9. Present and future of the photodynamic therapy in cervical cancer treatment

Eva Ramón-Gallegos

Abstract. Photodynamic Therapy (PDT) is considered a highly effective method to diagnose and treat a wide variety of pre-malignant and malignant processes characterized by the selective elimination of cancerous cells. PDT is based in the capability of some neoplastic cells to specifically accumulate a photosensitizer (Ps), which is photoactivated with local illumination of a specific wavelength. The Ps reacts direct and indirectly with the molecular oxygen present in cancerous cells by the generation of reactive oxygen species that are cytotoxic. In this review it will be briefly described the natural history of cervical cancer (CC) and its immediate precursor Cervical Intraepithelial Neoplasia (CIN), an analysis of the spontaneous regression of CINs and the management and treatment of CC. In the other hand it is also described the photochemistry and photobiology of the PDT, a review of PDT results on cervical carcinoma and premalignant lesions in others countries and a brief description of in vitro and pre-clinical studies in the treatment of cervical cancer that we realized, and the places where it is applied the PDT in Mexico. Finally it is presented an analysis of the perspectives of the CC treatment with PDT.

1. Introduction

Cervical Cancer (CC) is the second most common cancer among women worldwide, with an estimated of 529,409 new cases and 274,883 deaths in 2008. About 86-88% of the cases occur in developing countries, representing 13% of female cancers [1]. Mexico has a population of 37.45 million women of 15 years old or older who are at risk of developing CC. Current estimates indicate that every year 10,186 women are diagnosed with CC and 5,061 die from the disease. Cervical cancer ranks as the 2nd most frequent cancer among women in Mexico, only slightly below breast cancer [2], and the 2nd most frequent cancer among women between 15 and 44 years old [3]. The WHO reported that there are a projected number of cervical cancer deaths in 2025 of 8,374 in Mexico. Palacio-Mejia, et al. published a study in 2009 about regional differences in mortality for cervical cancer in Mexico between 1979 and 2006. They found that from the 32 states the following had the highest standardized mortality rates of cervical cancer: Chiapas (16.8), Colima (14.9), Morelos (13.8), Campeche (12.9), Veracruz (12.3), Baja California Sur (12.3) and Nayarit (12.3) and the entities with the lowest rates were Guanajuato (6.7), Nuevo León (6.9) and Distrito Federal (6.9) [4].

Human papillomavirus (HPV) infection is directly related to the development of Cervical Intraepithelial Neoplasia (CIN) and cervical cancer (CC) [5]. The treatment for CIN 1, 2 or 3 (Carcinoma in Situ-Stage 0) associated with VPH involves from cytoreduction, helping the immune system, to surgical methods, passing by cytotoxic agents and immune-modulation. The surgical treatments include the following: loop electrosurgical excision procedure (LEEP) or the transformation zone (LLETZ), CO₂ laser surgery, conization, cryosurgery, total hysterectomy for women with CIN 3 who cannot or no longer want to have children and internal radiation therapy for women who cannot have surgery [6]. The invasive cervical cancer involves the following treatments: radical surgery, radiotherapy and chemotherapy [7]. These therapies have a recurrence rates in 3 or 5 years that could be from 82 and 94% respectively [8] and have severe side effects or sometimes lead to preterm delivery during pregnancy or can not be applied during pregnancy. The Photodynamic Therapy (PDT) is very simple and requires a photosensitizer, light and oxygen, in addition is an alternative non-invasive method with minimal side effects. The purposes of this chapter are: 1. To briefly describe the natural history of cervical cancer, 2. To analyze the spontaneous regression of CINs and describe the management and treatment of CC, 3. To describe photochemistry and photobiology of the PDT, 4. A review of PDT results of on cervical
Photodynamic therapy and cervical cancer

2. The natural history of cervical cancer

38 years ago it was postulated that human papillomavirus (HPV) was linked to cervical cancer, now is established that the cervical cancer and its immediate precursor Cervical Intraepithelial Neoplasia are caused by HPV, other factors associated are: smoking, long-term use of contraceptives and multiparity [9]. Papillomaviruses are small non-enveloped icosahedral viruses of approximately 50–60 nm in diameter containing a circular, double stranded DNA genome (~7000–8000 bp) that exists in a chromatinized state [10, 11]. Their genome has three functional coding regions: “E” a gene coding early viral function, “L” a gene coding late viral function and “LCR” a long control region which lies between E and L [12], and encodes a maximum of eight genes, six of which encode nonstructural or the early proteins E1, E2, E3, E4, E5, E6 and E7 and two of which encode structural or the late proteins L1 and L2. This DNA of viruses belonging to *papillomaviridae* family, are highly epitheliotropic and this very heterogeneous virus family harbours important human carcinogens, causing not only the vast majority of cervical, but also a substantial proportion of other cutaneous or mucosal clinical manifestations ranging from benign hyperplasic epithelial proliferative innocuous lesions to cancer of the genital tract, skin, oropharyngeal [11]. The estimated worldwide HPV DNA point of prevalence is approximately 10%. The highest estimates were found in Africa and Latin America (20–30%), and the lowest in southern Europe and South East Asia (6–7%) [10]. Clifford *et al.*, 2003 made a meta-analysis and found that the most common HPV type in invasive cervical cancer were, in order of decreasing prevalence: HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70. In invasive squamous cell carcinoma, the HPV 16 was the predominant type followed by HPV 18, 45, 31 and 33 in all regions except Asia, where HPV type 58 and 52 were more frequently identified [12]. The International Agency for Research on Cancer (IARC) in its overall evaluation in the monographs on the Evaluation of Carcinogenic Risks to Humans of 2007 reported that several genotypes are defined as high-risk for invasive neoplasia; furthermore, they are subclassified as carcinogenic types (Group 1): 16, 18, 31, 33, 35, 39, 45 51, 52, 56, 58 and 59, probably carcinogenic type (Group 2A): 68, possibly carcinogenic types (Group 2B): 26, 30, 34, 64, 53, 66, 67, 69, 70, 73, 82, 85 and 97 and not classifiable as to their carcinogenicity to human (Group 3): 6 and 11 [7, 10].

Approximately 99.7% of cervical cancer cases or virtually 100% are associated with infection with one or more type of HPV [13], with 60% of
cancer-related infections due to HPV type 16 and an additional 10% due to HPV type 18. This DNA virus requires access to proliferating cells of cervical epithelium. It uses entry points created by micro-erosion of the overlying cell layers to reach the basal layer of the epithelium, where stem cells and progenitor cells reside. The virus may be held as a latent infection, expression of a limited set of latency-associated transcripts, but no production of infections virus, from outset, or it may have caused a subclinical or unnoticed clinical infection. In the last, the virus integrates its genome into the chromosomes of the host cells.

