Intralesional Tuberculin as an Immunotherapy in Warts

Thesis

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List of Abbreviations

AKs ····· Actinic keratoses

ALA ····· ٥-aminolaevulinic acid

APCs ····· Antigen-presenting cells

BCG ····· Bacille Calmette-Guerin

CIN ····· Cervical intraepithelial neoplasia

CO_2 ..... Carbon dioxide

DNA ····· Deoxyribonucleic acid

DNCB ····· Dinitrochlorobenzene

DPCP ····· Diphencyprone

E ····· Early region

Er:YAG ····· Erbium:Yttrium/Aluminum/Garnet

EV ····· Epidermodysplasia verruciformis.

FDA ····· Food and drug administration

FISH ····· Filter in situ hybridization

GM-CSF ····· Granulocyte-macrophage colony stimulating factor

HIV ····· Human immunodeficiency virus

HPV ····· Human papilloma virus

HSIL ····· High-grade squamous intraepithelial lesion

IFN ····· Interferon

IL ····· Interleukin

ISH ····· Tissue in situ hybridization

Kb ····· Kilobases

L ····· Late region

LCs ····· Langerhans' cells

Mw ····· Mycobacterium w

Nd:YAG ····· Neodymium:Yttrium/Aluminum/Garnet

List of Abbreviations (Cont.)

NF-Kb ····· Nuclear factor kB
NKCs ····· Natural killer cells
ORFs ····· Open reading frames
p٥٣ ····· Protein ٥٣
PCR ····· Polymerase chain reaction
PPD ····· Purified protein derivative
pRB ····· Retinoblastoma tumor suppressor protein
RRP ····· Recurrent respiratory papillomatosis
SADBE ····· Squaric acid dibutylester
SCC ····· Squamous cell carcinoma
STH ····· Southern transfer hybridization
STI ····· Sexually transmitted infections
TCA ····· Trichloroacetic acid
Th١ ····· T-helper ١
Th٢ ····· T-helper ٢
TLR ····· Toll-like receptor
TNF ····· Tumor necrosis factor
Tu ····· Tuberculin unit
UK ····· United Kingdom
URR ····· Upstream regulatory region.
US ····· United States
VLP ····· Virus-like particles
VV ····· Verruca vulgaris
٥-fu ····· ٥-Fluorouracil
٥-FU+LE ····· ٥-fluorouracil, lidocaine, and epinephrine

Introduction

Warts are tumours or growths of the skin caused by infection with Human Papillomavirus (HPV). More than ١٠٠ HPV subtypes are known ***(Pre'tet et al., ٢٠٠٧)***.

Warts are particularly common in childhood and are spread by direct contact or autoinoculation. This means if a wart is scratched, the viral particles may be spread to another area of skin. The incubation period varies from ١ to ١٢ months and averages ٢ to ٣ months ***(Karagas et al., ٢٠٠٦)***.

Warts have a hard warty or verrucous surface. Clinically, warts are classified by location into three main subtypes: cutanous, anogenital/mucosal and epidermo-dyspladia verruciformis (EV) ***(Pfister et al., ٢٠٠٣)***.

There are various types of viral warts including common warts, plantar warts, mosaic warts, plane or flat warts, periungual warts, filiform warts, oral warts and genital warts ***(Aubin and Laurent, ٢٠٠٦).***

There are many therapeutic modalities available for the treatment of warts including occlusion by duct tape, chemical treatment using salicylic acid or similar compounds, cryotherapy with liquid nitrogen, electrosurgery (curettage and cauttery), and laser vaporization, but none of them offers a guarantee of cure and recurrence is common ***(Scheinfeld and Lehman, ٢٠٠٦)***.

There are new trends towards the use of immunotherapy in treatment of warts, as the immune system seems to play an important role in the control of warts infection. Although the exact

mechanisms are unclear but most evidences suggest that cell mediated rather than humoral immunity plays an important role in control of HPV infection as the incidence of warts increases in subjects with cell mediated immune defects e.g. (HIV infection patients, malignant diseases…etc) ***(Frazer, ١٩٩٨ and Contant, ٢٠٠٠)***.

Various methods have been used to stimulate immunological response e.g. imiquimod, intralesional interferons, intralesional immunotherapy using mumps, Candida or trichophyton skin test antigens ***(Horn et al., ٢٠٠٥)***.

In the trial of mumps and candida antigens, patients with warts were tested for immunity to mumps and Candida using commercial antigens then according to the response to the test injection some patients did not respond and some patients had detectable immunity. The immune group received intralesional mumps and Candida antiserum. About ٧٤% of patients had complete clearance of the treated warts and about ٧٨% of patients had complete resolution of their immunotherapy treated warts and also complete resolution of the untreated distant warts so the intralesional mumps and Candida immunotherapy proved efficacy in treatment of warts ***(Johnson et al., ٢٠٠١)***.

Recently, intralesional tuberculin has been used for treatment of warts in Turkey, taking the advantage of vaccination schedule in this country ***(Kus et al., ٢٠٠٥).***

BCG is given routinely in vaccination in Egypt ***(Kotb and Azza, ١٩٩٣)***. No studies have been done to evaluate the efficacy of tuberculin use in treatment of warts in Egypt.

