■ ASPECTS OF CURRENT MANAGEMENT

Photodynamic therapy with acridine orange in musculoskeletal sarcomas

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Limb salvage involving wide resection and reconstruction is now well established for managing musculoskeletal sarcomas. However, involvement of major nerves and vessels with a large volume of muscle and skin may result in a useless limb, contributing to depression and a low quality of life. We have been studying alternative treatments for musculoskeletal sarcoma since 1990, and have recently established a regime using photodynamic surgery with cells labelled with acridine orange, photodynamic therapy with cells treated similarly and radiodynamic treatment using the effect of X-rays on such cells.

These techniques have been used after marginal or intra-lesional resection of tumours since 1999 and have enabled maintenance of excellent limb function in patients with sarcomas.

The use of photodynamic therapy (PDT) with haematoporphyrin or precursors such as porphyrin or aminolevulinic acid has been shown to prevent local recurrence, when employed with minimally invasive surgery in early-stage superficial tumours of the skin, lung, oesophagus and bladder. The principle of this treatment is that a photosensitiser, such as haematoporphyrin, should first specifically or selectively accumulate in the tumour cells. Photon energy (hv) from a light source then excites the photosensitiser which activates intracytoplasmic oxygen. The activated oxygen oxidises fatty acids on the organelle (lysosomes membrane or DNA and RNA in the cell, resulting in death of the tumour cells due to necrosis or apoptosis. The targets of the photosensitisers differ. For example, haematoporphyrin groups bind to mitochondria.

As light beams, even from a laser system, cannot reach deeper than the skin or mucosa, sarcomas localised in deep areas are not suitable for PDT. However, irradiation of the tumour with a light beam is easily performed during surgery for resection of the tumour. If an incomplete intralesional procedure is performed accidentally, PDT might be useful in killing tumour cells which have leaked into the surgical area. We considered that if PDT could kill tumour cells, they might be eliminated without damage to normal tissues, preserving limb function without local recurrence.

Limb salvage with wide resection of the tumour followed by reconstruction using

various types of endoprotheses, bone allograft or autograft in the treatment of musculoskeletal sarcomas has advanced markedly over the last 30 years.¹⁻⁵ However, satisfactory recovery of function may not be achieved and most patients are still not able to run or swim fast, jump confidently, or throw a ball a long distance.⁶⁻⁸ These disabilities limit the activity of the patients. Better means of achieving satisfactory recovery of function after limb salvage are needed. We have therefore been investigating new photodynamic therapies since 1990.⁹⁻¹³ The use of haematoporphyrin may result in dermatitis induced by light and it requires a relatively long time to accumulate in tumour tissue. PDT with haematoporphyrin also requires the use of expensive laser beams. We therefore employed acridine orange (AO) as a photosensitiser. This was extracted from coal tar in the late 19th century as a weak basic dye for staining clothes or microorganisms. Its biological properties include antitumour activity,^{14,15} photosensitising activity,^{16,17} and toxicity against bacteria, malarial parasites, and fungi.¹⁷⁻²¹ It has a low molecular weight of 463u, and is reported to be capable of rapid flow into the cytoplasm through the cell membrane to bind to DNA,²² RNA²³ and lysosomes.²⁴ Many experimental studies have shown that AO has properties as a photosensitiser and is useful for PDT in the treatment of cancer.²⁵⁻²⁹ However, the clinical application of AO in cancer therapy has not been described. This may be because of the potential

toxic effects of AO, which has been reported to induce mutagenic activity in bacteria.^{18,19} The carcinogenicity of AO has yet to be confirmed experimentally.³⁰ An International Agency for Research on Cancer (IARC) report³¹ in 1978 classified AO into group 3, meaning that the agent is not currently classifiable in terms of carcinogenicity in humans. The local administration of AO to patients for screening for gastric and cervical cancer has been described,³² but none have developed new cancers induced by AO during the current follow-up of 11 years.