HPV viral genes E6 and E7, oncoproteins E6 and E7 are major players in carcinogenesis. E7 bind to and triggers the degradation of pRB, thus preventing the sequestration of E2F. E6 forms a complex with an ubiquitin ligase. The complex bind to p53, and p53 degradation is triggered. This process lead to transformed cervical cells leading to tumors [14]. It has also been shown that chromosomal instability occurs in cells expressing these oncoproteins, including monosomy, chromatid gaps, and aberrant chromosomes. These chromosomal changes can lead to CIN, which is characterized by enlarged nuclei, irregularities in nuclear contour and decrease cytoplasmic content. CIN involves reversible changes in the cervical tissue from a normal state, in which no abnormal cells are observed on the surface of their cervix upon cytology, to varying states of cellular abnormalities that ultimately lead to cervical cancer. This sequence forms the premise on which cytology screening for cervical cancer is based and corresponds to an underlying multistep carcinogenic process in the development of cervical intraepithelial neoplasia (CIN). Low-grade squamous intraepithelial lesions (LSILs) may progress to high-grade (HSILs) and invasive cervical cancer or may regress to a normal state [15]. There is a lag time between HPV infection and cervical cancer of at least 15 and 20 years [16]. The International terminology in CC is the following [3]: In Europe three grades are recognized for cervical intraepithelial neoplasia (CIN 1, 2, 3), while in the United States, the Bethesda classification for cytology recognizes two classes low-grade SILs (LGSILs) and high-grade SILs (HGSILs). Then CIN and SIL are two commonly used terms to describe precancerous lesions or the abnormal growth of squamous cells observed in the cervix. SIL is an abnormal result derived from cervical cytological screening or Pap smear testing. CIN is a histological diagnosis made upon analysis of cervical tissue obtained by biopsy or surgical excision, then low-grade cervical lesions (LSIL/CIN 1) are defined by early changes in size, shape, and number of abnormal cells formed on the surface of the cervix and may be referred to as mild dysplasia, LSIL, or CIN 1. High-grade cervical lesions (HSIL/CIN 2/CIN3/CIS) are defined by a large number of precancerous cells on the surface of the cervix that are distinctly
different from normal cells. These lesions have the potential to become cancerous cells and invade deeper tissues of the cervix. These lesions may be referred to as moderate or severe dysplasia, HSIL, CIN 2, CIN 3, or cervical carcinoma *in situ* (CIS) or Carcinoma *in Situ*-Stage 0. This last is when the cancerous cells are confined to the cervix and have not spread to other parts of the body. Finally, invasive cervical cancer (ICC)/Cervical cancer is considered if the high-grade precancerous cells invade deeper tissues of the cervix or to other tissues or organs. Cervical cancer can be of two types: Invasive squamous cell carcinoma when is composed of cells resembling those of squamous epithelium, and adenocarcinoma when has a glandular elements and adeno-squamous when these element are intermingled. The clinical stages of cervical cancer are described as follows according to the International Federation of Gynecology and Obstetrics (FIGO) staging classification (Table 1).

**Table 1.** International Federation of Gynecology and Obstetrics (FIGO) staging classification for cervical cancer [17].

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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</table>
| Stage I | Carcinoma that is strictly confined to the cervix; extension to the uterine corpus should be disregarded. The diagnosis of both stages IA1 and IA2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion.  
**Stage IA**: Invasive cancer identified only microscopically. Invasion is limited to measured with a maximum depth of 5 mm and no wider than 7 mm.  
**Stage IA1**: Measured invasion of the stroma no greater than 3 mm in depth and no wider than 7 mm in diameter.  
**Stage IA2**: Measured invasion of stroma greater than 3 mm but no greater than 5 mm in depth and no wider than 7 mm in diameter.  
**Stage IB**: Clinical lesions confined to the cervix or preclinical lesions greater than stage IA. All gross lesions, even with superficial invasion, are stage IB cancers.  
**Stage IB1**: Clinical lesions no greater than 4 cm in size.  
**Stage IB2**: Clinical lesions greater than 4 cm in size. |
| Stage II | Carcinoma that extends beyond the cervix, but does not extend to the pelvic wall. The carcinoma involves the vagina, but not as far as the lower third.  
**Stage IIA**: No obvious parametrial involvement; involvement of up to the upper two thirds of the vagina.  
**Stage IIB**: Obvious parametrial involvement, but not to the pelvic sidewall. |
| Stage III | Carcinoma that has extended to the pelvic sidewall. On rectal examination, there is no cancer-free space between the tumour and the pelvic sidewall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or a non-functioning kidney are Stage III cancers.  
**Stage IIIA**: No extension to the pelvic sidewall, but involvement of the lower third of the vagina.  
**Stage IIIB**: Extension to the pelvic sidewall or hydronephrosis or non-functioning Kidney. |
| Stage IV | Carcinoma that has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum.  
**Stage IVA**: Spread of the tumour into adjacent pelvic organs  
**Stage IVB**: Spread to distant organs |
3. Cervical cancer and its treatments

3.1. Spontaneous regression of CINs

Before to describe the treatment for CINs is important to note that these premalignant lesions may regress spontaneously. CIN 1 has regression rates of 53-70%, whereas in adolescents and young women over 91% show regression [18]. Therefore in US a conservatively manage with observation, rather than treatment is recommended [19]. On the other hand, CIN 3 is considered a true precancerous lesion (Carcinoma in Situ-Stage 0) with potential to progress to an invasive cancer. Advanced CIN 3 lesions progress to cancer at a rate of 30–50% and a 47% regression at 18 month [20]. There is more information about CIN 2. The annual regression rate of CIN 2 in adult women is estimated to be around 40% [21]. Moscicki et al., 2010, made a prospective study of CIN 2 in adolescents and young women, and found that regression of CIN 2 was common with almost 70% regressing to normal within 3 years. However there are factors associated with CIN 2 regression and progression to CIN 3 that correlated with HPV persistence, specifically HPV-16/18 infections. They found that the combined hormonal contraceptive use and age at menarche support the premise that reproductive hormones are important influences on persistence and progression [18].

Figure 1. Regression comparison without therapy of CIN 1, 2 and 3 in women with ≤25 or >25 years old with an obtained minimum at the 8 month end point.
Castle et al., compared the cumulative occurrence of CIN 2 (n = 397) and CIN 3 or worse (n=542) diagnosed by the Pathology Quality Control Group in three trial arms immediate colposcopy, human papillomavirus (HPV) triage, and conservative management over the 2 year duration of the triage study trial, they evidenced that approximately 40% of undiagnosed CIN 2 will regress over 2 years, but CIN 2 caused by HPV16 may be less likely to regress than CIN 2 caused by other high-risk HPV genotypes. HPV16 positive CIN 2, the most strongly linked with CIN 3 and least regressive, probably warrants inclusion with CIN 3 for management [21].

McAllum et al., 2011 in a study with 98 women with CIN 2 diagnoses found that 62% showed spontaneous regression with a median of 8 months observation [22]. Regressions average of the CINs is shown in Figure 1.

### 3.2 Conventional treatment for CINs and invasive cervical cancer

The treatment of CIN 1, 2 or 3 (Carcinoma in Situ-Stage 0) associated with VPH goes from cytoreduction, to surgical methods, passing by cytotoxic agents (Trichloroacetic acid, podophyllin, podofilox, and 5-Fluorouracil) and immune-modulation (Imiquimod and interferons). The surgical treatments include the following: Loop electrosurgical excision procedure (LEEP) or the transformation zone (LLETZ), laser surgery, conization, cryosurgery, total hysterectomy for women with CIN 3 who cannot or no longer want to have children and internal radiation therapy for women who cannot have surgery. Invasive cervical cancer is treated by surgery, radiotherapy and chemotherapy [7]. All the therapies have recurrence rates that are describe in the table 2.

