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学 位 論 文 題 名

Clinical Application of the Near-Infrared Fluorescence-Guided Imaging

(近赤外線蛍光イメージングの臨床応用)

学位論文内容の要旨

General Background: Near-infrared (NIR) light, in the wavelength range of 700 to 900 nm, offers several significant advantages over presently available imaging techniques. NIR wavelength light take advantage of the "NIR window" where light absorption from blood and water (i.e., major light absorbers in the body) is minimal. Thus, NIR light can penetrate relatively deeply into the tissue. Namely it can generate relatively high photon penetration into, and out of, living tissue, due to reduced absorbance and scatter, and a relatively high signal-to- background ratio due to low tissue autofluorescence. Intraoperative use of NIR fluorescence-guided imaging would have a profound impact on the detectability of structures that need to be resected (e.g., tumors) and avoided (e.g., vessels and nerves). Our laboratory have previously developed a custom NIR florescence-guided imaging system, so-called FLARE™ (Fluorescence-Assisted Resection and Exploration) surgical imaging system. The features of this system included acquisition of NIR fluorescence emission in the context of surgical anatomy (i.e., provided by the color video camera) and no change to the look of the surgical field, because NIR light is invisible. We hypothesized the combination of an intraoperative NIR fluorescence imaging system and a clinically available NIR fluorophore could permit real-time, patient-specific identification of the extrahepatic bile duct and ureters, as well as assessment of perfusion, with the overlay of color video (surgical anatomy) and NIR fluorescence especially useful. Our system can also provide quantitative assessment via custom software, by which subjective optical brightness can be converted into the numerical fluorescence intensity. In this study, we evaluated clinical application of NIR fluorescence-guided imaging in the intraoperative extrahepatic bile duct imaging, ureter imaging, and perfusion imaging in the preclinical setting using large animals.

PART 1 - Extrahepatic Bile Duct Imaging

Background: Iatrogenic bile duct injuries are serious complications with patient morbidity. We hypothesized that the invisible near-infrared (NIR) fluorescence properties of methylene blue (MB) and indocyanine green (ICG) could be exploited for real-time, intraoperative imaging of the extrahepatic bile ducts during open and laparoscopic surgeries.

Methods: 2.0 mg/kg of MB and 0.05 mg/kg of ICG were intravenously injected into 35-kg female Yorkshire pigs and the extrahepatic bile ducts imaged over time using either the FLARE™ image-guided surgery system (open surgery) or a custom NIR fluorescence laparoscopy system. Surgical anatomy was confirmed using x-ray cholangiography. Contrast-to-background ratio (CBR), contrast-to-liver ratio (CLR), and chemical concentrations in the cystic duct and common bile duct were measured, and the performance of each agent quantified.

Results: Using NIR fluorescence of MB, the cystic duct and common bile duct could be identified with good sensitivity (CBR and CLR ≥ 4), during both open and laparoscopic surgeries, from 10 to 120 min post-injection. Functional impairment of the ducts, including constriction and injury were immediately identifiable. Using NIR fluorescence of ICG, extrahepatic bile ducts did not become visible until 90 min post-injection due to strong residual liver retention, however, between 90 to 240

min, ICG provided exquisitely high sensitivity for both cystic duct and common bile duct, with CBR ≥ 8 and CLR ≥ 4.

Conclusions: We demonstrate that two clinically available NIR fluorophores, MB fluorescing at 700 nm and ICG fluorescing at 800 nm, provide sensitive, prolonged identification of the extrahepatic bile ducts and assessment of their functional status. MB has the advantage of rapid excretion into bile and low signal intensity in the liver, but optical properties are modest and 700 nm emission is subject to higher autofluorescence background. ICG has the advantages of superior optical properties and lower dosing, but requires over 2 hr before imaging is optimal.

PART 2 - Ureter Imaging

Background: The aim of this study was to determine whether the invisible near-infrared (NIR) fluorescence properties of methylene blue (MB), a dye already FDA-approved for other indications, could be exploited for real-time, intraoperative identification of the ureters.

