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CO_2 LASER PLUS PHOTODYNAMIC THERAPY VERSUS CO_2 LASER IN THE TREATMENT OF CONDYLOMA ACUMINATUM: A RANDOMIZED COMPARATIVE STUDY

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To compare the efficacy and safety of CO_2 laser plus 5-aminolevulinic acid (ALA) photodynamic therapy (PDT) with CO_2 laser for the treatment of multiple condyloma acuminatum (CA), 120 patients with multiple CA were allocated into two groups — combined group (CO_2 laser plus ALA-PDT, $n = 60$) and CO_2 laser group (CO_2 laser plus placebo-PDT, $n = 60$). After CO_2 laser, a 20% ALA or a placebo solution was applied to the CA area 3 h before illumination with red light (635 nm, 100 mW/cm^2, 80 J/cm^2). The treatment was repeated seven days after the first treatment if the lesions were not completely resolved. The complete response rate, recurrence rate and adverse effects in the two groups were analyzed. After two treatments, the complete response rates in the CO_2 laser group and combined group were 100% (509/509) and 100% (507/507) in the CA ($p > 0.05$), respectively. The recurrence rates in the CO_2 laser group and combined group were 44.9% (229/509) and 10.6% (54/507) in the CA ($p < 0.05$), respectively. The adverse effects in CO_2 laser group was more than that in combined group. The combined group is a more effective treatment for multiple CA compared with CO_2 laser group. T/S. Style the highlighted text as abstract.

Keywords: 5-aminolevulinic acid; photodynamic therapy; condyloma acuminatum; CO_2 laser.

1. Introduction

Condyloma acuminatum (CA) is a common sexually transmitted disease caused by human papilloma virus (HPV). Up to date, since anti-HPV drug with high specificity and curative effect is not available, CA in subclinical stage or latent infection stage

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could not be cured completely yet. The conventional treatments for CA, including cryotherapy (freezing), surgical removal, carbon dioxide laser and electrofulguration, are often associated with an unsatisfactory response, a high recurrence rate and scarring. Also, all these treatments have limitations in that they can only remove visible warts. Latent HPV infection can still exist and serve as a source of recurrence and transmission.[1,2]

CO_2 laser surgery is one of the most effective treatment modalities of condylomata acuminata, but the limitation is that only the wart alone is physically destroyed, while the latent HPV in the surrounding tissue remain present with the potential of reactivation.

Topical photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) is a promising technique in local treatment of proliferative diseases. It was first described by Kennedy *et al.* in 1990.[3] However, the topical application of ALA results in a shallow penetration depth (<2 mm) into tissue.[4,5] Therefore, the limited penetration depth of the photosensitizer prevents topical ALA-PDT from sufficiently entering into and ultimately destroying multiple wart lesions.

ALA-PDT has shown efficacy as a technique for the treatment of superficial anogenital warts as well as latent HPV infections.[6,7] ALA-PDT treatment for urethral CA has achieved a satisfactory effect,[8] but for multiple CA, its efficacy was not satisfactory.[9] With the aim of improving the clinical outcomes and reducing the recurrence rate of multiple CA, we combined these two treatments which had not been systemic studied and conducted a randomized controlled trial to compare the efficacy and safety of laser plus ALA-PDT with those of laser in the treatment of patients with multiple CA.

2. Subjects and Method

2.1. *Subjects*

About 130 patients with at least three (maximum 20) anogenital CA or one CA lesion larger than 0.5 cm (maximum 2.4 cm) in diameter were invited to participate in the study, which includes 10 cases with lost visiting information and 120 cases with complete data. The diagnosis of CA was made based on clinical examination and also the acetowhitening test (the result of the test is defined as positive if the suspected lesions turn white after the topical application of a 5% acetic acid solution and a 3 min waiting time). All wart lesions were located in the anal area or external genitals. The patient group comprised 78 men and 42 women, the age range was 21−70 years, with an average of 43.65 years. Course of disease preceded 1 week to 2 years, average was 9.52 months. In the treated patients, recurrence times was 1−9 times, average was 5.07 times, recurrence cycle was 14−130 days, average was 33.55 days (Table 1). All the cases had exhibited the following: (1) no topical remedy in the preceding 2 weeks, (2) no systematic therapy in the preceding 4 weeks, (3) no systemic disease, no immunosuppression, no hypoimmunity, no pregnancy or breast-feeding, (4) no another sexually transmitted disease.