Management of cervical cancer patients is associated with high costs due to the utilization of several therapeutic strategies and more frequent appearance of disease progression/recurrence [23]. Recurrences in cervical carcinoma are 82% after the first three years of diagnosis and of 94% after five years [8]. Treatments have different limitations and complications. Radical surgery: pelvic sepsis, pelvic thrombosis and post-operative pneumonia, rectovaginal or vesicovaginal fistula [7], sometimes leads to preterm delivery during pregnancy. Radiotherapy: acute side effects including radiation-induced inflammation of the rectum (proctitis) and urinary bladder (cystitis). Late complications such as bowel obstruction and rectal-vaginal and vesicovaginal fistula formation may occasionally occur [7]. In chemotherapy some cytotoxic drugs are known to cause myelosuppression, pulmonary fibrosis, thrombocytopenia and neuropathy as well as unspecific toxicity [24].
Table 2. Treatment recurrences for premalignant lesions (CINs) and invasive cervical cancer. UI: unreported information.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Therapy</th>
<th>Time</th>
<th>Recurrences</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premalignant lesions (CIN 1, 2 and 3)</td>
<td>Podophyllin resin</td>
<td>6 weeks</td>
<td>30-70%</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>Tri- and bichloroacetic acid</td>
<td></td>
<td>10-70%</td>
<td></td>
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<tr>
<td></td>
<td>5-Fluorouracil</td>
<td>6 weeks</td>
<td>10-70</td>
<td></td>
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<tr>
<td></td>
<td>Cryotherapy</td>
<td>6 weeks</td>
<td>20-70</td>
<td></td>
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<tr>
<td></td>
<td>Electrosurgery or laser</td>
<td>3 weeks</td>
<td>6-51%</td>
<td></td>
</tr>
<tr>
<td>Invasive cancer at 5-year survival</td>
<td>Conization</td>
<td>UI</td>
<td>2%</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Chemoradiation therapy (IA2 to IB2)</td>
<td>67 month</td>
<td>11.5%</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Surgery or radiotherapy</td>
<td>UI</td>
<td>10-20%</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>IB-IIA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Radical hysterectomy and pelvic radiotherapy (RT)</td>
<td>19 month (local recurrence)</td>
<td>70%</td>
<td>[28]</td>
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<tr>
<td></td>
<td>IB-IIA</td>
<td>33 month (distant recurrence)</td>
<td>26%</td>
<td></td>
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<tr>
<td></td>
<td>25 month (both local and distant recurrence)</td>
<td></td>
<td>4%</td>
<td></td>
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<tr>
<td></td>
<td>Radiotherapy alone</td>
<td>UI</td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>stage IB</td>
<td></td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stage IIA</td>
<td></td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stage IIB, stage III, stage IVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pelvic exenteration</td>
<td>UI</td>
<td>30-60%</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Surgery Chemotherapy and radiotherapy</td>
<td>UI</td>
<td>23-94%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cisplatin-based chemotherapy</td>
<td>UI</td>
<td>50-83%</td>
<td></td>
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</table>

3.3 Photodynamic Therapy

Photodynamic Therapy (PDT) is considered a highly effective method to diagnose and treat a wide variety of pre-malignant and malignant processes. This therapy is characterized by the selective elimination of cancerous cells. PDT is based in the ability of some neoplastic cells to specifically accumulate a photosensitizer (Ps), which is photoactivated with local illumination of a specific wavelength. The scientific basis of photodynamic therapy were established by Professor Hermann von Tappeiner, who introduced the term "photodynamisagerscheinung" or photodynamic reaction as one that needed light, a photosensitizer substance and oxygen. The first report on the action of activated substances by light in biological systems was realized in 1900 by Oscar Raab, German student of medicine and pupil of the Professor von Tappeiner, who described the lethal effect on a
protozoan that causes malaria, treated with acridine orange dye [31]. He observed that neither the light or dye alone had an apparent lethal effect on cells, but together they were really cytotoxic. The first oncologic use of PDT was in 1903 carried out by Von Tappeiner and Jesionek both medical dermatologists who used 5% eosin dye and visible light to treat skin cancer, condyloma lata and lupus vulgaris. In 1911 Housmann initiated the experiments with hematoporphyrin observing that served to detect tumors. In 1924 red fluorescence was observed in certain malignant tumors when they were exposed to ultraviolet light (UV), assuming it to endogenous porphyrins. In 1942 Auler, Banze Finge, Weiland and Manganiello, described the hematoporphyrin located in neoplastic tissue. In 1958 the laser described by Einstein in 1917 was developed, allowing its use in PDT [32]. The modern era of PDT began in 1961 when Lipson et al. reported the use of hematoporphyrin derivative (HpD) in the Mayo clinic, to detect fluorescence of tumor tissue and then treat a patient with recurrent breast carcinoma, using hematoporphyrin derivative (DHp) and the localized exposure of tumor to light [33]. In 1972 Diamond et al. reported the destruction of glioma cells in tissue culture and in tumors of mice with white light after the injection of hematoporphyrin. In 1976, Kelly and Snell induced necrosis in papillary bladder tumors without damaging healthy tissue using a derivative of hematoporphyrin. In 1975 Dougherty et al. at Roswell Park Cancer Institute reported the radiation about 50% of transplants of subcutaneous tumors in mice and rates using intraperitoneal HpD and red light directly to the tumor. This was achieved without damage to surrounding tissue. Nevertheless, it was until 1979 that Dougherty et al. reported the first series of patients treated with HpD, encouraged by the responses of patients who had failed with the traditional therapies. This achievement stimulated the researchers to develop a suitable laser and fiber optical systems for radiation. In 1985 and 1986 Dougherty, Kessel and Moan described the development of sensitizers for the disease treatment [34, 35].

In the last 30 years the molecular mechanism and application of the PDT using DHp for the treatment of malignant tumors of humans and animals has been explored. Indeed, numerous clinical and experimental investigations have continued with DHp and new photosensitizers to examine his efficiency in a variety of tumor locations, as well as in the manufacture of an ideal system of irradiation for PDT. Nowadays the PDT is approved for his use in the clinical treatment of malignant intraoperative and intracavitary lesions in USA, European Community (EU), England, Canada, Russia, Japan, South Korea and Latin America in countries as: Mexico, Brazil and Argentina.
3.3.1 Photophysics and photobiology of PDT

PDT involves the simultaneous presence of a photosensitizing substance, light of an appropriate wavelength and the presence of oxygen in the tumor tissue. The interaction of these three components will be explained by Jablonski's diagram (Figure 1). The photosensitizer in its electronic basal state is in a singlet state ($S_0$). After absorbing light of an appropriate wavelength (Abs), it reaches a first excited singlet state of short-life ($S_1$ or $S_2$). Suppose that the excitation is in an electronic level, $S_2$. A nonradiative crossing from $S_1$ to $S_2$ is generally the dominant mechanism. This crossing between two electronic states of the same spin multiplicity is called internal conversion (IC). This IC process is then followed by a rapid vibrational relaxation where the excess vibrational energy is dissipated into heat. The photosensitizer in $S_1$ can return to its basal state ($S_0$) emitting the absorbed energy as fluorescence ($F$), useful in the Photodynamic Diagnosis (PDD), or by internal conversion (IC). Alternatively, the photosensitizer ($S_1$) can switch to a first triplet excited state ($T_1$) through a process known as intersystem crossing (ISC). This is a forbidden transition (spin forbidden); however, a good photosensitizer achieves a high yield of triplet state. $T_1$ state has a half-life long enough to take part in chemical reactions, so that the photodynamic action is mediated mostly by the photosensitizer in this energetic state. The photosensitizer in its triplet ($T_1$) can perform an electron or hydrogen transfer to neighboring molecules (water, biomolecules or oxygen), after this redox reaction the radical resultant can lead to the production of peroxy radicals and trigger other reactions resulting in the generation of free radicals and peroxides. If it is the oxygen who receives the electronic transfer it is formed directly the superoxide ($O_2^-$), this process is called reaction Type I. When the molecular energy transfer occurs directly between the photosensitizer in its triplet state and basal state molecular oxygen, forming highly reactive singlet oxygen ($^1O_2$), this is called reaction Type II [36, 37, 38]. Both reactions lead photodynamic action and can trigger cascades of biochemistry, biophysical, immunological, and physiological reactions, resulting in destruction of the irradiated tumor [36]. Also, the photosensitizer in its triplet state ($T_1$) can return to its basal state ($S_0$) emitting a photon and generating phosphorescence (Phos) [37, 39, 40]. Finally, the photosensitizer is degraded by light. This process is known as photobleaching [40].

