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Potential Efficacy of a Delta 5-Aminolevulinic Acid Bioadhesive Gel Formulation for the Photodynamic Treatment of Lesions of the Gastrointestinal Tract in Mice

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Abstract

A delta 5-aminolevulinic acid (ALA) bioadhesive gel has been developed and evaluated in an in-vivo mouse model for photodynamic treatment of gastric cancer or Barrett's oesophagus. Four gels were tested: noveon AA-1, keltrol T, lutrol and blanose.

An initial in-vitro study of gel adhesion showed that noveon and keltrol had longer polyethylene transit times than lutrol and blanose. In-vivo assays indicated that protoporphyrin IX was synthesized by gastric mucosa when ALA-noveon and ALA-lutrol were used (preferable results for noveon). Keltrol was eliminated from the study after these investigations. Only ALA-noveon gel was retained for studies of the relationship between ALA dose and fluorescence. Fluorescence measurements in-vivo showed that ALA concentration and application time had an influence on protoporphyrin IX synthesis. Maximum intensity (2091 counts s^{-1}) was found with 2 mg mL^{-1} ALA, and fluorescence intensities differed with application time, reaching 1805 counts s^{-1} after 240 min.

ALA-noveon, showing good adhesion and enabling efficient diffusion of ALA at a pH < 6, was considered the best formulation for maintaining ALA stability.

Barrett's oesophagus, characterized by the replacement of normal oesophageal squamous epithelium with metaplastic columnar epithelium (Barrett 1950; Allison & Johnstone 1953; Reid 1991), develops as a complication in 10–12% of patients with chronic gastrooesophageal reflux disease and predisposes to adenocarcinoma of the oesophagus and gastric cardia. Although oesophagectomy is recommended, new treatments are desirable to reduce the mortality associated with this procedure.

Photodynamic therapy, which has been used against superficial oesophageal cancer, might provide effective non-surgical treatment for Barrett's oesophagus or additive treatment; several authors have reported promising results (Hayata et al 1985; Overholt et al 1993; Sampliner et al 1993). The photosensitizers used were haematoporphyrin derivative or photofrin, both injected intravenously. To increase the efficacy of photodynamic therapy and limit damage to adjacent normal squamous epithelium, it would be preferable to apply the photosensitizer topically and improve tumour selectivity. The use of a delta 5-aminolevulinic acid (ALA) gel enables both objectives to be achieved. As mucosa constitutes a limited number of cell layers, local administration of a sensitizer is feasible. Delta 5-aminolevulinic acid is a natural precursor in haem biosynthesis; ALA, when administered in excess, is metabolized to protoporphyrin IX, an active photosensitizer which accumulates within the cell because of the relatively low activity of ferrochelatase, the enzyme responsible for the conversion of protoporphyrin IX into haem (Kennedy et al 1990). ALA has several advantages: protoporphyrin IX is eliminated rapidly from the body, reducing the risk of skin photosensitivity; selectivity seems to be better in terms of tumour-to-normal tissue uptake (Berlin et al 1956; El-Far et al 1990); and oral administration is convenient for patients. Photosensitization obtained in this manner has been successfully employed in treatment both of basal cutaneous carcinoma (Kennedy & Pottier 1992) and of tumour of the mouth (Grant et al 1993) by photodynamic therapy. Topical application of ALA-protoporphyrin IX has also been used successfully for the treatment of cutaneous lesions of various types (Wolf et al 1993; Robinson et al 1994).

Topical application of a sensitizer is highly preferable to systemic injection because the concentration is increased in the target and reduced in surrounding tissues. However, effective local application requires increased contact time between the sensitizer and mucosa. The use of a gel for transfer of the chemical to target cells would enhance oesophageal and gastric retention time and improve oral ALA absorption. A fluid gel could thus be easily introduced orally into the oesophagus. The purpose of this study was to develop an ALA bioadhesive gel and evaluate its efficacy for the treatment of Barrett's oesophagus in an in-vivo mouse model.

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Materials and Methods

Animals

Male OF1P IOPS mice, 30 g, were supplied by Iffa Credo (L'Arbresle, France). The animals were fed seeds for one week before the experiments to avoid parasitic fluorescence from chlorophyll contained in biscuits.

