Chapter IV

Materials & Methods

Patients:

1. Sample size : Since multiple warts is not a common condition and due to the open nature of the study, sample size is not calculated. However a number of 10 patients is expected to enroll in the study.
2. Diagnosis : Due to their typical clinical appearance, warts are easily recognized with naked eyes.
3. Inclusion criteria : All patients who present with multiple warts (more than 10 lesions of common and/or plantar warts) to Chulalongkorn hospital dermatologic clinic from August 1989 to January 1990 are included in the study, provided that they are able to attend for follow-up fortnightly over 2 months' time.
4. Exclusion criteria
   - 4.1 Patients with known immunosuppressive conditions (disease and/or medication).
   - 4.2 Patients with known hypersensitivity to Inosine pranobex or any ingredient of the compound.
   - 4.3 Patient with gouty arthritis and elevated serum uric acid (>7 mg/dL)
   - 4.4 Pregnant women

Methods

1. After understanding the procedures and purposes of the project, the patient is thoroughly questioned about duration of disease, symptoms and previous treatment(s).
2. Verbal consent is obtained.
3. Physical examination and mapping of lesions is done. Number of all lesions and size of one representative lesion are noted (measurement is done in millimeters). Number of lesions is used in statistical comparison but size of lesions is used only to indicate clinical regression. Together with physical examination, photography of all lesions is done.
4. If permitted, one lesion is biopsied, snap-frozen in liquid nitrogen or cryostat - sectioned and Kept at deep-freezing temperature until immunohistochemical study is performed.
5. Laboratory examinations include complete blood cell count and serum uric acid.
6. Inosine pranobex (Isoprinosine 500 mg tablet) is administered at the dosage of 50 mg/kg/day for pediatric patients and 6 tablets per day in 3 divided doses for adults for 2 months or until complete resolution takes place if it should occur before 2 months.

7. Follow-up is done at 2 week interval with complete mapping and measurement as done at the initial visit.

8. Compliance is assessed by questioning the patient and tablet counting.
9. Biopsy is performed on lesions showing evident regression (flattening and/or reduction in size or number) and tissue is processed as mentioned in (4)
10. If patient fails to attend for follow-up, he will be reminded by way of telephone or postcard.
11. At the discharge from the study, serum uric acid is repeated.
12. Response is considered as
    - 12.1 Cure : if all lesions disappeared
    - 12.2 Partial cure : if some lesions disappeared or showed flattening or reduction in size
    - 12.3 Failure : if lesions showed no change at all at the end of the 2 month study.
    - 12.4 Worsening : if more lesions developed.

Statistical Methods

Number of warts at the start and end of treatment are compared by Student's paired t-test.

Laboratory methods

After biopsy, sections are kept in liquid nitrogen until immunohistochemical staining is done.

Following cryostat sectioning, sections are fixed in cold (4`C) acetone for 10 minutes, then rinsed and bathed in Tris-buffered solutions. Sections are then incubated with primary monoclonal antibodies (DAKO-T4, M716, for T-helper cells; DAKO-T8, M707, for T-suppressor cells; DAKO-HLA-DR, M704, for HLA-DR antigen; and DAKO-IL-2-R, M731, for interleukin-2 receptors) at optimal dilutions. Then sections are incubated with linking antibody, APAAP reagent and color substrate (Fast Red) (DAKO-APAAP Kit, K 0671) sequentially. Counterstaining is done with hematoxylin.

Sections are then examined by light microscopy.