There are key parameters that determine the ability of a photosensitizing compound. These are the yield or production of singlet oxygen ($\Phi_d$) that is the probability that a photosensitizer having absorbed a quantum of light is converted to the triplet state, and then transfer their excess energy to
**Figure 2.** Photophysical basic process of PDT. After irradiation of the photosensitizer, a photon is absorbed, then the Ps goes from a basal state \( (S_o) \) to an electrically excited state \( (S_1 \) or \( S_2) \). If the electron is in \( S_1 \) can return to its basal state by fluorescence emission \( (F) \), this is used to realize Photodynamic Diagnosis \( (PDD) \) or may have an electronic rearrangement and perform an intersystem crossing \( (ISC) \) in order to generate the excited triplet state \( (T_1) \). The triplet can transfer its energy or charge to generate singlet oxygen \( (type II reaction) \) or reactive oxygen species \( (reaction type I) \), both reactions lead to action photodynamic \( (PDT) \).

molecular oxygen resulting in the formation of singlet oxygen. While the yield of the triplet state \( (\Phi_T) \) is the probability that a photosensitizer, after absorbing a quantum of light, convert to the triplet state \([40]\).

### 3.3.2 Photosensitizers

The effectiveness of photosensitizers is mainly based on the yield of triplet states \( (\Phi_T) \) of the molecule that leads to the generation of reactive oxygen species \( (ROS) \), these include the hydroxyl radical \( (\cdot OH) \), superoxide radical ion \( (\cdot O_2^-) \), hydroperoxyl radical \( (\cdot OOH) \) and hydrogen peroxide \( (H_2O_2) \), all products of oxygen reduction. These species are generated by a type I mechanism as mentioned above, in addition to the generation of singlet oxygen \( (1O_2) \) by a type II mechanism, both explained above \([41]\).
Other important variables to consider with regard to the effectiveness of the Ps are: concentration and intracellular localization (microtubules, membrane organelles, plasma membrane, nucleus and lysosomes and mitochondria especially) of these during irradiation, selectivity, pre-incubation period in cells target [42], wavelength, optical properties of tissues and tissue metabolic status.

### 3.3.3 Classification of photosensitizers for photodynamic therapy

**First generation:** this group includes hematoporphyrin (Hp) and analogs such as hematoporphyrin derivative (HPD) or Porfimer sodium or Photofrin ® developed in the 1970s and early 1980s [40, 43, 44]. The first generation of photosensitizers has been studied extensively in clinical and experimental studies. Unfortunately they have several disadvantages such as that prolonged exposure patient present photosensitivity (poor clearance) and lack of long wavelength absorption [45].

**Second generation:** to enhance the effect of photodynamic therapy several photosensitizers have been developed. These include porphyrin derivatives or modified tetrapyrrolic (porphyrin ®) as benzoporphyrin (Visudyne ®), chlorin (Temoporfirin ®), and porphycene. Metallated derivates have also been synthesized such as phthalocyanines, naphthalocyanines, texaphyrins, chlorins or bacteriochlorins. All of these have the advantage of being pure compounds and well characterized. These are also effective singlet oxygen generators, possess strong absorption peaks in the range of 650-800 nm, high selectivity targeting subcellular compartments, including the mitochondria, and a relatively rapid elimination [39, 40, 46].

**Third generation:** This generation of photosensitizers have properties that allow their selective accumulation in the tumor. This can be achieved through the conjugation of biomolecules such as monoclonal antibodies, single-chain monoclonal antibody, LDL and folate molecules [43, 44]. An alternative approach would be to use a molecular carrier as a liposome or targeted nanospecies. Shen et al. have synthesized photosensitizer-doped conjugated polymer nanoparticles that can act as novel photosensitizing agents for two-photon photodynamic therapy, making it a more efficient PDT [47]. All strategies that have been implemented have led the third generation of photosensitizers to generate selective photodamage without affecting normal tissue [46].

There are 6 hypotheses to explain the selective accumulation of porphyrin photosensitizers in malignant tissue [48]: 1. Neoplastic cells, in common with other rapidly proliferating cells, may have an increased
requirement of cholesterol for the membrane biosynthesis. The cells can therefore regulate the expression of receptors of low-density lipoproteins (LDL), which recognizes the lipoprotein apo B/E. It is known that most of these lipoproteins are lipophilic porphyrins conveyors into the blood stream and may therefore be the entrance of these compounds into cells; 2. An intratumoral decrease in pH can affect the ionization of porphyrin species with weakly acidic pKa being thus retained within the tumor; 3. Tumors often have an increased number of lipid bodies and vacuoles of neutral lipids. Also, their cell membrane can be more hydrophobic than normal cells. Both phenomena could explain the accumulation of hydrophobic photosensitizers; 4. A combination of "permeable" tumor vascularity and reduced lymphatic drainage could stimulate the accumulation of porphyrins in the interstitial space; 5. Tumor cells have increased capacity for phagocytosis or pinocytosis of porphyrin aggregates; and 6. Tumor-associated macrophages (TAM) can increase significantly the concentration of porphyrins in tumors. It has been found that TAM may contain up to 9 times more porphyrin levels in tumor cells than normal cells.

On the other hand it has been proposed the use of δ-aminolevulinic acid to induce one of the most active endogenous photosensitizer, protoporphyrin IX, which is present in low concentrations in normal cells, but at high levels in tumor cells [38, 49]. The advantages of this method, is that the ALA and its metabolites are eliminated within 24 h after administration, so the problem of skin and optical photosensitivity that produce the photosensitizers currently used in clinical treatments is eliminated. In addition, the endogenous photosensitizer PpIX has a defined molecular structure; the maximum absorption for this is at 630 nm [38, 50, 51, 52]. This photosensitization method is based on the following observations [38]: a) in each cell there is formation of porphyrin as heme precursors. b) The ferrochelatase enzyme, which converts protoporphyrin IX to heme, is decreased in several types of cancer and tissues in regeneration. The opposite was found for porphobilinogen deaminase (PBGD). Taking advantage of these two characteristics PDT has been applied using ALA to treat skin cancer.

Most of the current photosensitizers have some fluorescent emission. For PDT efficiency it is desirable to have a triplet state quantum yield as high as possible, which competes with the fluorescent quantum yield. However the Ps fluorescence may be utilized in a number of ways such as: in vivo lesion detection, in vivo lesion localization for therapy guidance, both PDT and surgical, quantification of the concentration of Ps in the target and other tissues in vivo for the purposes of pharmacokinetics and dosimetry, measurement of the photosensitizer photobleaching during PDT light
activation, Ps quantification in tissue samples \textit{ex vivo} and determining the microdistribution of Ps in tissues or cells by fluorescence microscopy [53].

3.3.4 Oxygen in PDT

The effects of photodynamic therapy for the majority of the photosensitizers used, except for some cyanines, are oxygen-dependent [51]. The dependence of $O_2$ in the tissues has been well established [38, 54, 55]. There are two mechanisms which can lead to a decrease in oxygen levels in tissue during photodynamic therapy: 1) the oxygen consumption during photodynamic process by the formation of singlet oxygen from molecular oxygen and 2) the vascular damage caused by treatment, which reduces blood flow and therefore the supply of molecular oxygen. The damage to blood vessels contributes to tumor control after photodynamic therapy on having produced severe hypoxia / anoxia [38].

