Quantification of in vivo Photofrin uptake by human pituitary adenoma tissue

USIAKIMI IGBASEIMOKUMO, F.R.C.S.(SN), M.D.
Department of Neurosurgery, Hamad Hospital, Doha, Qatar

Object. Photofrin is widely distributed in the body after intravenous injection. This study was designed to quantify the preferential uptake of Photofrin by pituitary adenoma tissue for intraoperative photodynamic therapy.

Methods. Eight patients (seven men) with recurrent pituitary adenomas who had undergone previous surgery and radiation therapy were recruited for a Phase I/II feasibility study of the application of photodynamic therapy to pituitary tumors. Photofrin was administered intravenously at a dose of 2 mg/kg body weight 48 hours before repeated transsphenoidal hypophysectomy was performed. At the time of the operation, pituitary adenoma tissue, muscle, fat, skin, and plasma were obtained for measurement of Photofrin content by fluorometric assay.

The mean Photofrin level in pituitary adenoma tissue was 6.87 ng/mg (95% confidence interval [CI] 3.99–9.75), which was significantly higher than the uptake by skeletal muscle (2.24 ng/mg, 95% CI 1.28–3.2; p = 0.008), or fat (2.54 ng/mg, 95% CI 0.66–4.42; p = 0.007). Nevertheless, the mean drug concentration in pituitary adenoma tissue was not significantly different from the level in plasma (7.65 μg/ml, 95% CI 5.38–9.90; p = 0.558). Skin specimens were available in four patients, and these showed a mean uptake of 2.19 ng/mg.

Conclusions. Photofrin is preferentially retained by pituitary adenoma tissue to levels both adequate for intraoperative photodynamic therapy and approximately 50% higher than those reported for gliomas.

KEY WORDS • PHOTODYNAMIC THERAPY • PORPHYRIN UPTAKE • PITUITARY ADENOMA

Despite considerable advances in pituitary surgery and the introduction of several potent drugs for hormone-secreting tumors, radiation therapy is still frequently required for the adjuvant treatment of pituitary adenomas. Although histologically benign, these tumors are locally invasive and may recur after surgery alone. Although radiation therapy is effective in reducing local recurrence, it is associated with significant long-term irreversible side effects. In particular, the high incidence of hypopituitarism following radiation therapy limits its application in women who want to get pregnant; hence, there is a need for a more innocuous adjuvant therapy for pituitary tumors.

Photodynamic therapy presents special advantages for the adjuvant treatment of pituitary tumors. First, pituitary tumors are outside the blood–brain barrier, allowing ready delivery of photosensitizer to the tumor. Second, the sella turcica is mostly surrounded by bone, which limits possible damage to adjoining structures from the photodynamic effect. Yano, et al., were the first to report the experimental application of photodynamic therapy in vitro and in vivo in rat pituitary tumors by using phophorhimbine a and white light. Marks, et al., subsequently demonstrated a dose-dependent cytotoxicity of photodynamic therapy on cultured human pituitary adenoma cells by using hematoporphyrin derivative as a photosensitizer and a quartz halogen lamp as a photoilluminator. Approximately 5 years after that study my colleagues and I reported the activity of photodynamic therapy against human pituitary adenoma cells implanted in nude mice; the cells were sensitized with polyhematoporphyrin. None of these studies quantified the uptake of the photosensitizers used, an important determinant of the photodynamic effect. Photodynamic therapy is a binary cancer treatment strategy that owes its selectivity to the preferential retention of photosensitizer by the tumor, which, when activated by an appropriate wavelength of laser light, leads to tumor destruction through the production of singlet O₂. The porphyrins are the most well known of these light-sensitive compounds (photosensitizers) and are characterized by the possession of a tetrapyrrole ring, in common with the ubiquitous “pigments of life”: hemoglobin and chlorophyll. More than four decades ago, Winkelman and Rasmussen-Taxdal argued that quantifying the uptake of porphyrins by tumor was a prerequisite for the investigation of photodynamic therapy. This assertion is still true today, especially given that the uptake of Photofrin varies from tissue to tissue; hence, there is a need to quantify the in vivo uptake of Photofrin by pituitary adenoma.