2.2. *Study design*

This was a randomized, masked-observer, double-blind, parallel group, placebo-controlled study designed to evaluate the efficacy and safety of CO_2 laser plus ALA-PDT with CO_2 laser for the treatment of multiple CA. The patients were randomly allocated into the combined group (CO_2 laser plus ALA-PDT, $n = 60$) or CO_2 laser group (CO_2 laser plus placebo-PDT, $n = 60$). Open-comparing method was adopted. Age, gender, course of disease, number of rashes of two groups was not statistically significant ($p > 0.05$). After a screening period, if the warts were not completely resolved one week after the first treatment, a repeat treatment was conducted once a week for a maximum of three weeks. The lesions were evaluated blindly after each treatment session by the same trained dermatologist. All patients gave oral and written informed consent. The study protocol was reviewed and approved by the Ethics Committee of TongJi Medical College Hospital.

Table 1. Characteristics of the patient population.

Characteristics	CO_2 laser plus placebo-PDT	CO_2 laser plus ALA-PDT
Total number of patients, n	60	60
Female/male	20/40	21/39
Mean age	43.1 + 8.77	43.9 + 8.05
Course of disease	9.48	9.56
Mean number of lesions	8.48 ± 4.71	8.45 ± 4.85

2.3. *CO_2 laser group*

After routine sterilization, the lesion was given topical anesthesia with Lidocaine. CO_2 laser (Shanghai Laser Institute, Shanghai, China) was used. We used continuous wave with an effect of 5−10 W and a laser beam with a spot diameter of 0.7 mm. The lesions and their adjacent normal skin (5 mm border) were vaporized with CO_2 laser one by one until all the lesions were completely removed. After patients were completely cleared of their baseline warts in the CO_2 laser group, an absorbent ball soaked in a placebo solution (sterile 0.9% NaCl without ALA) cover the wart and its adjacent area within 3 mm to ensure the complete cover of lesions and was fixed by plastic film. The lesions were occluded with a food-grade cling film and covered with thick gauze for light protection. Patients were asked to lie still until irradiation was performed. The time interval between drug application and irradiation was 3 h. Light irradiation of 80 J/cm^2 at 100mW/cm^2 was applied to the lesion and the adjacent normal skin (5 mm border) using a semiconductor laser machine (KDL-300, Beijing Kedian Microwave Electronic Co. Ltd) emitting a 635-nm laser light. If the warts were not completely resolved one week after the first treatment, a repeat treatment was conducted. Placebo solution and light doses used for the second course were same as the first treatment. The treatment was no more than three times.

2.4. *Combined group*

Lesions were sterilized and anesthetized routinely, then were treated by a CO_2 laser. We used continuous wave and a laser beam in the same way as those in the CO_2 laser group. When the surface of lesions after treatment was not higher than the adjacent skin, CO_2 laser treatment ended. The treatment merely caused the coagulated necrosis of the surface, forming a thin crust. After crust was washed by sterilized physiologic saline, ALA-PDT treatment was performed. Patients had their warts incubated in the same way as those in the CO_2 laser group, only this time their warts were incubated using 20% ALA (Fudan Zhangjiang Bio-Pharm Co. Ltd, Shanghai, China) for 3 h. Afterwards, light irradiation of 80 J/cm^2 at 100 mW/cm^2 was applied to the lesion and the adjacent normal skin (5 mm border) using a semiconductor laser machine (KDL-300, Beijing Kedian Microwave Electronic Co. Ltd, Beijing, China) emitting a 635-nm laser light in the same manner as described above for the CO_2 laser group. If the warts were not completely resolved one week after the first treatment, a repeat treatment was conducted. ALA and light doses used for the second course were in the same way as those in the first treatment. The treatment was no more than three times.

2.5. *Assessment*

Laboratory-based evaluation was conducted for all the patients for routine check of blood and urine, liver function and renal function before and after the treatment. The number, location and area of the warts and the adverse effects were evaluated for each patient at each follow-up. Follow-up evaluations were conducted at 4, 8 and 12 weeks after the treatments.

2.6. *Statistical analysis*

Data were analyzed by SPSS11.5 statistical analysis software. Difference between two groups was evaluated with the Chi-square test.

