].

Combined contraceptive pills may aggravate acne, for example, that contains norethisterone or levonorgestrel, but this is not the same for the entire cases, for example, those which contain desogestrel or gestodene [53].

Treatment of severe acne

Treatment of severe type of acne can be done in following ways [48] as shown in Fig. 5.

Isotretinoin

Isotretinoin decreases activity of SG, which may lead to decrease in sebum production that results into a noteworthy decline in the population of *P. acnes*. Retinol concentration is increased in the cutaneous region and may reflect a metabolic interference with endogenous vitamin A [54-56].

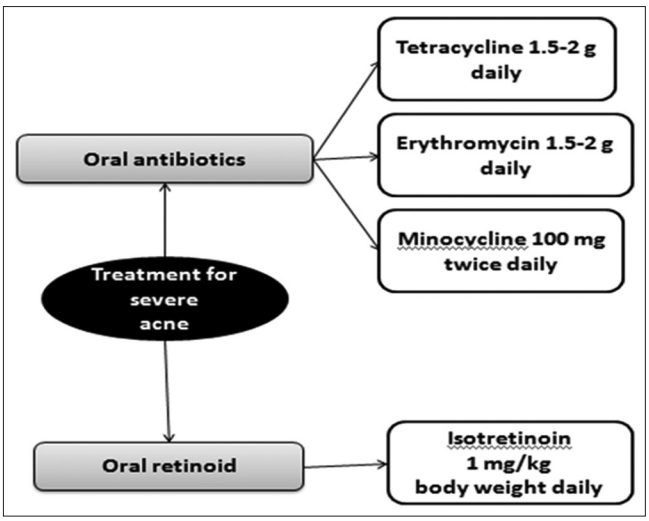

Fig. 5: Treatment of acne

Most of the patients require a therapy of 4 months at least but in 15% of the cases even more time period is required [57]. Out of all, 40% of patients are cured and need no further treatment, and a further 21% need topical treatment alone. In left, behind 39% relapse occurs within 3 years. Out of all, 16% require oral antibiotics and 23% require further courses of isotretinoin [58]. Irrespective to high cost of isotretinoin it is comparatively cheap to treat moderate or severe type of acne with it as compared to other available antibiotics [59].

CONCLUSION

On the basis of above literature, it is clear that the pathophysiology as well as treatment of acne is not simple. The major problem associated with available therapeutics is the adverse effect and resistance. Moreover, treatment criteria can vary as per the situation.

