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ORIGINAL ARTICLE

# Efficacy of inosine pranobex oral therapy in subclinical human papillomavirus infection of the vulva: a randomized double-blinded placebo controlled study

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**Summary:** A randomized double-blind placebo controlled study was carried out to assess the efficacy of inosine pranobex (1 g orally 3 times a day for 6 weeks) in the treatment of symptomatic subclinical human papillomavirus infection of the vulva. In a series of 55 women, 22 patients in the inosine pranobex group and 24 patients in the placebo group were suitable for analysis. A total of 14 (63.5%) of the inosine pranobex treated patients and 4 (16.7%) of the placebo treated patients showed significant vulval epithelial morphological improvement ($P$=0.005) at 2 months after initiation of treatment. Whereas 13 (59.1%) and 9 (37.5%) patients in the respective groups showed significant improvement in the severity of pruritus vulvae ($P$=0.435). Twelve (66.7%) of 18 patients with morphological improvement compared to 10 (35.7%) of 28 patients with no morphological improvement experienced significant symptomatic alleviation of pruritus vulvae ($P$=0.041). Similar results were seen at the second assessment 4 months after the initiation of treatment. Adverse drug reactions were reported by 2 patients in the treatment group and by 2 patients (skin rash) in the placebo group. These adverse reactions were mild and self limiting. It is concluded that inosine pranobex demonstrated a significant pharmacological activity in subclinical HPV infection of the vulva and should be considered an alternative treatment for the condition.

**Keywords: Human papillomavirus**

## INTRODUCTION

Human papillomavirus (HPV) is an ubiquitous virus in the female lower genital tract and an important aetiological factor in genital cancer development[1–4]. It can manifest in several forms such as condylomata acuminata, subclinical infection, latent infection or its associated neoplasia. Vulval HPV infection often induces recurrent and chronic irritation, itchiness or pain depending on the exact localization of the infection and the severity of the associated inflammation. The morbidity and sexual dysfunction associated with vulval HPV infection is therefore not insignificant and cannot be dismissed lightly.

Except for the exophytic condylomata acuminata, HPV infection on the vulva may be inconspicuous to naked eye examination. Colposcopy is a useful and one of the most practical diagnostic tools for HPV[5]. Its clinical management also imposes a dilemma. For the last decade laser vaporization has been a popular modality of treatment. Although effective, laser treatment is associated with a prolonged recovery period of several weeks and may result in post-treatment vulval pain syndrome or vulvodynia[6–8]. Surgical excision of the infected vulva is invasive and mutilating. This should be reserved as last resort treatment in specifically selected cases. Medical treatment of condylomata acuminata with topical application of podophyllin or its related compounds, or with tetrachloroacetic acid has a favourable response rate of 40–60%[9–11]. These treatments induce epithelial excoriation which limits their roles in cases with more widespread disease. Their efficacy and applicability in the treatment of subclinical HPV infection have not been elucidated.

In addition to the specific complications, all treatments are met with a high recurrence rate[7,12,13]. This may be a result of re-activation of virus existing in the tissues adjacent to the treated areas[14]. Recurrence of HPV disease is most commonly seen in immunosuppressed people. Local immuno-

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suppression at the site of HPV infection is commonly seen in patients without evidence of systemic immune compromise[15,16].

Reaction of the host immune system plays an important role in the regression of condyloma as witnessed by an increased T-cell infiltration in regressing cutaneous warts[17,18].

Generally speaking, cytotoxic immune killing of viruses and virally infected cells is most effective when the virus load in the host is low or when the ratio of effector immune cells to virus or virally infected cells is high. It has been shown that the virus load in subclinical HPV infection of the vulva is usually low[5]. The condition is therefore best suited for immunologically based therapy.

Clinical trials with interferons have elicited interesting results. The treatment is, however, costly and is associated with unpleasant adverse reactions[19–21]. More recently, inosine pranobex has been introduced as an orally consumed non-specific immune stimulant which increases the success rate in the treatment of HPV condylomata acuminata with conventional therapy[22,23]. Its clinical role in the primary treatment of HPV disease warrants further investigation.

The current study is a double-blinded placebo controlled trial designed to investigate the efficacy of inosine pranobex in the treatment of persistent subclinical infection of the vulva.

