Acta Derm Venereol (Stockh) 1992; 72: 39–40

Non-specific Immunity in Patients with Primary Anogenital Warts Treated with Interferon Alpha plus Cryotherapy or Cryotherapy Alone

J. M. HANDLEY[1], R. D. MAW[1], T. HORNER[1], H. LAWTHER[1], T. McNEILL[2] and W. W. DINSMORE[1]

Departments of [1]Genitourinary Medicine and [2]Immunology, Royal Victoria Hospital, Belfast, Northern Ireland

Combination treatment of primary anogenital warts with subcutaneous interferon alpha 2a plus cryotherapy was no more efficacius than cryotherapy alone. Patients with primary AG warts showed no in vitro or in vivo suppression of non-specific immunity. In patients treated with interferon plus cryotherapy non-specific cellular immunity was stimulated, both in vitro and in vivo compared with patients treated with cryotherapy alone.

(Accepted May 29, 1991)

Acta Derm Venereol (Stockh) 1992; 72: 39–40.

J. Handley, Department of Dermatology, Royal Victoria Hospital, Belfast, N. Ireland.

Interferon (IFN) has immunostimulative properties both in vitro and in vivo (1). Cryotherapy of warts lyses viral-infected keratinocytes, probably resulting in increased exposure of viral antigenic fragments to local components of the host immune system. A combination of cryotherapy and IFN might therefore prove efficacious in the treatment of anogenital (AG) warts.

The aims of this study were to determine whether:

(1) patients with newly diagnosed AG warts have impaired non-specific immunity (NSI),
(2) treatment with IFN plus cryotherapy, or cryotherapy alone, causes stimulation of NSI in patients with newly diagnosed AG warts,
(3) NSI is related to clinical outcome in patients with AG warts treated with either IFN plus cryotherapy, or cryotherapy alone.

MATERIALS AND METHODS

This trial was approved by the Medical Ethics Committee, Queen's University, Belfast.

Patients over the age of 16 years were recruited from among new genito-urinary (GU) clinic attenders with AG warts, and allocated by computerized randomization to IFN + cryotherapy or placebo + cryotherapy treatment groups. The randomization protocol did not include randomization by sex, which therefore resulted in significantly more females in the placebo group. Exclusion criteria were: treatment for AG warts within the preceding 3 years, immunosuppression for any reason past or present, and pregnancy. Controls were recruited from healthy clinic staff.

Prior to trial entry, patients had venous blood drawn for syphilis serology, and urethral, cervical, vaginal, and rectal swabs as appropriate to exclude non-specific urethritis, infection with *N. gonorrhoea, Chlamydia trachomatis,* Candida, *Trichomonas vaginalis,* and *Gardnerella vaginalis.* Any infections were appropriately treated before trial entry.

According to treatment group allocation, patients were given subcutaneous IFN alpha 2a (Roferon) in doses of three million units, or placebo (normal saline) injections, on 3 separate days in the first week, and thereafter twice weekly for the following 6 weeks (7 weeks' treatment course in total). In addition all patients had warts treated by weekly cryotherapy from weeks 2 to 7 inclusive. Cervical exophytic warts/subclinical intra-epithelial neoplasias were not specifically treated.

The study was observer blind; IFN/placebo injections were administered by H. L.; cryotherapy, clinical assessment were performed independently by J. H. Cryotherapy was with a standard cryoprobe, after prior application of KY jelly to the wart(s), for a single 60 s freeze. When required, local anaesthetic (EMLA) cream was applied instead of KY gel. Patients were instructed to take saline baths twice daily until healing of the treated area had occurred.

At the initial, and the 8-week (treament completion) assessment, patients underwent a clinical AG examination, including proctoscopy and examination with a vaginal speculum. Patients and controls had venous blood drawn for PHA LT analysis, white cell and lymphocyte counts, and serum immunoglobulin levels (IgM, IgG, IgA); analyses were carried out in hospital immunology, haematology, and biochemistry laboratories. CMI Multitest intradermal tests were applied as described.

At the 8-week review, patients clinically wart-free were asked to return 4 weeks later; those who had persistent warts were excluded from the study. Patients were advised to either avoid sexual intercourse completely, or if not possible, to use condoms during the study period.

Multitest CMI (Institut Merieux, Lyon, France) intradermal skin testing

Skin tests were applied as recommended by the manufacturer. Antigens in the multitest were 1) tetanus, 2) diphteria, 3) steptococcus group c, 4) tuberculin, 5) glycerin control, 6) *Candida albicans,* 7) *Trichophyton mentagrophytes,* 8) *Proteus mirabilis.* Tests were read 48 h after application, using calipers provided to estimate diameter of reaction. A test was deemed positive when induration exceeded 2 mm in diameter, if the reaction was not circular, the average diameter of induration was estimated. CMI score was calculated as the sum (in millimetres) of all positive reactions.

Phytohaemagglutinin lymphocyte transformation (PHA LT)

10 ml of venous blood anticoagulated with 100 µml of preservative-free heparin (Evans Medical, Liverpool) was used for this assay; the methodology has been described previously (2). Using blood from non-trial patients routine laboratory diagnosis and from trial controls, the dilution of PHA producing optimum lymphocyte transformation, i.e. greatest mean number of scintillation counts/minute of filter paper quadruplicates, was determined as 2 ug/ml. PHA LT results for patients and controls in this study were therefore taken as average scintillation counts/minute of filter paper quadruplicates at this PHA dilution.