REFERENCES

1. Kaur D, Prasad SB. Anti acne activity of acetone extract of *Plumbago indica* root. Asian J Pharm Clin Res 2016;9(2):285-7.
2. Gupta MA, Gupta AK. Depression and suicidal ideation in dermatology patients with acne, alopecia areata, atopic dermatitis and psoriasis. Br J Dermatol 1998;139(5):846-50.
3. Cunliffe WJ. Natural history of acne. In: Cunliffe WJ, editor. Acne. London: Martin Dunitz; 1989. p. 2-10.
4. Gollnick HP, Zouboulis CC, Akamatsu H, Kurokawa I, Schulte A. Pathogenesis and pathogenesis related treatment of acne. J Dermatol 1991;18(9):489-99.
5. Harris HH, Downing DT, Stewart ME, Strauss JS. Sustainable rates of sebum secretion in acne patients and matched normal control subjects. J Am Acad Dermatol 1983;8(2):200-3.
6. Blauer M, Vaalasti A, Pauli SL, Ylikomi T, Joensuu T, Tuohimaa P. Location of androgen receptor in human skin. J Invest Dermatol 1991;97(2):264-8.
7. Choudhry R, Hodgins MB, Van der Kwast TH, Brinkmann AO, Boersma WJ. Localization of androgen receptors in human skin by immunohistochemistry: Implications for the hormonal regulation of hair growth, sebaceous glands and sweat glands. J Endocrinol 1992;133(3):467-75.
8. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5a-reductase isozyme expression. J Clin Invest 1993;92(2):903-10.
9. Luu-The V, Sugimoto Y, Puy L, Labrie Y, Lopez-Solache I, Singh M, *et al.* Characterization, expression, and immunohistochemical localization of 5-a-reductase in human skin. J Invest Dermatol 1994;102(2):221-6.
10. Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K, Hagari S. Activity of the type I 5 a-reductase exhibits regional differences in isolated sebaceous glands and whole skin. J Invest Dermatol 1995;105(2):209-14.
11. Chen W, Zouboulis CC, Orfanos CE. The 5 areductase system and its inhibitors: Recent development and its perspective in treating androgen-dependent skin disorders. Dermatology 1996;193(3):177-84.
12. Zouboulis CC, Seltmann H, Hiroi N, Chen W, Young M, Oeff M, *et al.* Corticotropin-releasing hormone: An autocrine hormone that promotes lipogenesis in human sebocytes. Proc Natl Acad Sci U S A 2002;99(10):7148-53.
13. Orth DN, Kovacs WJ. The adrenal cortex. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, editors. Williams Textbook of Endocrinology. Philadelphia, PA: WB. Saunders; 1998. p. 517-664.
14. Zouboulis CC, Xia L, Akamatsu H, Seltmann H, Fritsch M, Hornemann S, *et al.* The human sebocyte culture model provides new insights into development and management of seborrhoea and acne. Dermatology 1998;196(1):21-31.
15. Leyden J, Bergfeld W, Drake L, Dunlap F, Goldman MP, Gottlieb AB, *et al.* A systemic type I 5 alpha-reductase inhibitor is ineffective in the treatment of acne vulgaris. J Am Acad Dermatol 2004;50(3):443-7.
16. Placzek M, Arnold B, Schmidt H, Gaube S, Keller E, Plewig G, *et al.* Elevated 17-hydroxyprogesterone serum values in male patients with acne. J Am Acad Dermatol 2005;53(6):955-8.
17. Zouboulis CC, Böhm M. Neuroendocrine regulation of sebocytes - A pathogenetic link between stress and acne. Exp Dermatol 2004;13 Suppl 4:31-5.
18. Weeks JG, McCarty L, Black T, Fulton JE Jr. The inability of a bacterial lipase inhibitor to control acne vulgaris. J Invest Dermatol 1977;69(2):236-43.
19. Downing DT, Stewart ME, Wertz PW, Strauss JS. Essential fatty acids and acne. J Am Acad Dermatol 1986;14:221-5.
20. Zouboulis C. Update on sebaceous gland physiology: Induction of inflammation and its clinical implications. JEADV 2001;15 Suppl 2:102.
21. Guy R, Kealey T. Modelling the infundibulum in acne. Dermatology 1998;196(1):32-7.
22. Ingham E, Eady EA, Goodwin CE, Cove JH, Cunliffe WJ. Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. J Invest Dermatol 1992;98(6):895-901.
23. Eller MS, Yaar M, Ostrom K, Harkness DD, Gilchrest BA. A role for interleukin-1 in epidermal differentiation: Regulation by expression of functional versus decoy receptors. J Cell Sci 1995;108:2741-6.
24. Akamatsu H, Horio T. The possible role of reactive oxygen species generated by neutrophils in mediating acne inflammation. Dermatology 1998;196(1):82-5.
25. Marples RR, McGinley KJ. Corynebacterium acnes and other anaerobic diphtheroids from human skin. J Med Microbiol 1974;7(3):349-57.
26. Holland KT. Microbiology of acne. In: Cunliffe WJ, editor. Acne. London: Martin Dunitz; 1989. p. 178-210.
27. Leyden JJ, McGinley KJ, Mills OH, Kligman AM. Age-related changes in the resident bacterial flora of the human face. J Invest Dermatol 1975;65(4):379-81.
28. McGinley KJ, Webster GF, Ruggieri MR, Leyden JJ. Regional variations in density of cutaneous propionibacteria: Correlation of *Propionibacterium acnes* populations with sebaceous secretion. J Clin Microbiol 1980;12(5):672-5.
29. Eady EA, Ingham E. Propionibacterium acnes – Friend or foe. Rev Med Microbiol 1994;5(3):163-73.
30. Webster GF, Leyden JJ, Musson RA, Douglas SD. Susceptibility of *Propionibacterium acnes* to killing and degradation by human neutrophils and monocytes *in vitro*. Infect Immun 1985;49(1):116-21.
31. Kirschbaum JO, Kligman AM. The pathogenic role of *Corynebacterium acnes* in acne vulgaris. Arch Dermatol 1963;88:832-3.
32. Eady EA, Cove JH, Holland KT, Cunliffe WJ. Erythromycin resistant propionibacteria in antibiotic treated acne patients: Association with therapeutic failure. Br J Dermatol 1989;121(1):51-7.
33. Holland KT, Holland DB, Cunliffe WJ, Cutcliffe AG. Detection of *Propionibacterium acnes* polypeptides which have stimulated an immune response in acne patients but not in normal individuals. Exp Dermatol 1993;2(1):12-6.
34. Layton AM, Morris C, Cunliffe WJ, Ingham E. Immunohistochemical investigation of evolving inflammation in lesions of acne vulgaris. Exp Dermatol 1998;7(4):191-7.
35. Herane MI, Ando I. Acne in infancy and acne genetics. Dermatology 2003;206(1):24-8.
36. Jeremy AH, Holland DB, Roberts SG, Thomson KF, Cunliffe WJ. Inflammatory events are involved in acne lesion initiation. J Invest Dermatol 2003;121(1):20-7.
37. Zouboulis CC, Nestoris S, Adler YD, Orth M, Orfanos CE, Picardo M, *et al*. A new concept for acne therapy: A pilot study with zileuton, an oral 5-lipoxygenase inhibitor. Arch Dermatol 2003;139(5):668-70.
38. Krowchuk DP, Stancin T, Keskinen R, Walker R, Bass J, Anglin TM. The psychosocial effects of acne on adolescents. Pediatr Dermatol 1991;8(4):332-8.
39. Degitz K, Placzek M, Arnold B, Plewig G. Endokrinologische Aspekte bei Akne. In: Plewig G, Degitz K, editors. (Hrsg): Fortschritte Der Praktischen Dermatologie Und Venerologie. Vol. 17. Berlin: Springer; 2001. p. 172-9.
40. Cunliffe WJ. Acne and unemployment. Br J Dermatol 1986;115(3):386.
41. Simpson NB. Social and economic aspects of acne and the cost-effectiveness of isotretinoin. J Dermatol Treat 1993;4 Suppl 2:S6-9.
42. Burke BM, Cunliffe WJ. The assessment of acne vulgaris--the Leeds technique. Br J Dermatol 1984;111(1):83-92.
43. Harkaway KS, McGinley KJ, Foglia AN, Lee WL, Fried F, Shalita AR, *et al.* Antibiotic resistance patterns in coagulase-negative staphylococci after treatment with topical erythromycin, benzoyl peroxide, and combination therapy. Br J Dermatol 1992;126(6):586-90.
44. Cunliffe WJ, Holland KT. Clinical and laboratory studies on treatment with 20% azelaic acid cream for acne. Acta Derm Venereol Suppl (Stockh) 1989;143:31-4.
45. Lipson AH, Collins F, Webster WS. Multiple congenital defects associated with maternal use of topical tretinoin. Lancet 1993;341(8856):1352-3.
46. Jick SS, Terris BZ, Jick H. First trimester topical tretinoin and congenital disorders. Lancet 1993;341(8854):1181-2.
47. Eady EA, Cove JH, Joanes DN, Cunliffe WJ. Topical antibiotics for

