EXPERIMENTAL STUDY

Kinetics of intraoperative fluorescence diagnosis of parathyroid glands

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Abstract

Objective: Identification of parathyroid glands is often a challenge even for experienced surgeons. The feasibility and efficacy of fluorescence diagnosis for localization of parathyroids has already been proven in an experimental setting. In preparation for a clinical application of this technique in patients undergoing surgery for hyperparathyroidism, we evaluated the kinetics of fluorescence diagnosis.

Design and methods: Fifty rats were randomized into eight groups with different photosensitization parameters using the photosensitizer aminolevulinic acid (ALA): a peritoneal lavage was performed with either 1.5 or 3.0% ALA solution and the induced photosensitization times varied from 0.5, 1 and 2 to 4 h. Under special fluorescence illumination, D-light, the exposed operative site with thyroid, parathyroid glands and neck muscles was examined. The identified parathyroid glands were studied according to fluorescence intensity by spectrometric measurement and compared with surrounding tissue.

Results: Photosensitizer accumulation in parathyroid glands, indirectly measured by spectrometry, was up to 3.2 times higher than in thyroid and 2.6 times higher than in muscle tissue (2 h photosensitization with 3.0% ALA). Using 1.5% ALA, the optimum fluorescence intensity and ratio/contrast was slightly lower (parathyroid-to-thyroid ratio $\times 3.0$, parathyroid-to-muscle ratio $\times 1.9$) but was reached earlier (1 h) and hence considered as the parameter of choice for a clinical application. **Conclusions:** In future clinical application, intraoperative fluorescence diagnosis is expected to increase the ease of identification of atypically located or supernumerary glands. In combination with preoperative diagnostics, this may result in reduced operation time and avoidance of persistent hypercalcaemia.

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Introduction

Identification of the parathyroid glands (PTGs) is often a challenge even for experienced surgeons. The glands are small, soft, well camouflaged, and variable in position and number, necessitating a meticulous dissection during surgery for hyperparathyroidism (HPT) (1).

Fluorescence diagnosis (FD) using the photosensitizer (PS) aminolevulinic acid (ALA) has been described for identifying PTGs during bilateral neck exploration for HPT in an experimental setting (2). When PTGs are atypically located, this method may be helpful intraoperatively to detect the glands. Hence, the technique may reduce high morbidity rates, long exploration time, and persistent hypercalcaemia.

The pilot study (2) proved the feasibility of FD in PTG surgery: the application of ALA, a precursor in the haem biosynthesis pathway, led to protoporphyrin IX (PpIX) accumulation within the PTGs. After fluorescence induction by blue light PTGs were detectable by emitted red light.

In the pilot study, photosensitization parameters, such as an ALA concentration of 3.0% and a photosensitization time of 2 h, were chosen. The following experimental study evaluated the variation of these two parameters to achieve optimal diagnostic results in the detection of PTG.

Materials and methods

All experiments were carried out in accordance with German laws and protocols approved by experimental animal welfare institutions.

Photosensitization

Fifty rats (Wistar; Janvier, Le Genest Saint Isle, France) (mean weight 244 g) were randomly divided into eight groups with a minimum of six each. The animals were anaesthetized i.p. with ketamine/xylazine and nitrous oxide. Photosensitization was achieved by peritoneal

lavage with two different concentrations of ALA solution (pH 6.5) (Medac, Wedel, Germany): 1.5% (50 mg/kg body weight (BW)) and 3.0% (100 mg/kg BW). Photosensitization times (interval between ALA application and fluorescence examination) of 0.5, 1, 2 and 4 h were chosen. The animals were then kept under subdued lighting conditions to avoid photobleaching and phototoxic reactions.

Operation

A longitudinal anterior collar incision was performed to expose thyroid, PTGs, trachea and muscles.

FD

First the operative site was observed under normal white light conditions to identify the right- and left-sided PTGs using the D-light system (Karl Storz, Tuttlingen, Germany). The system is based on a 300 W xenon short-arc lamp with special optical properties to focus high intensities of light. It allows two different illumination modes: (i) a conventional, white light mode, and (ii) a specific blue light (380–440 nm) mode for ALA fluorescence excitation. The modes can easily be switched any time by a footswitch or directly by a button on the charged coupled device camera head (3).

After white light illumination the operative site was examined under blue light fluorescence conditions (Fig. 1). Visualization was assisted by a modified CCD camera detection system (Telecam SL PDD; Karl Storz) within a cut-off filter to reduce interferences with the blue excitation light.

Spectrometry

Fluorescence intensities of PTGs, surrounding thyroid tissue and muscles were measured by spectrometry:

Figure 1 Operative site under blue light fluorescence conditions. The PTG is visualized by its red fluorescence, also observed under fluorescence microscopy.

the excitation light of the D-light was transferred to the tissue via one leg of a bifurcated fibre bundle (Avantes, Eerbeek, The Netherlands). The common tip of the fibre was brought into contact with the lesion and the second leg of the fibre was connected to an optical multichannel analyser (Avantes). Spectral analysis was achieved by a specific software (SpectraWin; Avantes). Background signals were subtracted from the spectra and fluorescence intensities measured at one typical, maximal emission wavelength of PpIX at 635 nm. From each tissue sample multiple spectral intensities were gained and averaged. A fluorescence ratio was determined by comparing the fluorescence intensity of PTGs with the score measured for thyroid and muscle tissue.