Chemicals

Noveon AA-1 (polycarbophil; Polyplastic, Rueil Malmaison, France), a poly(acrylic acid); keltrol T (xanthan gum; SPCI, La Plaine Saint Denis, France), a polysaccharide; and blanose (carmellose sodium; Aqualon, Rueil Malmaison, France) were

used at 1, 2 and 10%, respectively (as is the general practice), for their bioadhesive properties. The thermosensitivity of a fourth polymer—lutrol F127 (poloxamer 407; BASF, Levallois-Perret, France), a poly(oxyethylene)-poly(oxypropylene) block copolymer—enabled the preparation of a 15·5% gel with a swelling transition at 30°C for oral administration. Delta aminolevulinic acid (ALA) (ref. A 3785) and ponceau red (ref. P 3504) were provided by Sigma (Saint Quentin Fallavier, France).

Gel preparation

Polymers were gently poured into distilled water agitated by means of a rotating paddle. Mixing was continued at room temperature until complete swelling of the polymer, except for lutrol F127 which was handled at 4°C. The different polymer concentrations in water for preparation of noveon, blanose, keltrol and lutrol were respectively 1, 10, 1 and 15·5%.

Adhesion test

Ponceau red, the solubility of which is close to that of ALA, was added to the different gels at 1–2 mg g^{-1} to compare their adhesive properties. Like ALA, this dye reduces pH and thus affects gel viscosity. Each preparation was adjusted to pH 5·5 with sodium hydroxide (0·1 M).

Coloured gel (8–10 g) was injected by syringe into a horizontal flexible polyethylene tube which was placed in an upright position after 10 min. A peristaltic pump was then used to send a continuous flow of distilled water (37°C) through the tube at a rate of 7 mL min^{-1}. Depending on its adhesion, the gel was more or less thoroughly removed from the polyethylene tube. Washings were collected, volumes measured and the staining intensity evaluated by using a Unikon spectrophotometer to measure the absorbance of ponceau red at 520 nm. Results are expressed as the percentage of total ponceau red recovered as a function of time.

In-vivo studies

Optical-fibre spectrofluorimetry (Fig. 1). Light (514 nm) from an argon laser was used to excite fluorescence which was then attenuated by reflection on a glass plate and fed into a 600-μm core, plastic-clad silica fibre with perpendicular polished end-faces, by use of a small 45° metal mirror and an achromatic lens. The distal end of the fibre was brought into direct contact with the target tissue. This system provides simultaneous delivery of excitation light and gathering of fluorescence. An image of the light leaving the fibre is passed into a glass fibre bundle (Oriel 77402) through the above-mentioned achromatic lens, a second achromatic lens located behind the metal mirror and a high-pass filter (Schott OG 530) which virtually eliminates back-scattered laser light. The fibre bundle converts the circular beam into a rectangular beam. Convenient spectral resolution is thus obtained when this system is coupled to a monochromator (Jobin Yvon CP 200). Spectra were recorded using a cooled 1024-element diode array and a 386 personal computer (Jobin Yvon Quickview software). Electromechanical shutters placed in the excitation and detection paths eliminated excessive irradiation and enabled determination of the dark current of the diodes. Recording of a single spectrum required 20 ms, but the integration period was over 3 s. Power density was 0·5 mW cm^{-2} (514 nm) at the fibre tip.

ALA release and protoporphyrin IX synthesis. ALA was incorporated into noveon, lutrol, keltrol and blanose gels at a concentration of 0·75–2·5 mg mL^{-1} to produce ALA-noveon, ALA-lutrol, ALA-keltrol and ALA-blanose. Gel (1 mL) was instilled directly through an 18-gauge needle into the stomach lumen of mice after abdominal incision and pylorus ligature. After instillation for between 30 min and 4 h the gel was removed and the stomach washed with aqueous NaCl solution (0·9%). The fluorescence of protoporphyrin IX synthesized by the cells of the stomach mucosa and of protoporphyrin IX eliminated by the liver and gall bladder was detected by laser-fibre spectrofluorimetry. Measurements were performed by placing the optical fibre directly in contact with the stomach wall. A control experiment to determine the optical properties of the different gels had previously been performed using the same excitation wavelength. Results are expressed in counts s^{-1}. Fluorescence emission spectra were measured five times per tissue and per mouse, and at least four mice were used for

