

Original Article

## Allopurinol as a Potential Therapeutic Agent for Recurrent Herpes Labialis

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Recurrent herpes labialis (RHL) occurs in up to 40% of the population. Although the disease is usually self-limiting, patients seek treatment because of the significant pain and visibility of the lesion. Xanthine oxidase inhibitors (XOI) have been reported to have a potent antiviral effect against influenza-A virus. We examined the effect of the systemic xanthine oxidase inhibitor, allopurinol, on RHL duration of illness, severity of symptoms, number and frequency of recurrence during a 4-year follow up period in Egyptian patients. Duration of illness was shortened by about 25%, early disappearance of pain and other symptoms occurred. Also, aborted episodes were noticed when allopurinol was given just after beginning of common colds, at the prodromal stage of RHL or during severe stress conditions. Patients receiving 3 courses of treatment had markedly decreased recurrences during the follow up period. Ex vivo experiments to examine virus-induced plaque formation on Vero cells in the absence or presence of different concentrations of the drug could not prove any direct anti herpetic effect of the drug. However, allopurinol seems to be safe and effective in reducing duration of RHL and to abort lesion or

prevent its appearance in treated patients even when they experience immunosuppressive conditions.

**Key words:** HSV, allopurinol, RHL, treatment, antiviral agents.

### Introduction

Most human viruses known to cause oral diseases are DNA viruses that are contracted during childhood or early adulthood through contact with blood, saliva, or genital secretions. Herpes viruses seem to be the most important DNA viruses in oral pathology<sup>1</sup>. Human herpes viruses are found worldwide and are among the most frequent causes of viral infections in immunocompetent as well as in immunocompromised patients. The vast majority of the world population is infected with at least one member of the human herpes virus family. Herpes simplex virus (HSV) infections are the cause of cold sores and genital herpes as well as life-threatening or sight-impairing disease mainly in immunocompromised patients, pregnant women and newborns<sup>2</sup>. Oral HSV-1 infection affects about 75% of the general population and most people (up to 90% of the general population) have antibodies to herpes simplex virus suggesting that they may have latent infection<sup>4</sup>.

A main feature of oro-facial herpes simplex is the ability of herpes simplex virus (HSV) (generally type 1) to remain latent before erupting in response to stimuli

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such as stress, sunlight, fever, or respiratory tract infection<sup>2</sup>. After initial HSV-1 infection, there are 3 well-recognized sequels resulting from reactivation of HSV from latency; namely recurrent herpes simplex labialis (RHSL), asymptomatic shedding of HSV-1 in saliva and localized intra-oral recrudescence<sup>4</sup>.

Recurrent oro-facial herpes simplex is a common disease estimated to occur in up to 40% of the U.S. population<sup>5</sup>. RHSL is self-limiting with healing normally occurring in 7-14 days<sup>2</sup>. Lesions evolve rapidly within 12 hours of onset of infection or activation. Classic lesions are those that progress to the vesiculo-ulcerative stage before healing. Episodes that do not progress beyond the papule stage have been referred to as aborted or non-lesion episodes<sup>2</sup>. The window of time for treatment is therefore small, and it is essential that antiviral therapies be administered early.

Until a decade ago, the impact of acyclovir on the control of severe and life-threatening herpes virus infection was unprecedented. Acyclovir has been used to treat herpes virus infections for 15 years in over 30 million people. A large number of carefully designed clinical trials have clearly established acyclovir as a drug of choice for a wide range of mucocutaneous herpes virus infections in immunocompetent and immunocompromised patients<sup>6</sup>.

A 5% acyclovir ointment applied five times per day when symptoms first appear reduces slightly the duration of HSL and may abort some lesions. However, it does not prevent recurrence, and may be ineffective in some patients<sup>6</sup>. Oral acyclovir tablets (400 mg five times per day) for the treatment of HSL are more effective than the ointment form. It may not prevent recurrences, but it seems to shorten the course of the disease<sup>6</sup>. However, its limited oral bioavailability (range 15-30%) means that it is not optimal for orally administrated therapy of all susceptible herpes virus infections<sup>7</sup>.