Histology

Fluorescence visualization allowed safe biopsy sampling of each PTG. All random biopsies were confirmed as PTG tissue in haematoxylin and eosin staining and under fluorescence microscopy (Fig. 1). There were no false positives in this series.

Results

Photosensitization and fluorescence of the PTGs were seen as functions of time after ALA application and of ALA concentration. Spectrometry of fluorescence intensities served as an indirect parameter of PS accumulation in the examined tissue specimen.

The maximum uptake of ALA in PTGs, thyroid gland and muscle was 2 h after photosensitization using 1.5% ALA (Fig. 2) and 4 h using the 3.0% ALA concentration (Fig. 3). However, in the 3.0% ALA group, diminution of fluorescence was not evident even after 4 h of observation, indicating the maximum PpIX load in all three tissues was beyond the clinically relevant time frame of 4 h. Compared with the 1.5% concentration,

Figure 2 FD of PTGs with 1.5% ALA. Spectrometric fluorescence intensities of thyroid gland and muscle in ratio to parathyroid gland.

Figure 3 FD of PTGs with 3.0% ALA. Spectrometric fluorescence intensities of thyroid gland and muscle in ratio to parathyroid gland.

the 3.0% ALA solution reached slightly higher absolute fluorescence intensities in spectrometry.

The most effective diagnostic combination in terms of fluorescence ratio/contrast, was observed with 3.0% ALA after 2 h of photosensitization: here, the PTG showed a 3.2-fold stronger fluorescence than in thyroid tissue and a 2.6-fold higher fluorescence intensity than in muscle.

Under these criteria, an only 1 h photosensitization gave best results in the 1.5% concentration group. Despite lower absolute intensities compared with 2 h, fluorescence ratios were superior (PTG-to-thyroid $\times 3.0$, PTG-to-muscle $\times 1.9$).

A randomized number of PTGs, thyroids and muscle were resected after the examination and histologically examined to confirm the macroscopic classification.

Discussion

FD, formerly known as photodynamic diagnosis, is increasingly used to distinguish healthy from diseased tissue in various medical disciplines. The principle of FD is based on the almost specific accumulation of administered agents, PSs, in malignant cells. After i.v., oral or topical application, the drug predominantly concentrates in tumours and remains inactive until exposed to light of a specific wavelength. When light is delivered to the cancer site, either directly or indirectly through a fibre optic device, it causes fluorescence of the PS. Whereas earlier generations of PSs, such as porphyrin sodium, were already fluorescent at the time of application (exogenous PS), the PS ALA requires endogenous metabolism before it acquires fluorescence capabilities (endogenous PS). ALA is the natural precursor of the haem pathway. Administration of ALA, either systemically or locally, overloads the last step in the haem biosynthesis pathway of tumour cells due to missing negative feedback mechanisms and reduced enzymatic activities. This results in an increased accumulation of ALA's metabolite PpIX.

PpIX is a fluorescent agent when stimulated by light of a defined wavelength within its absorption spectrum. One main emission wavelength is within the visible light spectrum at 635 nm (red light). The positive, red fluorescence of PpIX is even detectable in macroscopically invisible tumour foci and can indicate lesions which would be missed when illuminated with conventional white light only.

Although theoretically available for several decades, fluorescence techniques became more attractive for clinical use when more effective generations of PSs such as ALA were clinically implemented and tested. They were intended to reduce common side effects of PSs such as skin sensitivity, nausea, vomiting and transiently elevated liver transaminase levels. ALA-induced PpIX photosensitization showed several advantages: (i) ALA, PpIX and other intermediates are rapidly eliminated from the human organism, thus minimizing the risk of skin phototoxicity; (ii) ALA can be applied locally, providing an acceptable and convenient route of administration for patients; and (iii) the fluorescence ratio between tumour and surrounding healthy tissue is superior to that of other PSs (4).

Our previous investigations were conducted to evaluate endoscopic, laparoscopic and thoracoscopic FD for tumour staging (4–8).

PTG surgery to correct primary HPT is successful in 80–97% of initial explorations. A careful exploration coupled with a thorough knowledge of PTG anatomy and embryology will produce surgical success. Failures are often linked to an inability to locate ectopic PTGs (9).

Preoperative imaging studies will identify the hyperfunctioning PTG in the majority of circumstances. The best types or combination of imaging tests have not been definitely established. However, because of their wide availability, ultrasound and technetium-99m-2-methoxyisobutylisonitrile (Tc-MIBI or sestamibi) scans have become the most commonly ordered preoperative localizing study, with sensitivity rates reported between 80 and 100% (1). But their usefulness is diminished by their inability to consistently identify smaller glands, with sensitivity rates around 50% (10). Because of the two-dimensional nature of the sestamibi scan, along with its inability to localize normal-sized glands and its occasional lack of uptake even in enlarged glands, additional tests including computed tomography or nuclear magnetic resonance may be useful. Based on the clinical setting, especially in circumstances when non-invasive imaging modalities are inconclusive, invasive imaging tests including selective angiography venous sampling and/or direct fine-needle aspiration should be used (11).