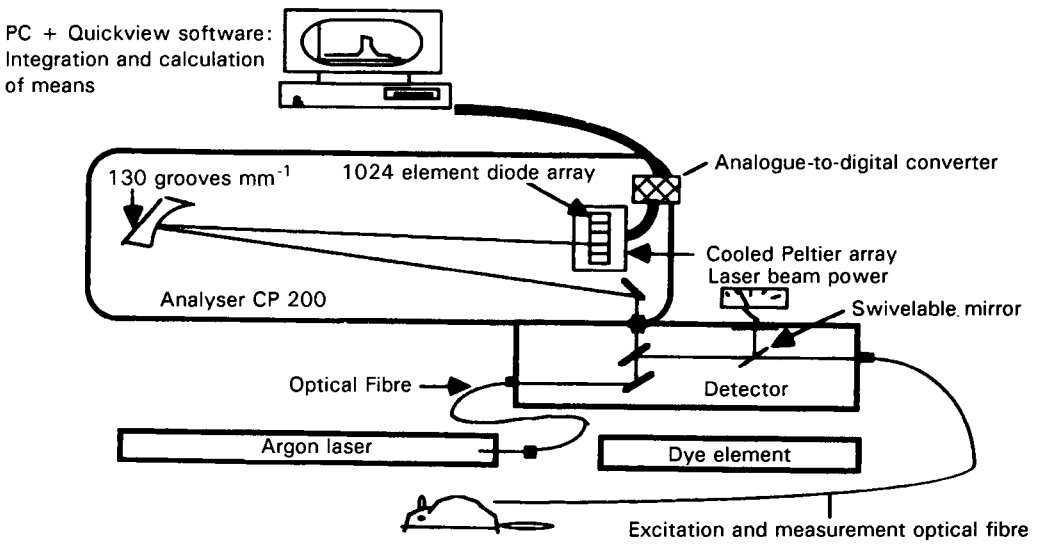

FIG. 1. Apparatus used for optical-fibre laser-spectrofluorimetry.

each experiment. Fluorescence intensities are the means of 20 recordings.

Results

In-vitro adhesion

Four coloured gels (noveon AA-1, keltrol T, lutrol and blanose) were injected by syringe into a flexible polyethylene tube. After 30 min 87% of ponceau red in noveon AA-1 and 75% in keltrol T were retained whereas only 28% and 16%, respectively, were retained in lutrol and blanose. Noveon AA-1 seemed to be the most adherent gel because 60% of the dye still remained in the polyethylene tube after 180 min, whereas none was left in the tubes containing the other gels (Fig. 2).

In-vivo release studies

Application of ALA to gastric mucosa led to protoporphyrin IX synthesis by cells. Protoporphyrin IX fluorescence emission was characterized by three peaks at 634, 675 and 700 nm. The most intense peak occurred at 634 nm; occasionally one of the other peaks was not detectable. Background fluorescence (gastric mucosa autofluorescence) was approximately 100 counts s^{-1} between 530 and 575 nm and was unmodified after application of ALA.

Three gels (ALA-noveon, ALA-lutrol and ALA-keltrol) each containing 1 mg mL^{-1} ALA were tested in-vivo (1 mL being instilled). As a control, water (1 mL) containing ALA (1 mg mL^{-1}) was injected into the stomach. After application to the gastric mucosa, protoporphyrin IX fluorescence at 634 nm was observed with an intensity of 1092 counts s^{-1} for ALA-noveon and 633 counts s^{-1} for ALA-lutrol. For ALA-keltrol, protoporphyrin IX fluorescence was observed (530 counts s^{-1}; Fig. 3). When ALA was dissolved in water and applied to gastric mucosa, 550 counts s^{-1} were obtained, i.e. the same intensity as with ALA-keltrol and half that with ALA-noveon (Fig. 4).

ALA-noveon was used in all further experiments because it was the only gel meeting our requirements.

Influence of contact time of ALA-noveon with mucosa on protoporphyrin IX synthesis

Administration of ALA (1 mg mL^{-1}) in noveon AA-1 led to protoporphyrin synthesis, with a difference in fluorescence intensities as a function of application time: 584 counts s^{-1} at 30 min, 1176 at 90 min, a transient decrease at 120 min and a subsequent increase to 1805 at 240 min (Fig. 5).