Clinical Material and Methods

Ethical Approval and Patient Selection

The quantification of Photofrin uptake was performed as part of a Phase I study of photodynamic therapy in patients with recurrent pituitary adenomas treated at the Leeds University Teaching Hospital Department of Neurosurgery. Ethical approval was obtained from the Leeds Research and Ethics Committee and a Doctors and Dentists Exemption

Abbreviation used in this paper: CI = confidence interval.
Quantification of Photofrin uptake by pituitary adenoma

### Table 1

Table showing the Photofrin uptake by pituitary adenoma and normal tissue 48 hours after intravenous injection in six patients.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Histological Findings</th>
<th>Pituitary (ng/mg)</th>
<th>Muscle (ng/mg)</th>
<th>Fat (ng/mg)</th>
<th>Plasma (mg/ml)</th>
<th>Skin (ng/mg)</th>
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<tr>
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<td>3.43</td>
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<td>8.80</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
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<td>3.82</td>
<td>0.69</td>
<td>0.59</td>
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<td>10.07</td>
<td>3.86</td>
<td>5.48</td>
<td>12.57</td>
<td>2.51</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>null</td>
<td>4.25</td>
<td>2.17</td>
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<td>6.62</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>FSH secreting</td>
<td>3.48</td>
<td>1.66</td>
<td>0.77</td>
<td>7.01</td>
<td>3.16</td>
</tr>
<tr>
<td>6</td>
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<td>7.61</td>
<td>1.65</td>
<td>1.74</td>
<td>4.59</td>
<td>2.44</td>
</tr>
</tbody>
</table>

* FSH = follicle-stimulating hormone; NA = not available.

Certificate was obtained in accordance with the relevant laws in the United Kingdom for the experimental use of Photofrin for photodynamic therapy in pituitary tumors.

After I received the appropriate regulatory approvals, eight patients between 29 and 69 years of age who had recurrent pituitary adenomas and who had undergone previous surgery and radiation therapy were recruited (Table 1). The experimental nature of the treatment and the possible side effects were discussed with each patient and his relatives and informed consent was obtained. Pregnant women and patients younger than 21 years of age were excluded.

**Drug Dosage and Administration**

Photofrin was supplied as a freeze-dried dark brown powder in light-proof boxes by QLT Pharmaceuticals, Katendijke, The Netherlands. It was reconstituted by mixing 75 mg dry powder (one vial) with 31.8 ml 5% dextrose, giving a final drug concentration of 2.5 mg/ml. This solution was administered by slow intravenous injection at a dose of 2 mg/kg body weight 48 hours before transsphenoidal hypophysectomy in all eight patients. After injection of the drug, patients were placed in rooms with subdued light and were advised to avoid direct sunlight and intense artificial light for approximately 8 weeks.

**Intraoperative Collection of Samples**

Transsphenoidal hypophysectomy was performed 48 hours after injection of the Photofrin solution. The surgical procedure followed the standard steps of endonasal transsphenoidal hypophysectomy, except that at the end of tumor resection intraoperative photodynamic therapy with 630-nm laser light was delivered to the tumor bed (75 mW/cm² over 1000 seconds). During the procedure, venous blood, pituitary adenoma tissue, muscle, fat, and skin samples were obtained for determination of Photofrin concentration. The muscle and fat were obtained from the right thigh and were used for packing the sphenoid and tumor bed; approximately 5 g of tissue were collected for measurement of the Photofrin level. Immediately after collection the specimens were placed in a cooled light-proof container and transferred to the laboratory. Specimens not analyzed immediately were stored in liquid nitrogen.