the treatment of acne vulgaris: A critical evaluation of the literature on their clinical benefit and comparative efficacy. J Dermatol Treat 1990;1:215-26.

48. Eady EA, Jones CE, Tipper JL, Cove JH, Cunliffe WJ, Layton AM. Antibiotic resistant propionibacteria in acne: Need for policies to modify antibiotic usage. BMJ 1993;306(6877):555-6.
49. Darley CR, Moore JW, Besser GM, Munro DD, Edwards CRW, Rees LH, *et al*. Androgen status in women with late onset or persistent acne vulgaris. Clin Exp Dermatol 1984;9:28-35.
50. Greenwood R, Brummitt L, Burke B, Cunliffe WJ. Acne: Double blind clinical and laboratory trial of tetracycline, oestrogen-cyproterone acetate, and combined treatment. Br Med J (Clin Res Ed) 1985;291(6504):1231-5.
51. Hammerstein J, Meckies J, Leo-Rossberg I, Moltz L, Zielske F. Use of cyproterone acetate (CPA) in the treatment of acne, hirsutism, and virilism. J Steroid Biochem 1975;6(6):827-36.
52. Bottomley WW, Yip J, Knaggs H, Cunliffe WJ. Treatment of closed comedones--comparisons of fulguration with topical tretinoin and electrocautery with fulguration. Dermatology 1993;186(4):253-7.
53. Vahlquist A, Rollman O, Holland DB, Cunliffe WJ. Isotretinoin treatment of severe acne affects the endogenous concentration of vitamin A in sebaceous glands. J Invest Dermatol 1990;94(4):496-8.
54. Rademaker M, Wallace M, Cunliffe W, Simpson NB. Isotretinoin treatment alters steroid metabolism in women with acne. Br J Dermatol 1991;124(4):361-4.
55. Perkins W, Crocket KV, Hodgins MB, Mackie RM, Lackie JM. The effect of treatment with 13-cis-retinoic acid on the metabolic burst of peripheral blood neutrophils from patients with acne. Br J Dermatol 1991;124(5):429-32.
56. Layton AM, Cunliffe WJ. Guidelines for optimal use of isotretinoin in acne. J Am Acad Dermatol 1992;27:S2-7.
57. Layton AM, Knaggs H, Taylor J, Cunliffe WJ. Isotretinoin for acne vulgaris--10 years later: A safe and successful treatment. Br J Dermatol 1993;129(3):292-6.
58. Cunliffe WJ, Gray JA, Macdonald-Hull S, Hughes BR, Calvert RT, Burnside CJ*, et al.* Cost effectiveness of isotretinoin. J Dermatol Treat 1991;1:285-8.
59. Walker BR, MacKie RM. Serum lipid elevation during isotretinoin therapy for acne in the west of Scotland. Br J Dernsatol 1990;122(4):531-7.