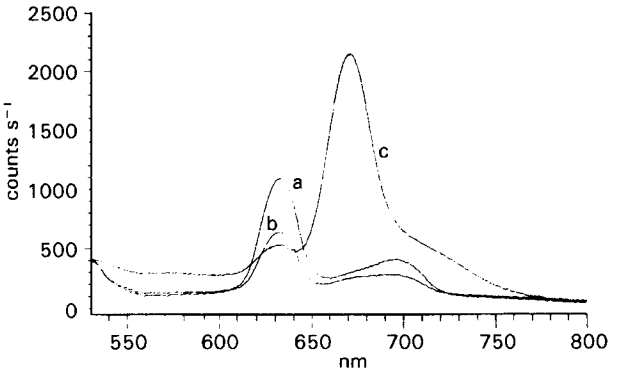

FIG. 3. Fluorescence of protoporphyrin IX in gastric mucosa cells 2 h after application of ALA: a, noveon AA-1; b. lutrol; c. keltrol. Results are expressed as counts s^{-1}.

Influence of ALA concentration on protoporphyrin IX fluorescence

Higher ALA concentrations in ALA-noveon produced an increase of protoporphyrin IX fluorescence in gastric mucosa. Application of a dose of 0·75 mg mL^{-1} for 3 h gave 1645·5 counts s^{-1}, increasing to a maximum of 2076 counts s^{-1} for 2 mg mL^{-1} (Fig. 6).

Elimination of protoporphyrin IX by the liver

Protoporphyrin IX fluorescence at 634 nm was detected in the liver 30 min after application of ALA-noveon to the gastric mucosa. A maximum intensity of 1542 counts s^{-1} was observed at 60 min; this decreased progressively to 274 counts s^{-1} at 240 min (Table 1).

Elimination of protoporphyrin IX by the gall bladder

Maximum protoporphyrin IX intensity at 634 nm in the gall bladder occurred 60 min after application of ALA-noveon to the gastric mucosa. Fluorescence was detected in the gall bladder 30 min after application; the intensity decreased from 60 to 180 min and then increased further to 4502 counts s^{-1} at 240 min (Table 1).

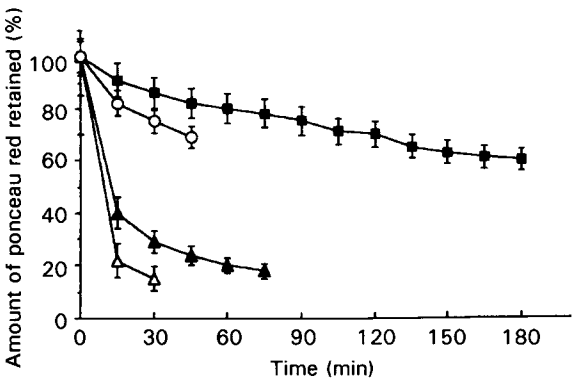

FIG. 2. In-vitro adhesion of noveon AA-1 (■), keltrol (○), blanose (△) and lutrol (▲) gels. Results are expressed as the percentage of ponceau red retained as a function of time.

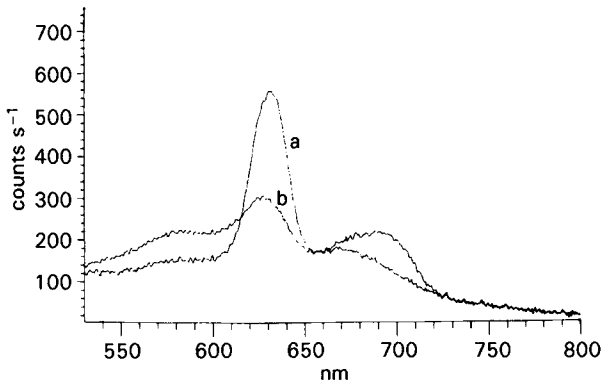

FIG. 4. Fluorescence of protoporphyrin IX in gastric mucosa cells 2 h after application of ALA: a. noveon AA-1; b. water. Results are expressed as counts s^{-1}.

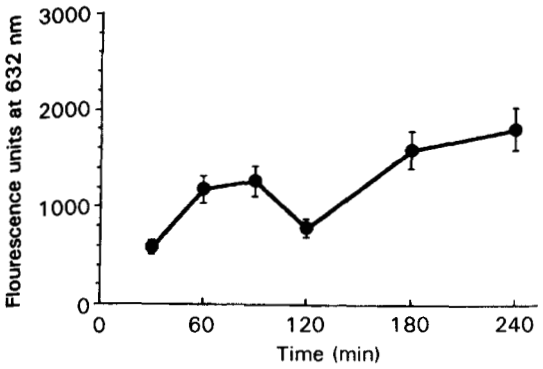

FIG. 5. Influence of ALA application time on protoporphyrin IX fluorescence in gastric mucosa cells. Fluorescence was obtained with noveon AA-1. Results are expressed as counts s^{-1}.