Some studies have shown that when oxygen is present at levels below 16 torr (2%), the cells are resistant to photodynamic therapy. \textit{In vitro} studies for Photofrin II, cell death is complete with 40 torr (5%) oxygen pressure and only half of this cell death with a pressure of 8 torr (1%) [51, 56]. Various techniques for photodynamic therapy have been proposed to facilitate tumor oxygenation. The flow velocity of light can be adjusted (reduced) for a slow consumption that allows the maintaining of the oxygen level in the tumor during treatment [38, 54, 55], or the modulation of light delivered in short periods (about 20-50 seconds), and periods of darkness to allow reoxygenation [38]. These procedures do not affect pre-existing hypoxic cells to treatment, so there are studies that use hyperoxygenation techniques to overcome cellular hypoxia and improve tumor response to photodynamic therapy [54].

3.3.5 Light sources used in photodynamic therapy

There are three main classes of clinical PDT light sources: lasers, Light Emitting Diodes (LEDs) and filtered lamps. Typically, 1.5 W of usable power are required in the 630-850 nm ranges at irradiances of up to several hundred mW cm$^{-2}$ in order to deliver treatments in tens of minutes [57]. The light delivery technologies more utilized are surface and interstitial irradiation with optical fiber, linear diffuser fiber into an endoscope instrument channel and balloon applicators for endoscopic esophageal PDT, intracavitary irradiation of brain tumor surgical resection bed using a balloon filled with intralipid to scatter uniformly the light delivered by an optical fiber, transcorneal delivery and nasopharyngeal applicator [57, 58].
3.3.6. Antitumor effects of the Photodynamic Therapy and cellular induced death

There are three principal mechanisms by which photodynamic therapy carries out tumor destruction: direct lethal effects on tumor cells caused by reactive oxygen species, damage to tumor-associated vasculature thereby limiting the blood supply to the region, and the activation of an immune response against cells [59]. Cells respond to photodynamic damage by initiating a rescue mechanism and/or lead to cellular death either by apoptosis or necrosis. In addition, autophagy is the 3rd pathway of cellular death induced by PDT [60].

4. Use of the PDT worldwide for cervical carcinoma and premalignant lesions

Since 1960 Lipson et al., [61] demonstrated HpD uptake in malignant tumors of cervix as well as in those of the bronchus and other sites. Later, Ward et al. (1982) made the first reports of therapeutic use of HpD to treat gynecologic tumors in humans [62]. With these works began the 1st stage of the efficiency investigation of PDT in gynecological cancers. In the decade of the 80s and early 90s there was great interest to demonstrate the efficiency of PDT in gynecological cancers and several works were published, such as Soma (vaginal and vulvar cancer, CIS of uterine cervix and metastatic to the vagina), Tokuda (Bowen’s disease) and Kubota in 1983 (stage I vaginal cancer, Bowen’s disease of the vulva, metastatic breast to the endometrium and metastatic adenocarcinoma to the vagina), Rettenmeier in 1984 (Cervical cancer recurrent to vaginal apex, stage III B), MacCaughan in 1985 (multifocal squamous cell cancer of the vagina), Lobraco in 1986 (cancer of the vulva, vaginal or perineal region) [63], Lele in 1989 (recurrent cervical cancer) [64], Huang en 1991 (genitourinary carcinomas) and Hetzel in 1993 (recurrent vulva carcinoma) [63]. In all of them total remissions were found with percentages ranging from 22 to 88.5% with the exception of the work of Tokuda (1983)[63] where complete response of Bowen's disease was achieved (100%) with no evidence of recurrence 18 months later. This may be due largely to the use, at the time, of the photosensitizers of the 1st generation, which have lacked long wavelength absorption [45] to allow deep penetration in tissue. In addition, it is important to notice that many of gynecologic cancers were in advanced stages. In some of the works the patients had already received traditional therapies and were multi-resistant. Nevertheless, the results were valuable and inspired the use of other photosensitizers with larger wavelength absorption peaks and different
strategies for administration, and also monitoring the fluorescence to determine the time of maximum accumulation in tissue and development of systems for efficient delivery of light into the lesion. The 2nd stage of investigation of the efficiency of PDT in gynecological cancers began with the work of Koren [65], who obtained a complete response to 100% in cervical cancer, although with a minimal number of samples. In the Table 3 the research that has been conducted in our group since 1996 with regards to PDT application to treat pre-cancerous lesions of cervix (CINs), early and non-invasive cervical cancer (CIS), squamous cell Ia carcinoma (invasive micro-invasive cancer according to FIGO classification) is summarized.

As can be observed, the most commonly used photosensitizers in PDT for CIN and premalignant and malignant lesions are ALA-PpIX and Photofrin. Of the rest there are mostly unique works. However all the Ps tested have a complete remission of more than 50% of the treated patients (Figure 2). Photofrin has shown to be effective in more than 80% for CIN I and CIN II, and over 90% for CIN III, achieving an impressive 100% remission in Squamous Cell Carcinoma Ia lesions. It is important to mention that the results of Photofrin are reproducible (Table 3). It has even been approved its use in Japan by the Japanese Ministry of Health and Welfare, and since April 1996, patients have been covered by Japanese National Health Insurance Program for treating cervical cancer in addition to gastric cancer [66, 67].

In turn the ALA-PpIX is the 2nd most tested photosensitizer in cervical cancer, as can be seen in Table 3. This Ps has given controversial results; for example in the works of [68, 69, 70, 71], the complete removal (CR) does not exceed 50%, but there are additional works such as [62, 72, 73] where the CR of the lesions is over 90%.

In none of the studies the spontaneous regression (SR) of CINs was considered. Taking into account that the average spontaneous regression for CIN I, II and III are 80, 65 and 43%, respectively, it can be inferred that the results found of PDT for CIN I cannot be distinguished if they correspond to PDT or SR, but for CIN II and III it can be clearly seen that the reference to the PDT exceeds the 40-50% spontaneous regression, which clearly favors the use of PDT to treat CIN.

Both photosensitizers seem to show the same efficiency (75-80%) in removing the lesions associated with HPV (Figure 3).

In order to improve the efficiency of both photosensitizers ALA-derivatives, such as hexyl-aminolevulinate (HAL) have been developed. These reach up to 63 % of efficiency for the complete elimination of CIN lesions. In addition, new Ps have been developed, such as Photogem, Photosen and Photolon. These eliminate 90 to 94 % of lesions (Table 3).
Table 3 shows the interest of the scientific community for the use of this therapy to treat the cervical cancer. This is reflected in an increase in the number of works each year (Figure 4).