During the past few years, we have witnessed approval of new therapeutic drugs for infections caused by HSV and VZV (penciclovir, valaciclovir and famciclovir), CMV (ganciclovir, cidofovir and fomivirsen) or HSV, VZV and CMV (foscarnet)<sup>8</sup>. While some of these antiviral therapies are considered safe and efficacious (acyclovir, penciclovir), some have toxicities associated with them (ganciclovir, foscarnet)<sup>9</sup>. In addition, the increased and prolonged use of these compounds in clinical setting, especially for the treatment of immunocompromised patients, has led to the emergence of viral resistance against most of these drugs. While resistance is not a serious issue for

immunocompetent individuals, it is a real concern for immunocompromised patients<sup>9</sup>. New anti-herpes agents are needed to face clinical issues such as drug resistance, increased use of anti-herpes prophylaxis in transplantation and safety concerns in young children or pregnant women<sup>8</sup>.

Kleymann et al.,<sup>10</sup> reported new inhibitors of the HSV helicase-primase enzymes with potent *in vitro* anti-herpes activity. These agents have a novel mechanism of action, a low resistance rate and a superior efficacy against HSV in animal models<sup>10</sup>. BAY 57-1293 (a well tolerated member of this class of compounds) significantly reduces time to healing, prevents rebound of disease after cessation of treatment and most importantly, reduces frequency and severity of recurrent disease. Thus, this class of drugs has a significant potential for the treatment of HSV disease in human including those resistant to recurrent medications<sup>10</sup>.

The use of guinea pig model of genital herpes has allowed investigators to evaluate carefully several vaccine and immunomodulatory strategies for the control of recurrent herpes virus infections. These investigations have clearly shown that immunotherapy can significantly decrease the recurrence rates. However, only moderate success has been reported for human trials, although the optimum strategies that were identified in the animal models have not yet been evaluated<sup>11</sup>.

Allopurinol is a tautomeric mixture of 1*H*-pyrazolo [3, 4-*d*] pyrimidin -4-ol and 1, 5 -dihydro-4 *H* pyrazolo [3, 4-*d*] pyrimidine-4-one. It is mainly used in treatment of chronic gout and hyperuricaemia due to its inhibitory action on the enzyme xanthine oxidase and the resultant reduction of the oxidation of hypoxanthine to xanthine and xanthine to uric acid<sup>12</sup>. It has other benefits and has been used in treatment of Duchenne muscular dystrophy<sup>13</sup>, epilepsy<sup>14</sup>, to reduce acute rejection after renal transplantation<sup>15</sup>, to improve postoperative recovery and reduce lipid peroxidation in patients undergoing coronary artery bypass grafting<sup>16</sup>, in treatment of chronic Chagas' disease<sup>17</sup> and for visceral and cutaneous leishmaniasis in AIDS patients<sup>18,19</sup>. Also, a similar XO inhibitor is reported to have an effect on Influenza-A virus<sup>20</sup>.

This study was conducted on 2 stages; the first stage was to study the possible effect of allopurinol on treating signs and symptoms of recurrent herpes labialis (RHL); and the second stage aimed to study the possible effect of the drug to delay, minimize or prevent the appearance of RHL.

## Patients and Methods

### Selection of patients:

The present study aimed to select 100 Egyptian patients diagnosed as having recurrent herpes simplex labialis (RHL) based on the past and present clinical manifestations. They were selected from patients who presented to the outpatient clinics of Oral Medicine and Periodontology department, Faculty of Dentistry and of Dermatology department Faculty of Medicine, Mansoura University in Mansoura city at the Northern part of Egypt during the period from March 1997 to January 2002. Patients who have had a clinical history of HSL with at least 3 recurrences during the past 12 months with a RHL history of more than 5 years were selected. Because there was no evidence in the literature that oral contraceptives influence RHL episodes, participants taking oral contraceptives were not excluded. Children, pregnant females, lactating mothers and subjects with known allergies were excluded from the study. Also patients with lesions inside the mouth, above the nares or below the chin were not included. Patients who used any approved antiviral agent, topical corticosteroid or any other non-specific therapy for HSL during or within 7 days before the study were excluded. The usage of systemic corticosteroids or other drugs known to induce immune stimulation or immune suppression was also an exclusion reason. Female patients agreed not to use cosmetics on or around the mouth during the treatment period. All subjects who met the criteria were enrolled into the study, after they signed an informed consent.