Aim of the Work

The aim of this study is to evaluate the effectiveness and adverse reactions, if present, of intralesional injection of tuberculin antigen (Purified protein Derivative, PPD) in treatment of warts.

١.٠.٠. Human Papiloma Virus (HPV)

١.١.٠. Virology of HPV:

HPV represents a complex group of small DNA tumor viruses that belong to genus A of the family Papovaviridae ***(Melnick et al., ١٩٧٤).***

Papillomaviruses are non-enveloped, double-stranded DNA viruses approximately ٥٥ nm in diameter. They are spherically shaped viral particles (virons) which consist of an outer protein shell (capsid) surrounding a single closed super coiled circle of double stranded DNA of about ٨٠٠٠ nucleotide base pairs (٨ kilobases kb) that comprises the HPV genome ***(Van Ranst et al., ١٩٩٦).***

The HPV genome is composed of three domains: the upstream regulatory region (URR), the early region (E), and the late region (L). The URR is approximately ١ kb in length, and lacks open reading frames (ORFs). It contains the origin of replication and many of the control elements for transcription and replication. The early region is approximately ٤ kb in length, and contains the ORFs for the viral genes that are principally expressed early in the papillomavirus life cycle. The late region is approximately ٣ kb in length, and encodes the viral capsid proteins ***(Howley and Lowy, ٢٠٠١).***

The HPVs have two genera, the genus α-papillomavirus containing mucosal and cutaneous HPV and the genus

β-papillomavirus containing the types associated with epidermodysplasia verruciformis (EV). Within each genus, HPV types are grouped according to the DNA sequence homology into species that often share similar biologic and pathologic properties (e.g.HPV-١٦ and -٣١) ***(DeVilliers et al., ٢٠٠٤).***

١.١.١. Early genes:

The early gene includes E١, E٢, E٤, E٥, E٦ and E٧.The E genes are transcribed at low levels in the basal cell layer and stratum malpighii. The first genes to be expressed following infection are the E١ and E٢ genes, which are viral regulatory proteins that are responsible for controlling the transcription of the viral genes and replication of the viral genome. The E٤ protein is expressed in the terminally differentiated keratinocytes where it causes cytokeratin collapse, thus facilitating the assembly and maturation of the virus. E٤ is probably a late gene because it is expressed late in the cycle of virus replication ***(Tyring, ٢٠٠٠).***

One major effect of HPV proteins E٥, E٦ and E٧ is that the epidermal cell cycle, which is normally blocked for cells that are supra-basal, continues so that HPV genome copy number can be amplified to high levels during viral replication for assembly into virions. The E٦ and E٧ proteins of the high-risk mucosal HPV types act as viral oncoproteins, but no such functions are associated with the corresponding proteins of the low-risk mucosal and EV types. There is limited understanding

of possible differences between the oncogenic EV HPV types (HPV-٥, -٨) and the non-oncogenic EV HPV types ***(Pfister, ٢٠٠٣).***

In combination with other cellular proteins, E٦ from high-risk mucosal HPV causes degradation of the cellular protein p٥٣, so that E٦-facilitated destruction of p٥٣ removes a brake on supra-basal cell cycling ***(Mantovani and Banks, ١٩٩٩).***

The E٧ protein binds the retinoblastoma tumor suppressor protein (pRB) leading to induction of DNA replication of HPV genome. Both E٦ and E٧ are multifunctional proteins and while their effects on p٥٣ and pRB are critical ones, they do have additional targets important to the oncogenic potential of the virus ***(Duensing and Munger, ٢٠٠٣).***

١.١.٢. Late genes:

The two late genes, L١ and L٢ encode the major and minor capsid protein respectively, these are the structural proteins of the virion. L١ is the major capsid protein which represents about ٩٥% of the total virion protein, and L٢ is a minor capsid protein which accounts for only a small portion of the virion mass. The function of L٢ protein is not yet clear ***(Kimbauer, ١٩٩٦).***

During virion assembly within cells, L١ multimerizes into pentamers called capsomeres, and ٧٢ capsomeres multimerize to

form the viral capsid with an icosahedral symmetry. The capsid surrounds the viral DNA, thereby protecting it from degradation, and it also enables the virus to bind efficiently to target cells ***(Kimbauer et al., ١٩٩٢).***

When expressed in cell culture, the L١ major capsid proteins of all papillomavirus types tested self-assemble into virus-like particles (VLP) that are morphologically similar to native virions. In addition, papillomavirus VLP display type-specific and neutralizing surface epitopes and are therefore useful as an antigen to detect serum antibodies and as a subunit prophylactic vaccine ***(Lowy and Frazer, ٢٠٠٣).***

١.٢.٠. Pathogenesis:

The papillomaviruses are highly species-specific, and productive infection has never been observed outside their natural host tissue (no known infection of man by animal HPV). HPVs are also tissue specific, i.e.the papillomavirus life cycle is completed only in fully differentiated squamous epithelia. This has impeded its study in monolayer tissue culture cells, where late gene expression and virion production do not occur ***(Howley and Lowy, ٢٠٠١).***

Infection begins with viral entry followed by one of three paths: Latent infection, in which there is no gross or microscopic evidence of disease, subclinical infection, in which microscopy reveals evidence of infection in the absence of