**Table 3.** Review of the PDT literature with different photosensitizers and light sources in premalignant lesions (CINs) and malignant cervical cancer in humans.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Ps</th>
<th>PDT conditions</th>
<th>n/CR (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical uterin cancer</td>
<td>Photosa III®</td>
<td>632 nm/200J/cm²</td>
<td>1 (100%)</td>
<td>[65]</td>
</tr>
<tr>
<td>(Stage T2 N0)</td>
<td>(2 mg/kg)</td>
<td></td>
<td>1 (Partial responses)</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer <em>in situ</em></td>
<td>HP (5 mg/Kg)</td>
<td>514.488 y 630</td>
<td>2 (100%)</td>
<td>[63]</td>
</tr>
<tr>
<td>CIN 3</td>
<td>nm/200J/cm²</td>
<td></td>
<td>1 (100%) CIN 3</td>
<td></td>
</tr>
<tr>
<td>Cervical Ca <em>in situ</em> and</td>
<td>Photofin®</td>
<td>630 nm</td>
<td>56 (96.4%) CIN and</td>
<td>[67]</td>
</tr>
<tr>
<td>dysplasia</td>
<td>(1 mg/Kg)</td>
<td></td>
<td>dysplasia</td>
<td></td>
</tr>
<tr>
<td>CIN 1, 2, 3</td>
<td>Photofin®</td>
<td>630 nm/100-140</td>
<td>22 (68%)</td>
<td>[74]</td>
</tr>
<tr>
<td>(4 mg/Kg)</td>
<td>J/cm²</td>
<td></td>
<td>CIN lesions</td>
<td></td>
</tr>
<tr>
<td>CIN lesions</td>
<td>ALA-PpIX</td>
<td>630 nm/100 J/cm²</td>
<td>8 (50%)</td>
<td>[68]</td>
</tr>
<tr>
<td>(20%)</td>
<td></td>
<td></td>
<td>CIN lesions</td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td>Photofin®</td>
<td>630 nm/100 J/mm</td>
<td>30 (96.8%)</td>
<td>[67]</td>
</tr>
<tr>
<td>CIS</td>
<td>(75 mg/vial)</td>
<td></td>
<td>Dysplasia</td>
<td></td>
</tr>
<tr>
<td>SCC Ia</td>
<td></td>
<td></td>
<td>93 (96.7%) CIN</td>
<td></td>
</tr>
<tr>
<td>Ec-Ad-ca Ia</td>
<td></td>
<td></td>
<td>3 (100%) SCC Ia</td>
<td></td>
</tr>
<tr>
<td>CIN 1 and 2 with HPV</td>
<td>ALA-PpIX</td>
<td>Red Light/100J/cm²</td>
<td>20 (95%)</td>
<td>[72]</td>
</tr>
<tr>
<td>(12%)</td>
<td>652 nm/50 J/cm²</td>
<td></td>
<td>CIN 1/2 12 (83%) low-risk</td>
<td></td>
</tr>
<tr>
<td>CIN 2 and 3</td>
<td>ALA-PpIX</td>
<td>635 nm/100 J/cm²</td>
<td>4 (0%)</td>
<td>[69]</td>
</tr>
<tr>
<td>(20%)</td>
<td></td>
<td></td>
<td>CIN 2 3 (0%)</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer <em>in situ</em></td>
<td>Photofin®</td>
<td>630 nm/100 J/cm²</td>
<td>31 (96.8%)</td>
<td>[67]</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>(2 mg/Kg)</td>
<td></td>
<td>Dysplasia</td>
<td></td>
</tr>
<tr>
<td>CIN 2 and 3</td>
<td>ALA-PpIX</td>
<td>630 nm/increments of 25</td>
<td>13 (31%)</td>
<td>[70]</td>
</tr>
<tr>
<td>(20%)</td>
<td>J/cm²; beginning at 50-150 J/cm²</td>
<td></td>
<td>CIN 2 13 (32%) CIN 3</td>
<td></td>
</tr>
<tr>
<td>CIN 2 and HPV</td>
<td>ALA-PpIX</td>
<td>630 nm/100 J/cm²</td>
<td>11 (91%)</td>
<td>[73]</td>
</tr>
<tr>
<td>CIN 1, 2, 3 and HPV</td>
<td>Photofin®</td>
<td>630 nm/100 J/cm²</td>
<td>31 (90%)</td>
<td>[75]</td>
</tr>
<tr>
<td>(2 mg/Kg)</td>
<td></td>
<td></td>
<td>CIN lesions 29 (76%) HPV</td>
<td></td>
</tr>
<tr>
<td>CIN 1, 2, 3</td>
<td>ALA-PpIX</td>
<td>635 nm/100 J/cm²</td>
<td>13 (33%)</td>
<td>[71]</td>
</tr>
<tr>
<td>(3%) Placebo</td>
<td></td>
<td></td>
<td>CIN lesion with ALA 13 (31%) With Placebo</td>
<td></td>
</tr>
<tr>
<td>CIN 1, 2, 3 and HPV.</td>
<td>Photofin II</td>
<td>635 nm/100 J/cm²</td>
<td>105 (95%)</td>
<td>[76]</td>
</tr>
<tr>
<td>(2 mg/Kg)</td>
<td></td>
<td></td>
<td>CIN lesions 64 (72-75%) HPV</td>
<td></td>
</tr>
<tr>
<td>CIN 1, 2 and 3</td>
<td>HAL-PpIX</td>
<td>633 nm/100 J/cm²</td>
<td>24 (63%)</td>
<td>[77]</td>
</tr>
<tr>
<td>(2 mg/Kg)</td>
<td></td>
<td></td>
<td>CIN lesions 24 (63%) HPV</td>
<td></td>
</tr>
<tr>
<td>CIN 3</td>
<td>Photogem®</td>
<td>56 (80.2%) CIN 3</td>
<td>56 (80.2%)</td>
<td>[78]</td>
</tr>
<tr>
<td>Cervical cancer <em>in situ</em></td>
<td>Photosen®</td>
<td>35 (94.2%) CIN 3</td>
<td>16 (68.8%)</td>
<td>[78]</td>
</tr>
<tr>
<td>CIN 3</td>
<td>Photosens®</td>
<td></td>
<td>12 (83.4%) CIN 16</td>
<td></td>
</tr>
<tr>
<td>CIN 2, 3 and HPV</td>
<td>ALA-PpIX</td>
<td>635 nm/100 J/cm²</td>
<td>5 (100%)</td>
<td>[79]</td>
</tr>
<tr>
<td>(118 mg/g)</td>
<td></td>
<td></td>
<td>CIN lesions 5 (100%) HPV</td>
<td></td>
</tr>
<tr>
<td>CIN 2 and 3</td>
<td>Photolense®</td>
<td>670 nm/100 J/cm²</td>
<td>104 (92.8%)</td>
<td>[80]</td>
</tr>
<tr>
<td>(1.0-2.5 mg/kg)</td>
<td></td>
<td></td>
<td>HPV</td>
<td></td>
</tr>
</tbody>
</table>

CR: Complete response; n: Number of patients; HP: Hematoporphyrin; HpD: Derivative Hematoporphyrin; HAL: Hemacinelevulinate; DHE: Deuteroporphyrin esterether; SCC: Squamous cell Carcinoma; Ec-Ad-ca: Endocervical adenocarcinoma; n: patients; Photosens: Sulfonated aluminum phthalocyanine. Photolense: combination of chlorin e6 potassium salt and low-weight polyglycerol. 

**Explanatory note:** though current nomenclature indicates that CIN III and CIS are the same thing, nevertheless it is respected the terminology used by the authors of each work.
Figure 3. Efficiency analysis of the PDT in: CIN lesions, SCC-Ia and VPH, using different photosensitizers and irradiation conditions. CIN III includes CIS.

Figure 4. Results of a PubMed and Scirus search showing the number of published original articles of PDT in patients with CIN and Cervical Cancer.
4.1 Application procedure of PDT in uterine cervix

In this review PDT protocols that presented the highest efficiency in the removal of cervical cancer are presented.

4.1.1 Treatment of cervical cancer patients with Ps

a) Photofrin (Porfimer sodium)

After confirmation of the diagnosis, Photofrin is administrated in doses of 2-4 mg/kg, with a drug-light interval of 24-72 h intravenously or using a cervical cap (Figure 5). For this, 2 mL of a 1% solution of Photofrin in a 4% Azone and isopropyl alcohol vehicle are applied to the cervix 24 h prior to PDT [74, 81]. The patient should stay in the room with a brightness of 10 lux immediately before the intravenous injection.

After treatment, all patients are hospitalized in a dark room with protection from light for 3 weeks. In the case of Photofrin patients became extremely sensitive to sunshine for about a week. When exposed to intense light, the exposed skin reddens and may develop optic hyperesthesia and edema. From the 5th day on, the intensity should be 15 lux and patients should be allowed to watch television. After the 8th day the intensity should be ≤ 30 lux and increased from 11th day the intensity to ≤60 lux. After the 16th day the intensity should be ≤ 80 lux and from the 19th day the intensity should be ≤ 100 lux. After the 20th day, the restriction on brightness is lifted and the patients may leave the hospital after sunset on that day. The patient should use UV screen make-up cream and sun-shield cream. When going out of the room, sunglasses, a hood, gloves, socks, a scarf, and a long sleeve dress should be worn, so not to be exposed to unnecessary light [67, 76].