During the first visit in which the patient accepts his/her enrollment in the study, we gave the patients placebo tablets and followed them till the healing of the lesion. This step was very important for many reasons: 1) many patients were lost from the start; 2) patients used to come to the clinic during a late stage of illness when eruptions are manifest; 3) most of patients could not define exactly the duration of the last attack. This visit aimed to define the duration of illness of each patient before treatment and also to educate patients about the prodromal symptoms or the provoking conditions. Then patients were asked to come to the clinic during any stress condition that used to induce his/her illness or as quickly as they can once they feel any of the prodromal symptoms. Only patients who came again at a very early stage of RHL were given the drug and included in the study.

We noticed that, not all participants experienced the same time to healing before starting treatment. The

time from start of the lesion till its healing ranged from less than 7 days to more than 10 days. Therefore, the studied subjects were subdivided according to their experienced lesion duration into 3 subgroups: first subgroup with a history of lesion duration  $\leq 7$  days, second subgroup with 7.5-9.5 days and a third subgroup with  $\geq 10$  days of lesion duration.

### The first stage of the study:

Patients who came for a second visit in a very early stage of the disease were selected. They were once more properly informed of the study purpose and after reading the pamphlet of the used drug they were asked to sign consent for accepting their enrollment in the study. We were able to recruit 100 patients (68 female and 32 male patients) with age ranging from 18-50 years. The study group consisted of 70 patients (48 female and 22 male patients) who received oral doses of allopurinol 300 mg (one tablet) three times a day after meals for 5 days. Allopurinol is available at the Egyptian market under 2 brand names [Zyloric manufactured and distributed by Glaxo company and Nouric manufactured and distributed by EIPICO (Egypt International Pharmaceutical Industries Corporation)]. In this study we used Nouric 300 tablets (which was kindly supplied by EIPICO).

A control group composed of 30 patients (20 female and 10 male patients) matched for age to the study group. The control group received a placebo tablets provided by EIPICO that did not contain any active ingredient. The clinician who did the evaluation (J.Y.) did not know the key that marked the study group from the control group.

Patients were asked to come every day for assessment by the investigators during the first week of the lesion, on every other day for another week, and then as frequent visits as they can for follow up. The initial lesion area was marked on a diagram in the case report form as the baseline clinical assessment. Localized signs and symptoms at that area were documented at each visit, including erythema, papule, vesicle, ulcer, soft crust, hard crust or healed skin. Also the patients' own reports of pain, burning, itching, tingling or any other symptoms were recorded.

### The second stage of the study:

Because of the relief of their symptoms, 46 patients from the study group (33 female and 13 male patients) decided to continue in the second stage of this study. In this stage patients took the drug for 5 days during any condition that used to cause recurrence of

their lesion. The evoking conditions included common colds (in more than 70% of patients), severe stress conditions (taking exams was considered stressing by most student patients) and after major physical efforts (going to Mecca for Muslim pilgrimage was the most mentioned event by our patients). The majority of patients in this stage were members of the authors' families, friends, dental students and medical staffs. Being close to us, we could follow up those patients easier, more frequent and for a long duration. Almost all participants reported that they normally experience prodromal symptoms before their RHL episodes. So, they were informed to contact us as early as they can, once they have any symptom suggestive of the prodrome of RHL or even when they pass stress conditions, as well as any endogenous or exogenous factors believed to trigger their RHL.

On reporting any burning, itching or hyperthesia sensation, and/or redness in the circumoral area they were asked to start the treatment course immediately. The disease signs and symptoms must not have been present for more than 12 hours and the episode must not have progressed beyond the erythema phase when they started therapy. Patients should visit the investigator for clinical assessment as soon as possible. They were regularly examined every other day for 7 days by the investigator for the appearance and duration of RHL eruption and the accompanying symptoms. No control group was included in this stage.