## PATIENTS AND METHOD

### Patients

The study subjects were patients with persistent pruritus vulvae for at least 3 months in the absence of bacterial or fungal vulvovaginitis or dermatosis. They all had colposcopically recognized and histologically confirmed subclinical HPV induced lesion. Patients with systemic diseases associated with immunosuppression, on immunosuppressant therapy, or either currently pregnant or who were planning to conceive were not eligible for the study. An informed consent was obtained from all the patients prior to recruitment to the study.

A detailed demographic, medical and symptomatology history was obtained from all the patients.

### Colposcopy

Colposcopy was performed on referral to the colposcopy clinic and then 2 and 4 months after the initiation of inosine treatment. Briefly, after soaking with 5% acetic acid for 5 minutes, the vulva was examined using a Zeiss colposcope. The criteria for diagnosis of subclinical infection were according to those described by Coppleson[24] and Tay *et al*[5]. Characteristically, the subclinical HPV infection manifested as acetowhite epithelium with well defined irregular and jagged edges. There might be satellite acetowhite papules surrounding the acetowhite epithelium. The location on the vulva, the size and colposcopic feature of the lesion were recorded diagramatically and photographically. A directed punch biopsy was taken, under local anaesthesia, with a Tischler forcep from a representative area within the lesion for histological diagnosis. The size of biopsy was generally small in comparison with the extent of the disease.

### Histology

All biopsies were fixed in formalin and processed for paraffin embedded sections for eosin and haematoxylin staining. The diagnosis of HPV infection was based on the presence of features similar to those found in cervical HPV infection, including koilocytosis, hyperkeratosis, parakeratosis and epithelial hyperplasia.

### Administration of drugs

Each patient was given a bottle of 252 tablets with an instruction to take 2 tablets (1 g inosine pranobex or placebo) orally 3 times a day for 6 weeks.

### Guideline for clinical management

All the patients were managed according to the routine clinical practice. Exacerbation of pruritus vulvae or occurrence of excessive vaginal discharge was investigated by microbiological and mycological tests prior to initiation of specific anti-microbial or anti-mycological therapy. Anti-pruritic therapy with topical Travacort cream (1 g contains isoconazole 10 mg and diflucortolone valerate 1 mg, Schering, Germany) was available upon patient's request. The frequency of its use was recorded.

### Assessment of treatment outcomes

The following parameters were assessed before and 2 and 4 months after commencing the treatment.

*Pruritus vulvae*

The patients were specifically enquired of their severity of pruritus vulvae which was graded in increasing severity into: grade 1=intermittent pruritus; grade 2=pruritus present in up to 50% of the time; and grade 3=continuous pruritus vulvae present most or all the time. A change in the severity of pruritus by one or more grades was taken as a significant change in the symptom, either improvement or worsening depending on the direction of change.

*Treatment efficacy*

The response of subclinical HPV infection to the treatment was assessed according to epithelial morphological changes revealed by colposcopy. The criteria for each type of response were: complete response=complete regression of the entire acetowhite lesion; partial response= regression of acetowhite lesion by 50% or more in size and with no development of new lesion on the vulva; progressive disease=extension of acetowhite lesion by 50% or more, or development of

condylomatous lesion; no change=changes of lesser than 50% in the extent of acetowhite lesion from the pre-treatment level.

*Adverse drug reactions*
Patients were enquired of any side-effects or drug reactions at each follow-up visit.

## STATISTICAL METHOD

### Sample size

Clinical experience in cervical HPV infection indicating that about 30% of the lesion regressed spontaneously was extrapolated to the subclinical HPV infection of the vulva in this study. It was considered a significant improvement if the active treatment produced at least a 30% positive response rate. Statistical calculation estimated that 25 patients were needed in each arm to detect a significant change of the regression rate of the lesion from 30% to 60% at $P$=0.05 level with a type II error of 10%.

### Randomization

Randomization of the patients into treatment and placebo arms was done at the drug supplier level. The placebo tablets were manufactured in identical shape, size, colour and taste to the active drug—inosine pranobex. The placebo and active drugs were packed into identical bottles and labelled with a series of random code numbers. The identity of these code numbers was kept by the drug supplier until completion of the study. On recruitment to the study, each patient was provided with a bottle of medication picked randomly from a box by the investigator.