Methods: The optical properties of MB were quantified *in vitro*. Open surgery and laparoscopic NIR fluorescence imaging systems were employed. Yorkshire pigs were injected intravenously with: 0.1 mg/kg MB (n = 8), 10 mg furosemide followed by 0.1 mg/kg MB (n = 6), or 0.5 mg/kg MB (n = 6). The contrast-to-background ratio (CBR) of the kidney and ureters, and MB concentration in urine, were quantified.

Results: Peak MB absorbance, emission, and intensity in urine occurred at 668 nm, 688 nm, and 20 μM, respectively. After intravenous injection, doses as low as 0.1 mg/kg MB provided prolonged imaging of the ureters, and a dose of 0.5 mg/kg provided statistically significant improvement of CBR. Pre-injection of furosemide increased urine volume but did not improve CBR. Laparoscopic identification of the ureter using MB NIR fluorescence was demonstrated.

Conclusions: Ureteral imaging using MB NIR fluorescence provides sensitive, real-time, intraoperative identification of the ureters during open and laparoscopic surgeries.

PART 3 - Perfusion Imaging

Background: Techniques currently used to determine flap perfusion are mainly subjective, with the majority of reconstructive surgeons still relying on clinical examination. In this study, we demonstrate the use of near-infrared (NIR) fluorescence angiography to directly quantify normal and abnormal perfusion in perforator flaps.

Methods: Indocyanine green was intravenously injected into anesthetized adult pigs (n = 38). A custom NIR fluorescence imaging system was employed for image acquisition and quantitation. Thirty-nine flaps were designed based on identified perforators, and post-operative imaging was performed for comparison. In select flaps, isolated occlusion of the arterial and venous pedicle was performed. In select flaps, vascular spasm was induced by local irrigation of the vessels with epinephrine. The fluorescence intensities of select regions-of-interest were quantified. From these data, we defined two indices for abnormal perfusion: the T_{max} ratio and the drainage ratio.

Results: We identified a normal pattern of perfusion prior to flap elevation, composed of a distinct fluorescence intensity peak at maximal arterial inflow followed by a smooth drop representing venous drainage. Delay of this peak after flap elevation, as indicated by T_{max} ratio, identified vascular spasm and arterial occlusion ($p < 0.0001$). Abnormal fall of fluorescence intensity after this peak, as indicated by drainage ratio, identified venous occlusion ($p < 0.0001$).

Conclusions: Quantitation of fluorescence intensity by NIR angiography accurately characterizes arterial and venous compromise. Our technique can assess perfusion characteristics during the intra-operative and post-operative periods, and therefore complements clinically-based subjective criteria now used for flap assessment.

学位論文審査の要旨

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Clinical Application of the Near-Infrared Fluorescence-Guided Imaging

(近赤外線蛍光イメージングの臨床応用)