RESULTS

We studied 10 healthy controls, and 40 patients with AG warts, 19 treated with cryotherapy + placebo, and 21 treated with IFN + cryotherapy. More females were treated with cryotherapy/placebo than IFN/cryotherapy (chi squared $p <$

Table I. *Demographic data of patients in the two treatment groups*

	IFN/CRYO	PLACEBO/ CRYO
No. of patients	19	21
Age (years)(median/range)	25/18–59	26/18–47
Sex:		
No. of males	13	6
No. of females	6	15
AG warts:		
1. No of pats. with past history	0	1
2. Median duration prior to 1st attendance (mths)	2	2
3. Median number	10	13
4. Median area/extent (cm^2)	1	1
5. % of patients with warts at at the following sites:		
Males:		
inner prepuce/glans coronal sulcus	38.4	50
outer prepuce/shaft	23.1	16.7
perianal/anal canal	7.7	50
terminal urethra	7.7	16.7
Females:		
introitus	33.3	26.7
vulva	66.7	80
vaginal	0	6.6
perianal/anal	33.3	66.7
Treatment efficacy:		
1. No./% patients wart-free at 8-week review	11/18 (61)	12/18 (66.7)
2. No./% patients wart-free at 12-week review	5/18 (27.7)	7/18 (38.8)

0.05); healthy controls (median age = 36,5 years) were older than patients in the IFN/cryotherapy group (Mann Whitney u $p = 0.005$) and patients in the cryotherapy + placebo group (Mann Whitney u $p < 0.001$) (Table I).

There was no significant difference in treatment efficacy between patients treated with IFN/cryotherapy and those treated with placebo + cryotherapy at either the 8- or 12-week review (χ^2, $p > 0.5$) (Table I).

In patients treated with IFN/cryotherapy, PHA LT was higher after treatment (PHA LT at week 8 = median 92,600 counts/min compared with (median) 68,254 counts/min at week 0, Mann Whitney u $p < 0.05$). CMI score at the 8-week review was higher in patients treated with IFN/cryotherapy than in those treated with placebo/cryotherapy (median 12 mm compared with median 7 mm respectively, Mann Whitney u $p < 0.05$).

No significant differences in any measurements of NSI (i.e. PHA LT,CMI score, white cell counts, lymphoctye counts, serum immunoglobin type IgM, type IgG, type IgA levels) were seen between: 1) controls and patients at trial entry, 2) patients in either treatment group at trial entry, 3) patients in the cryotherapy/placebo group at week 0 and at treatment completion, 4) patients who were wart-free at treatment completion compared with those who had persistent warts at this time.

DISCUSSION

The limitations of this study are: 1) small control group numbers 2) higher median age of the controls than in the treatment group, 3) greater number of females in the cryotherapy/placebo treatment group.

Patients with newly diagnosed AG warts had no measurable impairment of NSI in this study; other authors have reported similar findings (3–8). These findings suggest that host NSI is unlikely to be of primary importance in preventing AG warts in immunocompetent patients, while AG warts do not at least in the short term, cause measurable immunosuppression.

No measurable differences in NSI were seen between patients who were clinically wart-free, and those who had persistent warts at the 8-week review (treatment completion). This suggests that either 1) measurements of NSI in this study were too insensitive to detect subtle differences between patients who were wart-free and those with persistent warts, or 2) in immunocompetent individuals, stimulation of NSI is not important in eradicating AG warts.

Patients treated with IFN/cryotherapy showed some evidence of stimulation of non-specific cellular immunity; as this was not seen in the placebo/cryotherapy group, this effect was probably due to IFN. Two questions remain to be answered. Is this stimulation of cellular immunity IFN-dose-related? Would increasing doses of IFN in patients whose warts are concurrently treated with cryotherapy improve therapeutic efficacy? Further studies are required to answer these questions.

ACKNOWLEDGEMENTS

Thanks to Miss J. McKirgan and Mr S Macrory for help with PHA LT, and Roche UK Ltd. for supplying IFN alpha 2a (Roferon) used in this study.

This study is part of an MD thesis submitted to the Faculty of Medicine, University of London, and was supported by a Royal Victoria Hospital research Fellowship, awarded to Dr J. Handley for the year 1989–90.

REFERENCES

1. Trofatter KF. Interferon. Obstet Gynecol Clin N Amer 1987; 14 (2): 569–579.
2. Hughes D, Caspary EA. Lymphocyte transformation in viro measured by tritiated thymidine uptake. I. Lymphocyte culture techique. Int Arch Allergy 1970; 37: 506–531.
3. Thivolet J, Viac J, Staquet MJ. Cell mediated immunity in wart infection. Int J Dermatol 1982; 21: 94–97.
4. Mohanty KC, Roy RB. Thymus derived lymphocytes (T cells) in patients with genital warts. Br J Venerol Dis 1984; 60: 184–188.
5. Mohanty KC, Scott KS, Limbert HJ, Master PS. Circulating B and T lymphocyte subpopulations in patients with genital warts. Br J Clin Pract 1987; 41(2): 601–603.
6. Kienzler JL. Humoral immunity to human papilloma viruses. Clin Dermatol 1985; 3(4): 144–155.
7. Carotis S, Siliotti F, Carot A, Alborino F. Cell mediated immunity and condylomatosis. Clin Exp Obstet Gynecol 1989; 16(2/3): 79–84.
8. Obalek S, Glinski W, Haftek M, Orth G, Jablonska S. Comparative studies on cell mediated immunity in patients with different warts. Dermatologica 1980; 161: 73–83.