### Data analysis

The difference in the mean age and mean duration of symptomatic complaints between the active and placebo groups were analysed for statistical significance with the unpaired t-test. Chi-square test was used in the analysis of significant differences between the groups for the other parameters studied.

## RESULTS

A total of 55 eligible patients attending the colposcopy clinic were randomized between 1 March 1992 and 31 December 1992. Twenty-seven patients entered the inosine pranobex group and 28 entered the placebo group. The mean age (± SD) was 38.3 (± 8.6) and 37.8 (± 9.0) years respectively ($P$> 0.1). The reasons for referral to the colposcopy clinic, symptomatology and the colposcopic findings are summarized in Table 1. The clinical features were similar between the 2 groups of patients. On average, the observation period between the presentation of symptoms and recruitment into this study was 19.2 (± 13.4) months in the inosine pranobex group and 18.5 (± 13.8) months in the placebo group ($P$> 0.1). Five patients from the inosine pranobex group and 4 patients from the placebo group did not complete the treatment and were excluded from this analysis.

No patients in the whole study group developed bacterial or fungal vulvovaginitis that required specific anti-microbial therapy. No topical anti-pruritic therapy was used during the entire study period.

*Table 1. Characteristics of patients in the study and placebo groups*

| Characteristics | Inosine group ($n$=27) | Placebo group ($n$=28) | $\chi^2$-test ($P$ value) |
|---|---|---|---|
| Reason for referral to colposcopy | | | > 0.50 |
| Abnormal Pap smear | 11 | 13 | |
| Genital warts | 3 | 2 | |
| Vaginal discharge | 3 | 1 | |
| Pruritus vulvae | 6 | 8 | |
| Postcoital bleeding | 0 | 1 | |
| Suspicious cervix | 1 | 1 | |
| Others | 3 | 2 | |
| Severity of pruritus vulvae | | | > 0.50 |
| Grade 1 | 11 | 14 | |
| Grade 2 | 11 | 11 | |
| Grade 3 | 5 | 3 | |
| Associated genital tract pathology | | | > 0.50 |
| CIN 1 | 4 | 4 | |
| CIN 2 | 2 | 1 | |
| CIN 3 | 1 | 3 | |
| Cervical HPV infection | 7 | 3 | |
| Cervical HPV infection | 2 | 3 | |
| None | 11 | 14 | |

## TREATMENT EFFICACY

### Morphological response

Of the 22 patients in the active treatment group, 14 (63.6%) responded to the treatment at the first assessment 2 months after treatment (Table 2). This overall response rate was significantly different ($P$=0.005) from the response rate of 16.7% in the placebo group. However, all but one responders in

*Table 2. Morphological response of vulva subclinical HPV infection to inosine pranobex at 2 months*

| Type of response | Inosine group | Placebo group |
|---|---|---|
| Complete | 1 | 0 |
| Partial | 13* | 4 |
| Stable disease | 8** | 20*** |
| Progression | 0 | 0 |
| Total number | 22 | 24 |

*2 cases relapsed, **1 case converted to partial response, and ***2 cases converted to partial response at the second follow-up

the active treatment group showed a partial response. One additional patient among the non-responders from the active treatment group and 2 from the placebo group achieved a partial response at the second assessment at 4 months. On the other hand, the disease relapsed in 2 initial responders when assessed again at 4 months.

### Symptomatic response

Overall, 13 of the 22 (59.1%) patients in the active treatment group showed improvement in the severity of pruritus vulvae at 2 months (Table 3). In 4 patients, pruritus vulvae resolved completely. Of the 24 patients in the placebo group, 9 (37.5%) reported improvement in their symptoms. The difference did not achieve statistical significance. During the subsequent follow-up, one non-responder in the active treatment group showed significant improvement in her symptoms but symptomatic relapse also occurred in one initial responder. One non-responder from the placebo group achieved complete symptomatic relief at 4 months. The net results again did not show statistically significant difference between the 2 groups.

### Agreement between morphological and symptomatical responses

On the whole, colposcopically detected epithelial improvement was noted in 18 patients and no significant changes in 28 patients. Symptomatic improvement was recorded in 12 of the 18 (66.7%) patients with morphological improvement and 10 of the 28 (35.7%) patients without morphological changes. The statistically significant difference (Chi-square=4.21, $P$=0.041) indicated that the morphological improvement in these patients was associated with symptomatic improvement in pruritus vulvae.