近赤外線光は、生体において光の吸収に主として関与する水・ヘモグロビンに最も吸収されにくく、組織への高い透過性を実現できる。また、生体内に近赤外線蛍光を発する物質が存在しないため、背景蛍光を極めて低いレベルに保ち、対象のイメージングにおいて十分なコントラストを得ることができる。現在生体への投与が認可されている近赤外線蛍光物質は、methylene blue と indocyanine green (ICG)で、これらの使用に際し、methylene blue の Quenching、ICG の Saturation と呼ばれる現象に留意する必要がある。 実験は全て Yorkshire pig を用いて行った。イメージングシステムは、実際の術野映像に加え、2波長の近赤外線光イメージ、及び 3 者を superimpose したイメージを同時に表示させることが可能である、FLARE™ (Fluorescence-Assisted Resection and Exploration) System を用いた。また、 鏡視下用近赤外線蛍光イメージングデバイスの開発も行った。第 1 章・肝外胆管イメージングでは、胆汁排泄性である methylene blue と ICG を静脈内投与することで、肝外胆管の描出が可能であることが示された。胆管の蛍光強度を Contrast-to-Background Ratio (CBR)、Contrast-to-Liver Ratio (CLR)に変換し、定量的な解析を行った。Methylene blue では、投与後より CBR・CLR の速やかな上昇を認めたのに対し、ICG では、CBR は投与後早期より上昇したものの、CLR の上昇は緩徐であり、肝の残存蛍光が減弱し、胆嚢管・総胆管との十分なコントラストが得られるまで約 90 分を要した。臨床応用に際し、肝内に残存する蛍光を排除すべき状況、すなわち肝内胆管のオリエンテーションが要求される状況、あるいは可及的速やかに肝外胆管イメージングが要求される状況に対しては methylene blue が、それ以外の場合には、光学的に優れる ICG が第一選択と考えられた。鏡視下用デバイスを用いた肝外胆管イメージングでは、胆嚢管・総胆管の描出、及び総胆管に対する誤ったクリッピング・損傷の同定は可能であったが、用いた市販の光伝導ケーブルを介した近赤外線光の伝導が不良であり、対象の認識に際し、長いカメラ露光時間を要し、その結果画像が不鮮明となるなど解決すべき課題も提示された。第 2 章・尿管イメージングでは、腎排泄性である methylene blue を静脈内投与することで尿管の全走行の描出が可能であることが示された。腎・上部尿管・下部尿管の CBR を解析し、0.1mg/kg と 0.5mg/kg の methylene blue 投与群を比較したところ、0.5mg/kg 群において有意な CBR の増強を認めた。またループ利尿薬の前投薬群と非投薬群との比較では CBR に有意差を認めなかった。わずか 0.1mg/kg の methylene blue 投与にて、およそ 2 時間にわたる尿管の描出が可能であった。第 3 章では、穿通枝皮弁を用い、ICG の静脈内投与による近赤外線蛍光血管造影を施行し、有効な穿通枝の術前検索、及び皮弁環流の定量的評価を行った。同定した穿通枝の起始部を region of interest (ROI)とし、皮弁

作成前後で CBR 曲線の解析を行った。また環流障害モデルとして、穿通枝動脈・静脈の閉塞を行い、また穿通枝周囲に epinephrine を局所散布し spasm を誘発し、得られた CBR 曲線を解析した。皮弁作成前では、全例で CBR 曲線に鋭い inflow peak と、これに続くスムーズな CBR の減弱を認めた。皮弁の inflow、outflow を効果的に比較・検討するため、各指標となるパラメーターとして Tmax ratio (TR)、Drainage ratio (DR)を作成した。 TR、DR を各群にて検討し、inflow・outflow の異常を検出できる最大の正確度が得られるカットオフ値を設定し、異常環流を検出する診断アルゴリズムを作成した。第 1 章から第 3 章の検討を通じ、近赤外線蛍光イメージングは、低侵襲かつ簡便な検査法であり、対象を十分なコントラストをもって良好に描出することが可能であった。

口頭発表に続き，副査野々村克也教授より、第 3 章に関連し、本法が従来のドップラーを用いた組織環流の評価と比較し優れている点は何か、またどの程度の大きさの皮弁が本法の適応となり得るか、また第 2 章に関連し、尿管損傷が術後に診断される場合、通常患側尿管は損傷部で狭窄し、患側腎は低機能状態にあるため methylene blue の尿中排泄が期待できないが、そのような場合への対応策について，また副査山本有平教授より、第 3 章に関連し、近赤外線蛍光血管造影で描出できる血管の皮膚表層からの深度はどのくらいか、また術中の CBR 曲線のパターンや環流パラメーターが、どの程度鋭敏に術後起こりうる合併症を予測できるか、最後に主査近藤哲教授より、第 1 章に関連し、鏡視下手術における近赤外線蛍光肝外胆管イメージングの今後の展望、methylene blue と ICG の胆管描出における優劣・適応について、また近赤外線蛍光イメージングのセンチネルリンパ節描出への応用に関する今後の展望についての質問があった.

いずれの質問に対しても申請者はその主旨をよく理解し，自らの研究内容と文献的考察を混じえて適切に回答した.

審査員一同はこれらの成果を高く評価し，大学院課程における研鑽や取得単位なども併せ申請者が博士(医学)の学位を受けるのに充分な資格を有すると判定した.