INTRODUCTION
There are various imaging methods to detect malignant tumor in human body. PET is recently becoming so common for this aim in the world. However its accuracy is not perfect, because of some false positive and negative cases and it is also very expensive because of large equipments and valuable radio-isotopes. To improve this issue, we have attempted to develop new imaging technique of photodynamic diagnosis (PDD) with acridine orange (AO). We have already developed photodynamic therapy with AO (AO-PDT) after intralesional tumor excision and have applied to clinical cases with high grade musculoskeletal sarcoma, resulting low risk of local recurrence and excellent limb function. AO also has ability to specifically accumulate in vivo into malignant tumor and emit brilliant green fluorescence after blue light excitation (fluorovisualization effect) (Figure 1). In this study, we investigate imaging availability of PDD using fluorovisualization effect of AO, in mouse osteosarcoma inoculated in the soft tissues.

MATERIALS AND METHODS
Tumor cell line: The 1x10^6 mouse osteosarcoma cells of LM8 cell line which has high ability to metastasis to the lung were inoculated into soft tissue of the back of BALB/c nude mice (5-week-old males). After macroscopically detectable tumor formation (6 to 10 mm in diameter), the following studies were performed.

In vivo optimum dose study: Each group with 4 mice is intravenously administrated with 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/kg AO, respectively. After 2 hours, the tumor and surrounding normal tissue were illuminated by 5,000 luminance blue light (450-490nm) for AO excitation using 500W xenon lamp through a single fiber tube. The contrast of fluorescence between the tumor and normal tissue was detected using high resolution digital camera (Canon) equipped with an absorption filter (>520nm).

In vivo optimum timing study: Using 6 mice administrated with 1.0 mg/kg AO, fluorescence contrast between the tumor and normal tissue was also detected sequentially at 0.5, 1, 2, 3, 6 and 12 hours, in order to investigate AO exclusion speed from the tumor and normal tissue.

Fluorescence intensity detection method: After taking photographs of each view, fluorescence intensity (FI) of the tumor and normal tissue was measured as gray scale (darkness) converted by Scion Image Beta 4.02 software (Scion Co., Systems Co., Maryland, USA). The ratio (X) of FI which was indicator of contrast was calculated by the average FI of the tumor (B) / that of normal tissue (A).

RESULTS
At 2 hours after intravenous injection of various doses of AO, in the low dose groups (0.1 or 0.2 mg/kg), both of the tumor and normal tissue emitted weak green AO fluorescence, whereas in the high dose group (5.0 mg/kg), both emitted strong fluorescence. X value was almost 1.0 in these dosages. However, the injection of 0.5, 1.0 and 2.0 mg/kg AO provided a clear contrast between both areas, more than 1.5 of X value. In particular, 1.0 mg/kg AO provided most optimum contrast (X=2.5) (Figure 2). After injection of 1.0 mg/kg AO, at 30 minutes or one hour, FI of the tumor was higher than those at late phase. However the X value (X=2.70) was the highest at 2 hours (Figure 3).

DISCUSSION
The results of these studies demonstrated that AO-PDD with intravenous administration is available to detect osteosarcoma inoculating into mouse soft tissues. We believe that PDD using AO administered intravenously may be useful to detect musculoskeletal sarcomas in humans, especially those arising from the extremities, and that this technique might be less invasive, less expensive, quicker and more accurate imaging modality as compared to other imaging methods reported until now, including PET, although further improvements in devices and methodology are required.