#### **Ex vivo testing of the drug:**

The direct effect of allopurinol on HSV isolates was tested by the plaque-reduction assay in Vero cells according to Shier 1991<sup>21</sup>, as follows:

A) Virus Isolation: The fluid from the vesicular stage of the lesion of 3 different patients was taken by a swab that was broken immediately in a tube containing 1 ml of virus transport medium then kept at -40°C till being propagated on Vero cells.

Vero cells were cultured in 6-well tissue culture plates in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and penicillin, streptomycin, amphotericin B mixture (complete medium). The samples of different patients were added 100 µl/well to the semi confluent cell sheets of Vero cells and incubated for 3-4 days in 5% CO<sub>2</sub> humidified incubator. The culture supernatant was collected, filtered through 0.45 µm Millipore filter and tested again using the plaque assay method on Vero cells then kept at -40°C to be used as the virus preparation.

B) Drug dilution: Allopurinol was dissolved in dimethyl sulphoxide (DMSO) and diluted in DMEM to 10<sup>3</sup>, 10<sup>4</sup> and 10<sup>5</sup> µg/ml just before use.

C) Plaque assay: Vero cells were propagated in 3 plates as above for 2 days, and then the medium was replaced by 1 ml of complete medium. The virus preparation was added (100 µl/well) on the semi confluent sheet of cells in wells # 1 and 2. Well # 3 contained cells only as a control. The same amounts of the same virus preparation but mixed with 100 µl of allopurinol 10<sup>3</sup>, 10<sup>4</sup> or 10<sup>5</sup> µg/ml were added to wells # 4, 5 and 6 respectively. The virus and drug mixtures were kept on cells for 3h, and then the medium was replaced with 3 ml of complete medium containing 0.5% methyl cellulose and incubated for 3 more days under the same conditions. The plaques were stained, counted and compared for cultures infected with the virus only to those induced in cells infected with virus in presence of different drug concentrations. The test was done in triplicate using 3 different virus isolates obtained from patients enrolled in this study who showed *in vivo* response to the drug.

#### **Evaluation of the drug effects:**

In this study, we considered the lesion completely healed when there was no crust, with no evidence of active lesion regardless to the presence of any residual post-lesion skin changes that might include erythema or slight asymmetry. Time to healing was calculated from the time of the initiation of therapy until the time of complete healing of the episode. Percentage reduction was calculated for each patient as: duration before - duration after / duration before treatment (in days).

## **Results**

#### **Effect of allopurinol on the herpetic lesion:**

At the beginning, the study population consisted of 70 patients (48 female and 22 male) suffering from RHL who were seeking treatment because of the significant pain, and the visibility of the recurrent lesion. The percentage of female participants was 68.6 % or about two times greater than the percentage of male participants (31.4%). This is attributed mostly due to the fact that females are more aware about the visibility of the lesion, and may respond to the lesion visibility more dramatically seeking rapid treatment.

After accepting inclusion in the study and receiving the drug, 3 persons (4.2%) did not return to the clinics

for further evaluation. Although, the used medication is available in the market, and its safety is well documented, 2 other patients (2.8%) withdrew from the study after taking the drug for 3 days because they believed that they had had adverse experiences of the drug (nausea). Those participants were not replaced, so the study population finally consisted of 65 patients: 44 female (67.7%) and 21 male (32.3%) patients with RHL. Nine patients (13.8%) from the total studied population did not show any improvement in time to healing of their lesions while they had using the drug protocol for a single course (5 days). Table 1 shows the number of patients and their response during the study.

Among the responding 56 patients (86.2%), 43 patients (76.8%) showed a marked promising effects of allopurinol in the form of well defined shortening of the lesion duration (percentage reduction ranged from 27-31%) and 13 patients (20%) showed moderate shortening of disease duration (reduction of about 22% of their lesions' duration) as shown in table 2. The times to healing of the episodes were markedly shortened in those patients who used to experience long episode duration of more than 8 days (the 2<sup>nd</sup> and the 3<sup>rd</sup> sub-

groups) as shown in table 3.

Not all participants showed the same improvement but 43 of the 65 patients (66.1%) had well-defined marked shortening of the disease period duration compared to their own previous experience. Thirteen patients (20%) had moderate shortening of the disease period duration compared to their own previous experience.