Our basic studies have shown that AO binds densely to lysosomes and acidic vesicles, emitting orange fluorescence after excitation by blue light in viable cultured mouse osteosarcoma cells and binds sparsely to RNA, emitting green fluorescence after excitation. AO has a unique feature in that the polymer type emits orange fluorescence, whereas the monomer shows green fluorescence under excitation by blue light.¹³ Osteosarcoma cells transplanted into mice emitted green fluorescence after intraperitoneal or intravenous injection of AO followed by blue light excitation, while normal muscle and adipose tissue cells did not. Hence, tumours could be visually localised under a fluorescence surgical microscope, through an effect of fluorovisualisation.¹² We have confirmed that most human malignant bone and soft-tissue tumours are sensitive to staining with AO, as specimens which have been resected emit intense green fluorescence after exposure to a solution of AO with blue light excitation. Although the mechanisms underlying selective binding of AO to musculoskeletal sarcoma are unclear, staining with AO is useful for visualising the tumour during surgery under fluorescence microscopy. We also found that AO had a strong cytocidal effect on mouse osteosarcoma cells after blue light excitation, both *in vitro*⁹ and *in vivo*,¹⁰ suggesting that it might be useful for PDT against musculoskeletal sarcoma. Since the concentration of AO solution (1 µg/ml) used in our clinical study was very low, and it was only administered locally, we believe that the risk of carcinogenesis induced by AO in patients may be significantly lower than that occurring from most other known anticancer agents.

When using porphyrin or its derivatives for PDT,³³ a laser beam with high energy focused over a narrow area is commonly used as the excitation light source. With AO we have used a high-power xenon lamp (SANEI Electric MIG Co., Ltd., Tokyo, Japan), since blue light illumination over a wide area is necessary for fluorovisualisation of AO and for the strong cytocidal effect of AO-PDT on the tumour cells spread widely throughout the surgical field during curettage. Xenon lamps are much cheaper than lasers. A recent study has shown that the cytocidal effect of AO-PDT is dependent not only on the wavelength (blue light, 466.5 nm), but also on the lux (lx) value of the light. Hence, while blue light needs to be used for microscopic curettage, full-wave light obtained from the xenon lamp without an interference filter is more effective than blue light alone for AO-PDT, because of the much higher lux of the lamp. We have therefore

slightly modified our technique of AO-PDT during the last two years by using full-wave light.

Before the application of AO-PDT to human sarcomas, we carried out a simulation study of curettage supported by AO-PDT, using a mouse model.¹⁰ This showed that AO-PDT after macroscopic and microscopic curettage of a mouse osteosarcoma significantly inhibited local recurrence of the tumour. The rate of recurrence in the control group was 80%, compared with only 23% in the group-treated using AO-PDT. We also observed that low-dose X-ray irradiation of 5 Gy to mouse osteosarcoma after exposure to AO (radiodynamic therapy with acridine orange: AO-RDT) showed the same strong cytocidal effect as that of AO-PDT.¹¹ Such irradiation has the advantage of reaching deeper areas of the human body compared with a light beam, although the deleterious effects on normal tissues are greater. The results of these basic studies suggest that AO-PDT and AO-RDT might be applicable for limb salvage in patients with malignant tumours of bone and soft tissue. If effective, patients might recover almost full limb function, with only a low risk of local recurrence.

We therefore conducted a clinical study to determine the feasibility and usefulness of AO-PDT with AO-RDT in human musculoskeletal sarcomas. We found an overall rate of recurrence of 10%, almost the same as that after wide resection of the tumour.³⁴⁻³⁶ None of the five patients who had received AO-PDT with AO-RDT had recurrent tumour during a follow-up of more than two years. In the one case treated with AO-PDT alone the tumour recurred after 21 months, longer than would have been expected after macroscopic curettage of high-grade malignant sarcoma. Although the duration of follow-up was relatively short, we are convinced that the treatment employed was beneficial. The use of AO-PDT with, or without AO-RDT, inhibits local recurrence of musculoskeletal sarcomas, as most high-grade malignant sarcomas will recur within six months after intralesional excision.³⁴⁻³⁶ However, it is uncertain for how long this treatment might remain effective.

With the exception of one patient, the function of the limb was restored to the pre-operative level, and all patients were satisfied with the recovery of function. Compared with the results after wide resection of the tumour followed by limb reconstruction, recovery of function after AO-PDT (with or without AO-RDT) was superior in our study. We expect these patients to spend the remainder of their lives as normal, non-handicapped individuals. Local administration of AO, AO-PDT or AO-RDT was not associated with any complications, such as skin hypersensitivity to light, which is often encountered with PDT using porphyrin or its derivatives,³³ and the patient does not need to avoid exposure to the sun even in the early phase after surgery.

AO-PDT with or without AO-RDT appears to be a promising new regime for the preservation of limb function in patients with a musculoskeletal sarcoma. This approach may also be applicable to other solid tumours, although larger studies with follow-up are required.

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