**Method of Photofrin Assay**

The photofrin level in the pituitary, muscle, fat, skin, and plasma was determined using the method described by Vernon, et al.43 In common with other fluorometric assays, this method involved the extraction of Photofrin oligomers from tissue or plasma, which are then hydrolyzed to monomeric porphyrins and the amount is measured using fluorescence analysis. Unlike other methods, however, this one calibrates for the acid-stable fraction, which may constitute up to 40% of the drug, and the monomeric species, which are invariably cleared shortly after injection. This method, therefore, provides an accurate measure of the amount of drug in plasma or tissue. The details are reported in Vernon, et al., but the essential steps for the use of this method for plasma start with precipitation of the proteins out of 100 ml plasma with 5 ml of a mixture of ethyl acetate and acetic acid (4:1 vol/vol). After centrifugation, 4 ml of the supernatant is mixed with 1 M hydrochloric acid, which separates the mixture into two phases: an upper layer containing contaminants and bilirubin and a lower aqueous layer containing the porphyrins. The top layer is discarded and the oligomeric porphyrins in the aqueous layer are hydrolyzed to monomers by boiling in a water bath for 30 minutes. The aqueous mixture is cooled to room temperature and the fluorescence is measured (400 nm excitation with 596 nm emission). A blank control is prepared by treating the aforementioned reagents in a similar fashion but without the plasma. A standard solution of Photofrin from the same batch as that given to the patient is mixed with 1 M hydrochloric acid and used to calibrate the extraction process. The procedure is similar for the tissues, but 100 mg of tissue is first dissolved in 1 ml of 1 M NaOH by incubating the mixture overnight in a shaking water bath at 40°C. The alkali is neutralized with 50 μl of phosphoric acid. The rest of the procedure is similar to that used with the plasma. Calibration curves that accommodate for the acid-stable component as described by Vernon, et al., were used to calculate the amount of Photofrin in the plasma and tissues.

**Data Analysis**

The SPSS program for Windows (version 11) was used to analyze all data. The paired (dependent) Student t-test was used to compare the mean Photofrin levels in different tissues and the measured levels of Photofrin were adjusted for within-subject design for the error bars as suggested by Loftus and Masson.31 A 5% level of significance (α-error, two-tailed) was accepted for differences between means, and the 95% CIs for the means are indicated in parentheses.

**Results**

Eight patients were recruited for this Phase I/II feasibility study. Transsphenoidal hypophysectomy was abandoned.
in two of them, leaving six patients with a complete set of samples (pituitary, muscle, fat, and serum) that were analyzed for this study. The clinical outcome has been reported elsewhere.\textsuperscript{32} The mean of two determinations in plasma and tissue was taken as the final level of Photofrin. The clinical characteristics of the six patients are shown in Table 1. The Photofrin level in pituitary adenoma tissue, in nanograms of oligomer per milligram of tissue, ranged from 3.48 to 11.99 (mean 6.87; 95% CI 3.99–9.75). In muscle the level of Photofrin ranged from 0.69 to 3.86 ng/mg of tissue (mean 2.24; 95% CI 1.28–3.2) and in fat the drug level ranged from 0.59 to 5.48 ng/mg of tissue (mean 2.54; 95% CI 0.66–4.42). The mean drug level in pituitary adenoma tissue was therefore significantly higher than the concentration in muscle (mean 2.24, p = 0.008; Fig. 1 upper) and fat (mean 2.54; p = 0.007; Fig. 1 center). The drug concentration in pituitary adenoma tissue was not significantly different, however, from the level in plasma (range 4.59–12.57 \textmu g/ml, mean 7.65; p = 0.558); the adjusted error bars for plasma and pituitary adenoma tissue were almost identical (Fig. 1 lower). The mean uptake ratio of pituitary adenoma tissue to muscle was 3.3 (range 1.96–5.54) and for fat it was 4.02 (range 1.84–6.47). Skin specimens were available in four patients and these showed a mean uptake of 2.19 ng/mg (range 0.67–3.16) with a mean pituitary skin uptake ratio of 3.4 (range 1.1–5.7).

