Photoradiation therapy with protoporphyrin disodium for human hepatocellular carcinoma transplanted nude mice

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ABSTRACT
In the present report, we studied about in vivo anti-tumour effect by photoradiation therapy (PRT) with proroporphyrin disodium (PPNa) and hematoporphyrin derivative (HpD). To investigate anti-tumour effect by PRT, human hepatocellular carcinomas transplanted to the flanks of BALB/c/nu nu mice were injected intratumorally 2 mg of HpD, PPNa or 0.9% NaCl 48 hours before laser irradiation (514.5 nm, 0.1W, 30 minutes.) The results revealed that laser irradiation alone had no anti-tumour effect, while PRT with PPNa had intense anti-tumour effect (p<0.05) more than PRT with HpD. These results showed that PRT with PPNa may have clinical use.

INTRODUCTION
Recently, considerable interest has been aroused by possibility of inducing the regression of tumours in experimental animals and human by PRT. In order to extend PRT to wide clinical use, it will be worthwhile to find out the new photosensitizer which is less toxic and rather strong. This report describes the experiments in this field and demonstrates that human hepatocellular carcinomas in nude mice can be remarkably destoryed by the combination of PPNa and an argon laser.

MATERIALS AND METHODS
Cell cultures and animals: Human hepatocellular carcinoma cells maintained in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) containing 10% fetal calf serum (Microbiological Associates Inc.) Cells were rinsed and suspended in 0.9% NaCl and 1x10^7 cells were injected to flanks s.c. of BALB/c/nu nu mice. After tumour cell injections, the volume of the tumour was determined from caliper measurements of length, width and height of the tumour and the empirical formula (Loney, et al., 1975) V=1/2LWH.
Photosensitizer: Protoporphyrin disodium (TOKYO TANABE, Co., Ltd., Japan) was adjusted 10mg/ml in distilled water. Hematoporphirin derivative (5mg/ml) was obtained from Roswell Park Memorial Institute (Buffalo, N.Y.)
Laser irradiation: An argon laser (COHERENT, Supergraphite CR-8) was transmitted by a flexible quartz fiber and irradiated through 23G needle intratumorally with an energy of 0.1W and the irradiation time was 30 minutes.

RESULTS
Tumour regression curve: As shown in Fig. 1, neither laser irradiation group (514.5 nm, 0.1W, 30 minutes) nor PPNa treatment group, 2 mg/animal, intratumoral injection were different from control group, while photoradiation groups had tumour regression after treatment. And PRT with PPNa group had marked anti-tumour effect more than PRT with HpD group (Fig. 2).

Histological study: After experiments, all animals were sacrificed and histological studies were performed. Irradiations at 0.1W for 30 minutes produced no histological change in three tumours on mice which not injected PPNa, whereas injection of a total dose of 2mg PPNa or HpD resulted in tumour regression or complete disappearance of tumour.

DISCUSSION
We have already reported that PPNa might be effective as a photosensitizer. In vitro $^{51}$Cr release assay had revealed that PPNa alone had no cytocidal effect, while laser irradiation in the presence of PPNa had intense cytocidal effect more than HpD. Furthermore, this study revealed that PRT with PPNa had remarkable anti-tumour effect in vivo. Therefore, PPNa may be used as a new photosensitizer for photoradiation therapy for cancer.

REFERENCES