Intraoperative diagnostic modalities are useful adjuncts to the safe and successful conduct of PTG surgery. In the 1980s, miniature semiconductor electrodes of high sensitivity and special shape were applied to characterize diverse tissues during operation.

The measured radioactivity of phosphorus reflected its enrichment and storage in the PTG tissue (12). However, the method remained experimental.

More recently, the intraoperative use of sestamibi and a gamma probe has been advocated by some authors as a means of facilitating PTG exploration (13–16). Rubello *et al.* (14) performed minimally invasive radio-guided surgery in preselected patients with primary HPT with a success rate of 97.9%. Using a hand-held gamma collimated probe, which was inserted through a 2–2.5 cm skin incision, even ectopic PTG adenoma in the upper mediastinum and at the carotid bifurcation, as well as glands deep in the para-oesophageal/para-tracheal space, was rapidly found (14). In a prospective series by Nichol *et al.* (16), radio-guided surgery for secondary and tertiary HPT was associated with a cure rate of 100%. It decreased the duration of surgery and length of hospital stay, as well as alleviating the need to obtain frozen sections (16). However, radio-guided parathyroidectomy remains controversial as several studies comparing preoperative imaging and intraoperative sestamibi scans did not recommend the routine use of radio-guidance during surgery (17, 18). In unselected patients with primary HPT, preoperative sestamibi imaging was more accurate than intraoperative gamma probe detection in localizing abnormal PTGs (18). The only advantage of the intraoperative gamma probe was its capability to evaluate the radioactivity in the tissue that was removed and to determine whether it was an enlarged PTG, lymph node, thyroid tissue, or fatty tissue (19). Radio-guided surgery did not substantially influence the surgical procedure in terms of surgical approach and operating time.

The quick intraoperative parathormone assay (qPTH) has proven its reliability and applicability as a predictor of successful surgical treatment of primary and secondary HPT (20, 21). The qPTH can accurately facilitate unilateral, directed neck exploration for uniglandular PTG disease, as well as guide the extent of gland resection for multiglandular disease (22).

The idea of staining PTGs for visual detection is not completely new. Before we used fluorescent markers, intraoperative staining of abnormal PTGs with methylene blue as dye was first described by Dudley in 1971 (23). Since then several studies have proved the effectiveness of this technique (24–27). The staining technique allows the visualization of 85–100% of hyperplastic PTGs; however, it stains normal glands only occasionally. This differentiation can help surgeons to preserve normal PTGs more easily. Perhaps forgotten in the wake of higher-tech developments, methylene blue is readily available, safe, devoid of significant side effects, and inexpensive.

Our experimental studies proved that FD was technically feasible with little expenditure of time and training. For surgery of HPT, a short photosensitization time with subsequent high fluorescence of the PTGs is essential. In case of intraoperative difficulties to localize the

PTGs, immediate application of the PS with consecutive fluorescence of the PTGs would thus allow their detection without further delay. In this regard the data suggest photosensitization with 1.5% ALA solution and 1 (–2) h of PS exposure. These parameters appeared most effective in reaching high fluorescence intensities and ratios, which are essential to discriminate the PTGs in the surrounding tissue by their fluorescence contrast. The 3.0% ALA groups proved to be clinically impracticable, as the maximum fluorescence intensity and optimal ratio was reached later than in the 1.5% groups.

Owing to its distinct red colour, ALA-induced fluorescence helps PTG tissue stand out in contrast to background soft tissue, further speeding identification and dissection. It remains unelucidated why PTGs are almost selectively fluorescent after ALA application. However, it is hypothesized that due to increased blood supply and prolonged retention of PpIX the fluorescence is higher in PTGs compared with the thyroid and other tissues in the neck. This implies an even higher fluorescence in abnormal, hyperplastic PTGs where vascularization and perfusion is increased.

The benefit of the routine use of intraoperative modalities in PTG surgery, besides the qPTH assay, has yet to be critically determined.

Based on the experience of the present study, it is believed that the application of FD is not obligatory in all cases of surgery for hyperparathyroidism. The use of the fluorescence technique should be limited to selected cases, such as minimal invasive operations, or to cases where preoperative ultrasound or a sestamibi scan has suggested abnormal localization and number of glands. In these cases, the start of the photosensitization with 1.5% ALA would be 1 (–2) h before surgical incision leading to an optimal PS accumulation before PTG exposure.

In conclusion, FD is not a substitute for careful and meticulous dissection, as well as knowledge of normal PTG anatomy and its potential variations. However, when used together with preoperative ultrasound and a sestamibi scan, FD may justify unilateral neck exploration and allow a minimally invasive approach in selected patients. The combination of intraoperative monitoring of parathormone and FD can reduce the operative time even further and contribute to the prevention of persistent hyperparathyroidism or surgically induced hypoparathyroidism.

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