3. Results

3.1. *Clinical efficacy*

A total of 120 patients with CA were treated by CO_2 laser plus placebo-PDT or CO_2 laser plus ALA-PDT (Tables 2 and 3). After two treatments, the complete response rates in the CO_2 laser group and combined group were 100% (509/509) and 100% (507/507) in the CA ($P > 0.05$), respectively. The recurrence rates in the CO_2 laser group and combined group were 44.9% (229/509) and 10.6% (54/507) in the CA after 12 weeks of follow-up ($p < 0.001$), respectively. The recurrence rate of CA in the anal (11.5%) in the combined group is higher than that of external genital (9.8%), and the recurrence rates of 3−5 lesions in the combined group were 4.7%. The average recurrence cycle became 40.19 days by combined treatment, which was delayed compared to only laser treatment (27.80 days).

3.2. *Safety evaluation*

There is no obvious change in the reports of routine blood and urine checkup, liver function and renal

Table 2. Response rates and recurrence rates of patients with CA in combined group or CO_2 laser group.

	Combined group						CO_2 laser group					
	3−5 lesions		6−10 lesions		11−20 lesions		3−5 lesions		6−10 lesions		11−20 lesions	
	Anal area	External genital	Anal area	External genital	Anal area	External genital	Anal area	External genital	Anal area	External genital	Anal area	External genital
Total cases	10	11	11	10	9	9	10	10	11	11	9	9
Number of lesions	40	44	82	78	130	133	41	42	81	82	131	132
Reponse rates												
1[a]	92.5% (37/40)	95.4% (42/44)	90.2% (74/82)	92.3% (72/78)	90% (117/130)	90.2% (120/133)	95.1% (39/41)	97.6% (41/42)	95% (77/81)	96.3% (79/82)	90.5% (118/131)	91.7% (121/132)
2[b]	100% (40/40)	100% (44/44)	100% (82/82)	100% (78/78)	100% (130/130)	100% (133/133)	100% (41/41)	100% (42/42)	100% (81/81)	100% (82/82)	100% (131/131)	100% (132/132)
Recurrence rates[c]%	5% (2/40)	4.5% (2/44)	8.5% (7/82)	7.7% (7/78)	15.3% (20/130)	12% (16/133)	29.2% (12/41)	24.3% (10/42)	44.4% (36/81)	39% (32/82)	53.4% (70/131)	52.2% (69/132)

[a]Response rates after one treatment.[b]Response rates after two treatment.[c]During the follow-up period. ALA, aminolevulinic acid; PDT, photodynamic therapy.

Table 3. Total response rates and recurrence rates of patients with CA in combined group or CO_2 laser group.

	Combined group			CO_2 laser group		
	Anal area	External genital	Total	Anal area	External genital	Total
Number of lesions	252	255	507	253	256	509
Reponse rates	100% (252/252)	100% (255/255)	100% (507/507)	100% (253/253)	100% (256/256)	100% (509/509)
Recurrence rates	11.5% (29/252)	9.8% (25/255)	10.6% (54/507)[a]	46.6% (118/253)	43.3% (111/256)	44.9% (229/509)[a]

[a]The recurrence rates in the two groups were evaluated with the Chi-square test. (Pearson Chi-square value = 149.04, $P = 0.000$).

function tests of all the patients before and after the treatment.

The adverse reactions of the treatment by ALA-PDT are: In the beginning of laser irradiation, most patients experienced a stinging sensation or intense pain, and the intensity of pain gradually dissipated. However, for some patients, the pain did not dissipate until 24 h after the treatment. The first day after PDT treatment, the wart was grey and mild erythema appeared around it. In the following days, CA was gradually responding to regression without scarring. In all cases, no adverse reactions occurred during the ALA-PDT treatment. Mild edema occurred in one or two days after treatment and dissipated in three to four days, with some tissues having effusion, but without the ulcer formation. The adverse reaction rate was 100% in control group. The side effects in CO_2 laser group mainly included pain, erosion, ulcer, hyperpigmentation and hypopigmentation. Scar formation was seen in three cases in control group. The adverse reaction rate was significantly higher in CO_2 laser group than that in the combined group.

4. Discussion

CA caused by HPV infection ranks third in the sexually transmitted diseases in China. HPV infection also plays a part in some types of periungual, head and neck squamous cell carcinomas. HPV-related squamous cell carcinomas show a higher risk of local recurrence than non-HPV-related squamous cell carcinomas, which can lead to metastasis if not detected early.[10] For the long period of HPV infection and high recurrence which easily causes cancer, it was very important to reduce the recurrence rate of CA in the treatment.