With respect to the lesion-associated symptoms, it was noticed that all participants reported itching and/or burning before treatment. Patient's feelings of pain were still present while using the drug, but for a shorter duration and to a less magnitude than their previously experienced symptoms. This improvement in lesion-associated symptoms reported by the participants after treatment was parallel with improvement of signs noticed by the investigator.

From the control group 3 patients did not return to the clinic after the first visit. Among the 27 control patients who were followed up, 6 persons (5 females and one male) reported improvement of their symptoms, but the investigator noticed no change in the duration of the lesion.

**Table 1.** Patients included in the study:

Patients	First stage		*Second stage	
	Number	%	Number	%
<b>The study group</b>				
At the start	70	100	46	100
At the end	65	92.9	38	82.6
Responding	56	86.2	31	81.6
Non-responding	9	13.8	7	18.4
Lost patients	5	7.1	8	17.4
<b>The control group</b>				
At the start	30	100		
At the end	27	90		
Responding	00	00		
Non-responding	27	100		
Lost controls	3	10		

\*Patients of the 2nd stage are from the 56 responding patients in the 1st stage of the study.

**Table 2.** Degree of response of RHL patients to allopurinol:

Stage of the study	Degree of response	Patient number (%)
1st(one course)	Marked shortening of duration	43 (66.20%)
	Moderate shortening	13 (20.00%)
	No Response	9 (13.80%)
Total number		65 (100.0%)
2nd(3 courses)	Aborted or no lesions	31 (81.60%)
	Same frequency with shorter duration	7 (18.40%)
Total number		38 (100.0%)

\*Patients of the 2nd stage are from the 56 responding patients in the 1st stage of the study.

**Table 3.** Shortening of duration of illness in responding subjects:

Responding patients Number (%)	Mean duration of illness	
	Before treatment	After treatment
56 100		
09 16.1	12.7	8.8
31 55.3	9.1	6.6
16 28.6	6.2	4.8

### Effect of allopurinol on recurrence of the herpetic lesion:

We started the second stage of this study to evaluate the possible preventive effect of allopurinol on RHL. During the second stage, 4 women were excluded from the study since they became pregnant. Also, 3 men and one woman were excluded from the study as they traveled abroad. Consequently the patients group during the second stage included 38 patients: 28 female patients (73.7%) and 10 male patients (26.3%) as show in table (1).

After 3 courses of allopurinol, starting early in the prodroma or erythema stage or just after the appearance of early symptoms of common cold, 31 patients (81.6%) had aborted lesions. Those patients had erythema and itching in the usual site and small papules without proceeding to vesicles. Further follow up of those treated patients revealed that, they experienced the same stress conditions or factors used to trigger the activation of their RHL more than once without eruption of RHL. Those patients were advised to start the allopurinol course once they have any evoking conditions and most of them are not experiencing a single attack of RHL till now. On the other hand, 7 patients (18.4%) experienced only shortening of the duration of the disease and milder symptoms even after 3 courses of allopurinol; without complete prevention or abortion of the lesion as shown in Tables 1 and 2.

### Ex vivo effect of allopurinol on HSV:

The plaque assay on Vero cells showed no significant difference between cells infected with HSV alone and cells infected with HSV in the presence of different concentrations of allopurinol. The mean number of plaques induced in Vero cells infected with 3 different isolates of HSV in the absence or presence of different

concentrations of allopurinol was almost the same as shown in Table 4. These findings may suggest that allopurinol is acting *in vivo* through an indirect mechanism. Xanthine oxidase inhibitors have antioxidant effects that may improve the immune status of the host.

## Discussion

The impact of herpes virus diseases on human health continues to grow. Infections caused by herpes viruses not only have an impact on an individual physical well being, but are also linked with the psychology and sociality of infected patients. It is therefore important to consider treatment of RHL, although it is a self-limited disease, not only for the physical symptoms but also for the psychological well being the patients. Identification of improved antiviral agents and immunological interventions till a vaccine is developed are necessary.