Discussion

Photodynamic therapy has been proposed in recent years for the treatment of superficial oesophageal cancer; it could provide a non-surgical alternative to oesophagectomy for Barrett's oesophagus. Some authors have performed photodynamic therapy in Barrett's oesophagus using haematoporphyrin derivative (Laukka et al 1993) or photofrin (Overholt & Panjehpour 1995) as photosensitizer. With photofrin, a reduction in the extent of Barrett's mucosa was achieved in all patients, and much of the treated glandular mucosa was replaced by squamous epithelium. Eight patients (four with dysplasia) presenting early oesophageal adenocarcinoma in Barrett's oesophagus were treated with photofrin (2 mg kg^{-1}) and laser treatment (630 nm, 300 J cm^{-2}), together with omeprazole. In two patients, complete replacement of Barrett's mucosa occurred after photodynamic therapy. In the study of Laukka et al (1993), low doses of photodynamic therapy were used to treat Barrett's oesophagus in 10 patients who received haematoporphyrin derivative (1·5 mg kg^{-1}) and laser treatment (630 nm, 150–200 J cm^{-2}). The length of the Barrett's segment was reduced, but no change occurred in dysplasia grade.

Exogenous porphyrins such as photofrin and haematoporphyrin derivative are not ideal photosensitizers because their long lifetimes entail a high risk of skin photosensitivity. ALA, a natural precursor in haem biosynthesis, can increase the efficacy of photodynamic therapy in the treatment of Barrett's oesophagus and eliminate photosensitivity. ALA has been administered orally to patients with oesophageal cancers in

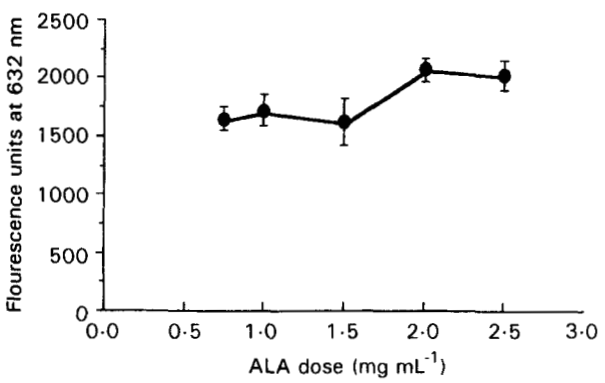

FIG. 6. Influence of ALA concentration on protoporphyrin IX fluorescence in gastric mucosa cells. Fluorescence was obtained with noveon AA-1. Results are expressed as counts s^{-1}.

Table 1. Protoporphyrin IX fluorescence (counts s^{-1}) in liver and in gall bladder after application of ALA-noveon AA-1 to gastric mucosa. Results are expressed as counts s^{-1}.

Time (min)	Fluorescence in liver	Fluorescence in gall bladder
30	284	297
60	1542·5	12694
90	282	6110
120	342	960
180	338·5	1024
240	274	4502

clinical trials. Mlkvy et al (1995) gave ALA dissolved in orange juice at fractionated doses of 30 to 60 mg kg^{-1} at hourly intervals. Fluorescence microscopy measurements at 630 nm showed that both neoplastic and normal tissue could synthesize protoporphyrin IX from ALA. Greater selectivity between tumour and normal tissue was found for a dose of 60 mg kg^{-1}. Photodynamic therapy provided superficial necrosis to a depth of 1·5 mm, and no skin photosensitivity was detected 48 h after administration of ALA. Other authors (Kennedy & Pottier 1992; Robinson et al 1994) have considered the use of ALA-protoporphyrin IX after topical application for the treatment of various skin cancers. If feasible, this approach would have several advantages over systemic infusion: an increase in the dose delivered to the target, reduced amounts of chemical to other tissues (potentially fewer adverse effects), increased efficacy, and lower costs of chemicals and patient care.