CO_2 laser for genital condylomata is a rapid and effective mode of treatment and can be done on an outpatient basis, but it can easily cause ulcer and scar. CO_2 laser usually targets only visible wart lesions, and high recurrence rates (10%−65%) result partly from the location of HPV as far as 1 cm from the clinical border of the warts.[11] One reason postulated for frequent wart recurrence is the lack of antiviral activity. Thus, only the wart alone is physically destroyed, while the latent HPV in the surrounding tissue remain present with the potential of reactivation.[12]

ALA-PDT is based on light-induced oxidation reactions that lead to cell death. The mechanism of cellular damage includes a variety of biochemical and molecular reactions, generating a complex process that leads to tissue destruction at both macrostructural and microstructural levels.[13−15] Some basic investigations proved that ALA-PDT could induce various host immune responses.[16−19] Giomi *et al.*[20] suggested that rapid activation of specific immunity, CD4+ T lymphocytes and dendritic cells induced by ALA-PDT could be responsible for healing the recalcitrant CA. Moreover, AIA-PDT can destroy the latent HPV in the surrounding tissue.

The ALA-PDT is a promising treatment for CA, but it can be affected by many factors, such as the light, penetration depth of drugs, etc. The visible spectrum part of the light is useful in PDT, especially the red light (> 600 nm) have better penetration in tissues, and it can reach 5 mm in depth. However, energy dropped sharply when penetrating deeper, consequently, it produces inferior photodynamic effect.[21]

In our study, the combined treatment group had the merit of CO_2 laser in vaporizing the lesions, moreover, the recurrence rate was lower and side effects were less. The recurrence rate of CO_2 laser is consistent with Schultz's study,[11] and the higher recurrence rate was related with the lack of antiviral activity and the failure to eliminate the latent HPV. After two treatments, the recurrence rate in the combined group was 10.6%, which was significantly lower than the CO_2 laser group (44.9%). There are three potential reasons to explain the difference between two groups. First, CO_2 laser therapy in the combination vaporize only the visible lesions above the surface of skin, which reduce the thickness of the lesion and do not cause the scar, meanwhile, it can increase the permeability of the skin, penetration depth of ALA and valid therapy depth of laser. Second, compared to CO_2 laser treatment, which may miss some of the subclinical human papillomavirus-infected cells of CA, the combination of CO_2 laser and ALA-PDT results in the destruction of visible wart lesions as well as the destruction of surrounding latent HPV infected cells. Third, ALA-PDT could induce various host immune responses, which is an effective way to inactivate intracellular viruses.

The response rate in the combined group (100%) in our study is equal to what was reported in the ALA-PDT group (100%) by Chen and colleagues.[22] The recurrence rate for the combined group by our

study was 10.6%, which are higher than the recurrence rate of the ALA-PDT group (6.3%) by Chen and colleagues.[22] The subjects enrolled in our study are multiple CA patients, which may explain the increased recurrence rate. The average number of warts (8.45 ± 5.5 in the combined group) in our study is significantly higher than that in Chen and colleagues' study (1.5 ± 0.7 in the ALA-PDT group). However, the recurrence rate of 3−5 lesions for the combined group in our study was 4.7%, which is lower than that of the ALA-PDT group (6.3%) in Chen's study, which may be related to the better vaporization of the lesions and further increasing the effect of ALA-PDT. Compared with Mi *et al.*'s study,[23] the response rate in the combined group (100%) in our study is higher than that of Mi *et al.*'s study(70.4%) for the cryotherapy plus ALA-PDT group, but the recurrence rate of the former (10.6%) was lower than that of the latter (12.5%). Although we and Mi *et al.* (the average number of warts 7.0 ± 5.0) are all study the multiple CA, the ability of vaporizing the lesions by CO_2 laser is better than that of cryotherapy, which may be a reason to explain the difference between the two studies. The recurrence rate of CA in the anal (11.5%) is higher than that of external genital (9.8%) in our study, which is consistent with the result of Mi and colleagues.

The present study has some limitations that should be considered. The influence of anesthesia on ALA-PDT is unclear, which is expected to further study. In the combined treatment group, there are still some relapsing patients. This effect can be correlated to the insufficiency of exposure of the lesion to treatment and the limited irradiation scope which is not big enough. It is expected to further the therapeutic effect through making some improvements in the instruments.

5. Conclusion

This study suggests that, compared with CO_2 laser treatment, CO_2 laser plus ALA-PDT is a simple, safe and more effective treatment for multiple CA.

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