In this study, we used clinic-initiated treatment trial to evaluate the clinical efficacy of allopurinol in treatment and prevention of HSL. Despite the self-limiting nature of the disease, the drug was able to decrease the duration of illness by 2-4 days (20-29% percentage reduction) that was considered significant by many patients. Reduced time to healing was accompanied by quicker relief of pain, burning and itching that is also important to patients. The time of the most severe stage (ulcer /soft crust) of the lesion was significantly reduced. These stages are considered the most active infectious stage since they represent the peak period of viral replication and inflammation and hence a role of the drug in inhibiting virus replication and spread could be suggested.

Some patients (13.8%) showed no improvement in time to healing while they had using the drug for a single course. Those patients might actually have early, established lesions before the commencement of therapy, which may explain the lack of responsiveness to the drug. The probability of drug-resistant isolates of HSV or even isolates with low susceptibility to allopurinol is a remote possible cause as the drug has no direct anti herpetic effect and should be active through the patient own immune system.

In addition to the favorable promising clinical results of allopurinol on both the healing and symptom components of RHL, there was a marked trend to prevent or reduce episodes after receiving three courses. However, the limited number of our participants and the difficulty to define the provoking condition in different

**Table 4.** Effect of different concentrations of allopurinol on numbers of plaques produced in HSV-infected Vero cells:

Allopurinol concentration µg/ml	Plaque mean number (+/- SD)		
	HSV isolate #1	HSV isolate #2	HSV isolate #3
0	198 (18)	142 (16)	223 (24)
10 <sup>2</sup>	196(24)	138 (30)	234 (12)
10 <sup>3</sup>	190(24)	156 (20)	194 (22)
10 <sup>4</sup>	202(16)	128 (18)	204 (30)

patients may not support the use of the drug for lesion prevention. To date, agents prescribed for lesion prevention has never been unequivocally demonstrated. Nevertheless, the clinic-initiated treatment before lesion onset used in this study offers the potential for trying different agents for disease prevention or suppression.

There are recognized limitations in this study. The small number of subjects provided low statistical power and made it more difficult to capture differences that might have been more apparent with a larger sample. Also, laboratory confirmation of the direct effect of the drug on herpes virus could not be achieved. The difficulty in demonstrating direct antiviral effect is increased by the fact that viral cultures must be obtained to test the specific antiviral efficacy of the drug against different isolates of HSV. Virus culturing has often not been pursued in HSL, because sampling is very painful and may have a possible effect on delaying healing, which may in turn contribute to lack of sensitivity of this parameter in HSL studies<sup>22</sup>.

Ex vivo experiments to evaluate antiviral agents depend on direct action of the agent on the virus or the infected cell. XO1 was shown to act on influenza-A through its modified super oxide dismutase activity as a scavenger of O<sub>2</sub> or what is called antioxidant effect<sup>20,23</sup>. This effect has an indirect systemic antiviral action through raising the immune status of the host and control of the viral pathogenesis. Samples from patients' lesion and infection of Vero cells with HSV isolates in the absence or presence of different concentrations of allopurinol did not give a conclusive direct antiviral effect of this agent *in vitro* (table 4). We think that allopurinol is producing its effect through its oxygen radical removal and augmentation of the adenine nucleotidase activity as reported previously<sup>24</sup>.

In conclusion, simplification of the five-times a day dosing regimen of acyclovir to three-times a day dosing regimen of allopurinol for treatment of herpetic infections is considered to be an important issue especially with the big difference in the cost of both drugs as a good advantage for allopurinol. Systemic use of allopurinol was shown to be effective to hasten the resolution of the episode that develops into classic lesions, and also to shorten the duration of all associated lesion symptoms. Also, allopurinol should be used as a prophylactic measure when patients with history of RHL pass a stress condition or are exposed to any factor believed to trigger RHL activation in order to abort the lesion or suppress it. Patients' awareness of their precipitating factors is so important in any preventive

strategy directed toward aborting viral reactivation. Further studies should be done to study a double-blind comparative trials of allopurinol versus acyclovir to clarify which has superior efficacy, or even if they are equivalent. Then, it will be possible to extend the study to examine the mechanism of action of allopurinol as an immunomodulator or an antiviral agent.

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