The topical use of a sensitizer requires that the chemical migrates from the solvent to the cellular target. This transfer depends on several factors: the solubility of the solvent, retention of the chemical by its solvent, and tissue uptake capacity. Thus, increasing the time of contact of the chemical with the target would probably increase the amount transferred to tissues. The purpose of our study was to develop an ALA bioadhesive gel augmenting ALA uptake and protoporphyrin IX synthesis, and to evaluate its potential efficacy for treatment of Barrett's oesophagus in an in-vivo mouse model.

In the absence of an in-vivo model of Barrett's oesophagus, we conducted our experiments on stomach mucosa to evaluate the efficacy of the bioadhesive gel of ALA. It was easier to apply the gel and control its efficacy on the stomach than on mouse oesophagus. Moreover, Loh et al (1992) have already used a stomach mucosa model to evaluate the efficacy of photodynamic therapy on gastrointestinal cancer. They compared the efficacy of two photosensitizers, ALA and a disulphonated aluminium phthalocyanine, by measuring fluorescence on the rat stomach wall and then treating the stomach mucosa. However, gastric secretions probably reduced adhesion and transit for mucosa as compared with oesophagus.

Candidates for the gel to be used in our study were selected according to their clearness in solution and adhesion to mucosa. Noveon has been developed in the form of topical, vaginal and oral gels (Longer 1985; Knuth et al 1993), and lutrol has been used in vaginal, rectal and buccal applications (Garcia et al 1992; Morimoto et al 1984; Nagai 1985).

The transfer from gels to mucosa was easy to study because the non-fluorescent ALA carried by gels was transformed into fluorescent protoporphyrin IX only in tissues, providing a perfect correlation between the rate of transfer and fluorescence. Moreover, the pylorus was ligatured so that gels could

not escape from the stomach, mimicking the use of a balloon for future clinical applications. The ligature also prevented contamination of stomachal protoporphyrin IX fluorescence by protoporphyrin IX eliminated through the gall bladder. In clinical applications, a lightly inflated balloon would be sufficient to retain the gels. However, a tighter-fitting balloon, though poorly tolerated by patients, would be necessary for ALA dissolved in water.

In-vitro studies have shown that noveon and keltrol have longer polyethylene transit times than lutrol and blanose and that 50% of noveon gel is not eliminated after 180 min. Our in-vivo experiments showed that protoporphyrin IX was synthesized by gastric mucosa when lutrol and, particularly, noveon were used. Keltrol was eliminated from the trials because of weak protoporphyrin IX synthesis, suggesting retention of ALA in the gel. Use of ALA-noveon gel resulted in an increase of protoporphyrin IX fluorescence by 92% compared with that measured when ALA was dissolved in water and instilled into the stomach under conditions similar to those for ALA-noveon. Finally, only ALA-noveon was retained for studies of the relationship between ALA doses and fluorescence because this gel had the best adhesion and led to the best diffusion of ALA.

Our fluorescence measurements in-vivo showed that ALA concentration and application time had an influence on protoporphyrin IX synthesis. The optimum concentration was 2 mg kg^{-1}, and the optimum application time between 3 and 4 h. These results must be confirmed in man because transfer kinetics could be slightly different. The optimum interval is too long for application in man, and a compromise would have to be found between efficacy and clinical feasibility.

Topical administration of ALA to the gastric wall also led to a rapid increase in liver and gall bladder fluorescence at 634 nm. In previous work, we used optical-fibre spectrofluorimetry to study biodistribution and elimination pathways in mice after systemic injection of ALA and found that elimination was both hepatic and urinary (Vonarx et al 1994). Fluorescence in the liver was maximum at 60 min with ALA-gel compared with 3 h after intraperitoneal injection. Although anatomical factors could partly account for this faster elimination, the results indicate that elimination was quicker after application of gel than after intraperitoneal injection of ALA. This suggests that application of gel could be more convenient for patients, involving less risk of skin photosensitization as a result of faster elimination. This study, based on the development of an in-vivo mouse model to study gel bioadhesion, found that transparent noveon AA-1 was the best choice for avoiding interference with laser treatment and maintaining ALA stability (pH < 6·0). Studies of gel adhesion and ALA diffusion properties in-vivo confirmed that ALA-noveon was preferable. Clinical trials are now required to study the efficacy of this formulation in local treatment of Barrett's oesophagus or other gastrointestinal lesions which could benefit from local photodynamic therapy.

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