## ADVERSE DRUG REACTIONS

Four patients reported adverse drug reactions, 2 each from the active treatment and placebo groups. The 2 patients from the placebo group reported macular rash 2 and 3 weeks after initiation of medication respectively. One patient receiving active inosine pranobex reported tightness in the chest during the second week of treatment and the other patient reported undue generalized malaise after 3 weeks on the treatment. These adverse reactions resolved spontaneously upon cessation of drug consumption. No other adverse effect was reported in the remaining 46 patients who completed the entire treatment.

*Table 3. Symptomatic response to inosine pranobex*

| Type of response | Inosine group | Placebo group |
|---|---|---|
| Complete response | 4 | 3 |
| Improved | 9** | 6 |
| No change | 9* | 14*** |
| Worsened | 0 | 1 |
| Total number | 22 | 24 |

*1 case converted to improved, **1 case relapsed, and ***1 case converted to complete response at the second assessment at 4 months

## DISCUSSION

This small randomized double-blinded placebo controlled trial suggested that inosine pranobex was superior to placebo in improving the vulval epithelial disorder caused by subclinical HPV infection. Furthermore, the epithelial morphological improvement appeared to be associated with symptomatic improvement of pruritus.

The population of this study was women with chronic and frequent recurrent pruritus vulvae. Most patients had previously been treated unsuccessfully with either anti-mycotic, anti-inflammatory or both medications. All the patients displayed characteristic epithelial morphological changes for the clinical diagnosis of subclinical HPV infection on colposcopy and conventional histology. HPV DNA, although not tested in the present study, was detected in 85% of similar types of lesions in a separate previous study in which more than one-third of the cases were HPV type 16[5]. Information on the subtyping of HPV infection into the common oncogenic types 16 and 18 and low oncogenic types 6 and 11 was, however, not obtained in this report.

It has been shown that HPV load in subclinical vulval infection was low[5]. This represented an ideal situation for optimal immune mediated anti-virus treatment. Inosine pranobex, an immunostimulant, should theoretically be beneficial in the treatment of this disease. The finding here that 63.6% of the patients treated with inosine pranobex, compared to 16.7% of the placebo group, attained at least 50% regression in the epithelial lesion demonstrated the significant pharmacological activity of inosine pranobex in this disease. The finding in a previous study that inosine pranobex reduced recurrence of genital condyloma after conventional treatment but did not show any direct activity on the primary condyloma concorded with this study[22,33]. It indicated the importance of low virus load in the action of inosine pranobex. Whether the exact mechanism of action of inosine pranobex was mediated via immunological stimulation as claimed or via a direct anti-virus activity cannot be answered by this study.

It is noteworthy that colposcopically detected epithelial morphological improvement appeared to be associated with reduced frequency of pruritus vulvae. This supported the aetiological role of the epithelial disorder in the patients' symptomatology. However, pruritus vulvae is a subjective symptom found in numerous gynaecological and dermatological diseases as well as psychological disorders.

The placebo effect of any intervention becomes more pronounced. This could explain the finding in this study that the overall symptomatological improvement, though commoner in the inosine pranobex group (59.1% versus 37.5%), was not statistically significant between active treatment and placebo groups. One other explanation was that the sample size might be inadequate for the detection of a real difference of 20% in the effect of inosine pranobex on the symptomatology.

Both the morphological and symptomatological responses to inosine pranobex were sustained in the subsequent period of observation. This made the overall response clinically significant in this group of patients with long-term history of pruritus vulvae.

Persistent subclinical HPV infection with chronic pruritus vulvae is a common clinical condition causing significant suffering in the patients, posing difficult diagnostic and treatment decisions for the doctor. The present study revealed that inosine pranobex induced significant regression in the disease in nearly two-thirds of the treated patients and reduced the severity of the symptom in more than half of the patients. The ease of the oral medication and the infrequent adverse drug reactions make this an attractive treatment in women with symptomatic subclinical vulval HPV infection. Further clinical experience with this treatment is needed before it can be advocated as a first line therapy for this disease.

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