**DISCUSSION**

**Photofrin Uptake**

I chose Photofrin as the photosensitizer because nearly four decades of clinical use\textsuperscript{29} attest to its safety and it is one of the most widely used and studied photosensitizers in that time.\textsuperscript{8} Laws, et al.,\textsuperscript{25} remarked on the use of hematoporphyrin derivative to localize pituitary adenomas in vivo, but as far as I am aware this study is the first report of the quantitative determination of the in vivo uptake of Photofrin by human pituitary adenoma tissue. I have demonstrated that weight for weight, pituitary adenomas take up Photofrin to more than three times the level in normal tissues such as muscle and fat. Several authors have confirmed that the normal brain takes up less Photofrin than muscle;\textsuperscript{2,3, 13,27} hence it would be reasonable to expect that the drug level in the normal brain of the patients presented here would be less than in muscle. The effect of photodynamic therapy on the normal brain has been the subject of considerable controversy, however: in early reports it was suggested that there was very little uptake (and hence no photodynamic therapy–induced damage),\textsuperscript{20} yet several authors have demonstrated dose-dependent brain tissue damage from Photofrin photodynamic therapy.\textsuperscript{5,26} It was not possible or ethically desirable to compare the uptake in pituitary adenoma tissue with normal pituitary glands, because in this group of patients there was very little normal pituitary tissue left after repeated surgery and radiation therapy. The levels of Photofrin we obtained in pituitary adenoma tissue were nearly one and a half times those reported for gliomas in vivo.\textsuperscript{15,19} This is not surprising because Photofrin uptake in the central nervous system parallels blood–brain barrier permeability.\textsuperscript{17,19} Thus my findings indicate a need to consider reduced doses of Photofrin for photodynamic therapy in pituitary adenomas, with the potential benefit of reduced skin sensitivity and reduced risk of damage to the adjacent normal brain.\textsuperscript{12}

**Mechanism of Preferential Uptake**

It has been suggested that porphyrins are taken up by tumors via low-density lipoprotein receptor–mediated endocytosis.\textsuperscript{18} In addition, several other factors have been proposed, including the decreased conversion of porphyrins to heme in tumors (due to low ferrochelatase activity in tumor) and reduced or absent lymphatic drainage in tumors.\textsuperscript{21,42} Nevertheless, it is much more likely that the nearly identical levels in plasma and pituitary adenoma seen in this study...
are the result of free equilibration along a concentration gradient, given the very porous capillaries in pituitary tissue. The lower levels in muscle and fat, however, could be the result of both lower uptake and conversion of Photofrin to heme. Attempts to identify the subcellular localization of Photofrin in pituitary adenoma tissue by using fluorescence microscopy and immunocytochemical markers for factor VIII and macrophages did not yield consistent results (U. Igbaseimokumo, unpublished data). Reliable data from this procedure would have offered important clues to the site of action and mechanisms involved in Photofrin-photodynamic therapy in pituitary adenomas because singlet O₂ generated during photoactivation invariably acts at the site of production and does not last long enough to diffuse far.34,40

Limitations of Fluorometric Assay

Photofrin is a complex mixture of monomeric and oligomeric porphyrin molecules.9 The highly fluorescent monomeric species (10–20%) in Photofrin are cleared shortly after intravenous injection (within 1 hour or so), leaving the nonfluorescent oligomeric portion in plasma and localized to tissue.21 Most fluorometric methods depend on the extraction and hydrolysis of the oligomers to monomeric porphyrins, which are measured using fluorometric analysis. Unfortunately, up to 40% of the oligomeric porphyrins in tissue or plasma are acid-stable and nonfluorescent in aqueous solution. The method published by Vernon, et al.,43 accounts for these limitations by using Photofrin from the same batch of drug as that administered to the patient to calibrate the extraction process as well as correct for the monomers; hence, this modified process is an accurate method of Photofrin assay for clinical use. Furthermore, drug determination by this method has been shown to have a linear relationship with drug determinations made using radioactivity.