![Figure 5. Cervical cap or Cervix-Adaptor, disposable, for drug delivery at the cervix, threat length: 330 mm from Wisap (Sauerlach, Germany). This was developed by Professor Dr. Kurt Semm (also known as the father of laparoscopic surgery). The image is courtesy of Dr. Isolde Semm of Wisap Company.](image-url)
b) ALA-PpIX

After confirmation of the diagnosis, 2-3 g of 5-ALA gel (118 mg/g) is topically applied on the cervix and covered with a special plastic cap or cervical cap (Figure 5) for 3-4 h [79]. Alternatively, 5 to 10 mL of 12% w/v solution of 5-ALA (and EDTA 1% w/v dissolved in either 0.9 sodium chloride) is sterile filtered or dissolved in Lutrol F-127, thermolabile bioadhesive 19% poloxamer 407 gel which is liquid at 4°C and sticky at temperatures >31°C, are topically applied to the cervix canal using an appropriately sized cervical cap (Figure 5). After 4 to 8 h, the cervical cap is removed [72, 82].

The toxicity of ALA is tolerable and only consists of spotting, vaginal discharge, mild cramping, and vaginal warmth [70]. As ALA is a natural body compound, which is completely metabolized within 24 h, the problem of prolonged skin sensitivity is avoided by its use [83].

4.2. Preparations of patients before irradiation

1. Check patient’s vital signs and confirm that patient has finished urinating. 2. Patients should wear a cloth to cover entire body and then proceed to the laser treatment room. 3. After putting on socks patient should get on table and then use paper tape to fix bath towels to minimize exposed areas. 4. Patient should cover genitals, other than the parts for irradiation by using gauze, and fix it by paper adhesive plaster. 5. Patients should wear sunglasses during the entire treatment period. 6. A speculum should be inserted in a manner such that mobility is maintained, and fix the position slightly so as not to slide. 7. After sterilization of vagina using a disinfectant and a 3% acetic acid should be applied [67].

4.3. Estimation of shape and area of pathologic lesions at cervix by photodiagnosis (PDD)

After ALA or Photofrin application the shape and area lesions at cervix are estimated by means of conventional white-light and fluorescent colposcopy, for the fluorescent images registration the colposcope should have a high sensitivity CCD camera and LED light source for fluorescent excitation [80]. The fluorescence in CINs lesions showed a threefold for CIN 3 than normal cervix and a ratio of 1.3 for CIN 1 and 1.21 for CIN II [83].

4.4 Conditions of irradiation (PDT) with Photofrin and ALA

After removed the ALA or Photofrin gel, 1. The lesions located at the uterine cervix should be irradiated, particularly around the worst lesions at
Figure 6. Cylindrical light catheter for ecto and endocervical application of PDT, this system is effective for homogenous Light distribution (Biolitec GMBH, Germany) [84].

an energy intensity rate 100-200 J/cm² with 100 mW/cm² 630 nm of wavelength for Photofrin [67] and 100 J/cm² with 100-150 mW/cm² 635nm of wavelength for ALA-PpIX per shot from non-thermal laser like semiconductor laser [79] or YAG-OPO laser [75] or excimer dye laser. Alternatively, a laser with a special cylindrical light catheter with a backscattering surface for homogenous light distribution (Figure 6) can be used. This applicator is able to illuminate both ecto- and endocervical canal homogenously for 1000 s, administering a light dose of 100 J/cm² [77]. Is possible to use a thermal light source (150 W halogen lamp) emitting a broadband of red light (total energy: 100 J/cm², fluency rate: 90 mW/cm²) for superficial illumination of the portion and an Nd:YAG pumped dye laser to illuminate the cervical canal (total energy: 50 J/cm², fluency rate: 300 mW/cm²) [72]. 2. A large amount of cervical mucus is produced by irradiation. It should be removed using swabs or a 1 cc syringe for cervical mucus extraction whenever needed. 3. When is not possible use a light applicator for homogeneous light distribution to ecto and endocervical canal, a spot irradiation should overlap. The pattern should resemble the Olympic Game logo. The uneven surfaces and angles such as the cervical canal should be irradiated in all direction and use a cervical probe. Irradiation should be repeated as the inserted fiber is drawn from the cervical canal in 1 mm increments till it drop out from the external uterine orifice [67].

4.5 Evaluation of the effectiveness of PDT in cervical cancer

A colposcopic analysis, cytological and histological examinations, immunohistological of molecular markers of cellular death, HPV-DNA by
PCR diagnostics or hybrid capture and bacteriological examination are necessary after PDT. The PDT efficacy is evaluated by gynecological examinations in 3, 6 and 12 months and then every year alternatively after PDT [76, 80, 84]. The patients should be classified on the basis of clinical and morphological finding using the following common criteria [80]:

- Complete remission or complete response (CR): When the examinations shows no abnormal findings, in other words all lesions are completely cured in terms of cytological, colposcopic and histopathological finding.
- Partial remission or partial response (CR): Almost all lesions are cured, but cytological, colposcopic, and histopathological findings suggest that some lesions may not yet be cured. At least 50% reduction of a pathologic lesions area or absence of clinical signs of CIN but positive results of morphological examination of the cervical epithelium.
- Stabilization or stable disease (SD): Less than 50% reduction of pathologic lesions area for a stabilization of the cytological differentiation.
- Progression or progressive disease (PD): At least a 20% increase in the sum of the longest diameter for all target lesions.

4.6 Advantages of PDT in Cervical cancer

The current treatments for CIN are invasive. Unfortunately, these treatments often lead to adverse effects such as hemorrhages, endometriosis, and traumatization of subjacent tissues with a formation of rough scraps, stenosis and structures of the cervical channel. Changes in the anatomical structure of the cervix uterine lead to the loss of its functionality and obstetric problems, e.g. reduction of cervical secretion, resulting in a decrease of successful conception probability and increase of spontaneous abortion and perinatal mortality, as well as impediment of normal delivery [77, 80]. Contrary to this, PDT is minimally invasive with no surgical excision; it should be a cervix-sparing treatment, which may be particularly attractive to women desiring to preserve fertility [76]. Other advantages with regard to conventional methods are the high selectivity of tissue destruction, absence of surgical interventions and low risk of severe systemic complications after treatment [75]. As to the disadvantages, cutaneous photosensitivity with some photosensitizers is important since patients cannot be exposed to sunlight from 2-3 days to 4 weeks, but when using ALA-PpIX and its derivatives this effect is eliminated.
A comparison of PDT using topical 5-ALA with cold-knife conization yield similar clearance rates (75%) of human papillomavirus (HPV) at 3 months and disease-free rates at 12 months (PDT, 91%; cold-knife, 100%). PDT preserved the function and structure of the cervix better than did surgery [73].

In addition the PDT is also economically appealing, for example Dr. Soergel in Germany evaluated the economical aspect of CIN treatment including associated pregnancy complications. He found that the cost of treatment for CIN with a conization procedure alone was 1,473 €/year, whereas the PDT procedure alone accounted for 1,386 €/year in Germany private practice. This is equivalent to US $1943.9 and $1829.1 ($24,805.1 and $23,340.2 Mexican pesos), respectively. Nevertheless, Soergel et al. (2011) added the collateral conization effects on women in reproductive age, such as premature births, which led to a real cost of a conization of 2,046 € as compared to the 1558 € of PDT (US $2,705 and $2,060 or $34,720 and $26,441 Mexican pesos, respectively). In this work the authors concluded that in Germany the PDT has the potential to be a cost-effective treatment for high-grade CIN compared to conization procedure and the increased perinatal morbidity, perinatal mortality and associated costs after conization procedures are significant and may be reduced by implementation of PDT in CIN treatment.