Variation in Photofrin Level From Patient to Patient

Although the variation in the level of Photofrin from patient to patient as seen in this study (3.48–11.99 ng/mg of tissue, mean 6.96) is a common observation, there are as yet no satisfactory explanations.2,15 Several reasons, however, may apply: Photofrin is a complex mixture of monomeric and oligomeric porphyrins whose biodistribution is determined by the hydrophobicity of the monomeric species, the charge distribution, and the pKₐ value along with the aggregation/dissociation constant and molecular weight of oligomers. After intravenous injection, Photofrin is more than 90% protein bound, with the monomeric species primarily bound to albumin and the oligomers bound to low- and high-density lipoprotein. Thus, the variations in the amount of Photofrin from patient to patient that were seen in this study and other reports2,27,35 probably reflect variations in the interaction between different patients and the drug due to differences in the aforementioned properties. Interpretation of data is further complicated by the complexity of Photofrin, a mixture whose composition may vary slightly from one batch to another, in addition to possible alteration occurring during transportation, storage, and reconstitution.4 In a pharmacokinetic study involving 12 patients with lung cancer who were given 2 mg/kg of Photofrin intravenously, the peak serum concentration varied from 39.3 to 222 μg/ml (these data appear in QLT Phototherapeutics, Inc., Clinical Investigator’s Brochure, December 1994).

Long-Term Follow Up

Although the primary purpose of this study was to establish the scientific basis for the feasibility study reported earlier,32 these six patients represent the longest follow up to date; hence, their long-term clinical course is summarized here. All six patients had undergone surgery and radiation

**Fig. 2.** A: Coronal post-Gd T₁-weighted MR image obtained 24 hours after photodynamic therapy, revealing the infiltrated enhancing dural capsule (arrows) that marks the outline of the tumor preoperatively. Note the seroma (S) that frequently fills the resection cavity. B: Follow-up MR image demonstrating the collapse and destruction of the dural capsule 6 months after photodynamic therapy; disease has remained stable for more than 5 years.
therapy initially and then developed recurrent tumors. The mean time from the last treatment to recurrence was reported to be 2 years (9–66 months) by Marks, et al.32 It is highly remarkable that at 6 years of follow up after repeated surgery and intraoperative photodynamic therapy there has been no recurrence or progression in any of the five surviving patients. The progressive collapse and destruction of the infiltrated dural capsule, which marks the outline of the preoperative tumor mass (arrows in Fig. 2A) on early serial imaging, indicates that this was the effect of photodynamic therapy (Fig. 2B). Although the current work provides a clear scientific basis for the application of photodynamic therapy as adjuvant treatment for pituitary tumors, long-term efficacy will need to be confirmed in a randomized study in which the long-term outcome (clinical and radiological) in untreated patients is compared with those who received adjuvant intraoperative photodynamic therapy. One patient died of unrelated causes in January 2000, 3 years after receiving intraoperative photodynamic therapy. In the remaining five patients, all of whom suffered hypopituitarism prior to photodynamic therapy, there has been no change in hormone replacement needs and the improvement in vision has been maintained.

CONCLUSIONS

In this study I have shown that the in vivo uptake of Photofrin by pituitary adenoma tissue is three times the concentration in normal tissues such as muscle and fat and is higher than the uptake reported for gliomas. Because the concentration in normal tissues such as muscle and fat and is higher than the uptake reported for gliomas. Because the concentration in the pituitary tissue was virtually identical to the level in plasma, it appears likely that in the pituitary tissue was virtually identical to the level in plasma, it appears likely that in the pituitary. Because the concentration in normal tissues such as muscle and fat and is higher than the uptake reported for gliomas. Because the concentration in normal tissues such as muscle and fat and is higher than the uptake reported for gliomas.

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REFERENCES

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Address reprint requests to: Usiakimi Igbaseimokumo, M.D., Department of Neurosurgery, Hamad Hospital, P.O. Box 3050, Doha, Qatar. email: usiakimi@qatar.net.qa.