Table 4. Cost in American dollars of diagnosis and treatment in patients with CIN at a private setting in Mexico [85]. *The patient is called to the doctor’s office every 6 months during a period of two years for a cytology test and colposcopy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average cost</th>
<th>Cost range</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Conservatively management with observation only.</td>
<td>$1,161.00</td>
<td>$774-1,548</td>
</tr>
<tr>
<td>Cryosurgery or cryotherapy</td>
<td>$1,548.80</td>
<td>$1,032-2,065</td>
</tr>
<tr>
<td>Electrosurgery without excision</td>
<td>$2,066.63</td>
<td>$1,377-2,755</td>
</tr>
<tr>
<td>CO₂ laser therapy without excision</td>
<td>$2,337.00</td>
<td>$1,558-3,116</td>
</tr>
<tr>
<td>Loop electrosurgical excision procedure (LEEP)</td>
<td>$2,333.40</td>
<td>$1,555-3,116</td>
</tr>
<tr>
<td>CO₂ laser therapy with excision</td>
<td>$2,604.00</td>
<td>$1,736-3,472</td>
</tr>
<tr>
<td>Conization with diathermy or Loop electrosurgical excision the transformation zone (LLETZ procedure)</td>
<td>$3,210.40</td>
<td>$2,140-4,280</td>
</tr>
<tr>
<td>Conization with CO₂ laser</td>
<td>$3,479.00</td>
<td>$2,319-4,638</td>
</tr>
<tr>
<td>Conization with a scalpel (cold-knife conization)</td>
<td>$5,037.40</td>
<td>$3,358-6,716</td>
</tr>
</tbody>
</table>
In 2008 Glaxo SmithKline Company determined the costs in Mexico City private medicine that a Mexican woman has to face with CIN or precancerous lesion of cervix uterine, which depends on the diagnosis and therapeutic monitoring scheme that requires the patient, are presented in Table 4.

The conization treatment for CIN III including the diagnosis and follow-up to two years goes from U.S. $1,667.3 to $5,232.1 ($21,403 to 67,165 Mexican pesos) (Table 4). So the implementation of PDT in the treatment of CIN III cheapens the treatment of this disease in Mexico.

5. PDT in Mexico

PDT has been applied in Mexico for 10 years to treat noncancerous lesions and macular degeneration at the Hospital Conde de Valenciana and ISSSTE since 2001 [86, 87], Barrett's esophagus in the hospitals Manuel Gea Gonzalez and Instituto Nacional del Cancerología [88] and actinic keratosis and basocellular carcinoma with cosmetological ends in private institutions as Hospital San José del Tecnológico de Monterrey and Hospital Médica Sur [89]. Nevertheless, cancer is still not in use for treating cervicouterine carcinoma.

Our research "Photodynamic Therapy for Cancer Treatment" began in 1997. Since then, we have carried out projects focused on the application in Mexico. Here are the achievements both in vitro and in vivo in this area in Mexico.

In our first paper we determine the accumulation of Pp IX induced by ALA in cervical cancer cells [90]. We used HeLa and CaLo cervical cancer cells, and normal uterine cervix cells. These were exposed to various concentrations of ALA; we found that these cells accumulated from 1:5 to 1:8 times more PpIX than normal cells of the cervix. Subsequently we determined the time of photobleaching of PpIX in the skin of mice, the location of PpIX in mouse skin by photoacoustic spectroscopy, the pharmacokinetics of PpIX induced by d-aminolevulinic acid (ALA) in the skin of mouse, the possible side effects of ALA by of the ethylene determination as a biomarker, the efficiency of using PDT in cervical carcinoma cells using pulsed light and continuous light and also the time photodecay of PpIX to know the time of each pulse irradiation. We determined that the production of PpIX in the skin of mice is a function of exposure time to ALA. We found two maximum peaks, one at 15 min and another at 2 h after administration of ALA [91]. There is an overlap: we concluded that the first peak corresponds to the PpIX of blood vessels and
the second one to the PpIX of the skin; in addition in this work it was found that the PpIX accumulates it in the dermis. This was confirmed by determination of PpIX in blood, obtained by cardiac puncture. The removal of PpIX could be found at the 480 min. It was also found that the disodium salt solution of PpIX irradiation reaches its photo-elimination maximum at 400 s, therefore a longer irradiation in PDT would be ineffective, since over 90% of the concentration reaches its triplet states at this time [92]. With regard to the lipoperoxidation it was found that ALA should not be utilized above 40 mg/kg since large doses cause important lipoperoxidation. It was also demonstrated that the viability of the cells after treatment with PDT depends on the type of irradiation (pulsed or continuous) since we found a decrease in the viability of HeLa cells after irradiation with an argon laser, when hertz decreased from 1850 to 20 [93].

In the study of the effect of PDT in vitro using CC cells infected with papillomavirus (HPV), we found that SiHa cell line infected with HPV type 16 had sensitivity similar to HeLa with HPV 18, as death in them was 97% whereas C33 showed more resistance to PDT, with a maximum cell death of 84.7%. It is noteworthy that 3% of HeLa and SiHa cells and 15% of C33 cells that apparently survive, die within 48 h after treatment, thus achieving 100% mortality in these cells [94]. Finally, the effectiveness of PDT in a cervical carcinoma model in nu/nu mice was demonstrated. In this, PDT is capable of diminish the volume of a tumor up to 53%, and provoke the death of 90% of tumor cells. It was found that the PDT fulfills in vivo satisfactorily the antitumor function demand of the National Cancer Institute as part of the preclinical studies of phase I [95].

6. Conclusions

With the antecedents discussed in this chapter we can conclude that photodynamic therapy has shown that is effective in eliminating cervical cancer and its precancerous lesions (CIN). It has been even included in Japan as an option to treat gynecological injuries. So far the photosensitizers of the 1st and second generation have achieved on average a complete response from 90 to 97%. This could be improved with the use of 3rd generation photosensitizers that are more selective, with the use of a cervical adapter, a light delivery that could be performed in pulses and with a catheter system that irradiates simultaneously the endo and ecto cervix. On the other hand, PDT has the potential to become an important treatment modality for cervical cancer in women who want to preserve their fertility, in addition to the fact that it exceeds the most common method for CIN in aesthetics and costs. However, more clinical studies are needed to establish treatment
protocols with Ps and use of novel delivery system of drugs that so far have not been tested.

**Future trends**

As we observed with the increasing number of articles per year of the application of PDT in the treatment of cervical cancer worldwide there is a global interest to treat this type of cancer, as a therapy to preserve fertility in young women, non-invasive and side effects are minimal. Works are being published where PDT is combined with other therapeutic strategies to achieve higher levels of efficiency. For example at the beginning of 2012 Tae-Gyu et al. [96], published a paper where the PDT is combined with chemotherapy to what they called chemo-photodynamic therapy (CCPD) to treat cervical cancer 1B1 and 1B2 staged, demonstrating its effectiveness because the women who received this treatment its fertility was preserved and have had no recurrence of the disease for over 6 years. So this could be an alternative to treat cervical cancer and improving the low penetration to tissues that have the photosensitizers such as Photofrin (<5 mm) and low light penetration in tissues, leaving only PDT for in situ tumors. Other option that could improve PDT is to aim to achieve greater accumulation of the photosensitizer in tissues with the use of local systems, such as the cervix-adapter described in this chapter.

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**8. References**


