

# Contrast-Enhanced MRI with Gadodiamide Injection in Rabbit Carcinoma Models

Kazutaka YAMADA<sup>1,2,3)\*</sup>, Takeshi JINBO<sup>2)</sup>, Kazuro MIYAHARA<sup>1)</sup>, Motoyoshi SATO<sup>1)</sup>, Tsuneo HIROSE<sup>1)</sup>, Hiroto KATO<sup>3)</sup>, Yukio TATENO<sup>3)</sup>, Hiroo IKEHIRA<sup>3)</sup>, Hiroshi SUGIHARA<sup>2)</sup>, and Kazuhisa FURUHAMA<sup>2)</sup>

<sup>1)</sup>Department of Clinical Radiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, <sup>2)</sup>Daiichi Pharmaceutical Co., Ltd., Tokyo 134, and <sup>3)</sup>National Institute of Radiological Sciences, Chiba 263, Japan

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**ABSTRACT.** This study was designed to demonstrate the broad utility of contrast-enhanced magnetic resonance imaging (MRI) for diagnosis of carcinoma. Twenty-six New Zealand White rabbits of either sex (1.7–3.4 kg) were used for the investigation. VX2 carcinoma (squamous cell carcinoma) was implanted in either the brain, lung, ovary, bone or muscle of rabbits. Contrast agent, Gadodiamide Injection, was administered intravenously at a dose of 0.1 mmol/kg. MR images were obtained by a 1.5 T, or a 2.0 T magnetic field strength super-conductive MRI unit. The intensity of the signal for the carcinoma was increased after administration of contrast agent, and the detectability or diagnostic information of post-contrast images was superior to that of pre-contrast images in all models. In addition, no significant side effects were observed during the MRI examination. After diagnosis using MRI, morphological damage in each model was assessed by gross and histopathological examinations. In contrast-enhanced MRI, though there were variations between the models employed, contrast effects in brain and muscle carcinoma models were generally related to differences in capillary permeability, while imaging in lung, ovary and bone carcinoma models was dependent on differences in blood flow rate and the size of interstitial spaces. Overall, our results demonstrate that contrast-enhanced MRI is a useful and safe method for diagnosing tumors. — **KEY WORDS:** contrast-enhanced MRI, gadodiamide injection, MRI, rabbit, VX2 carcinoma.

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Magnetic resonance imaging (MRI) has come into wide use in the veterinary field recently [7, 9, 20, 21]. An inherent characteristic of MRI (T2 weighted image) is that it frequently permits the detection of even small neoplastic lesions without the need for a contrast agent [11]. However, to obtain the T2 weighted image requires a long scan time owing to the long repetition time (TR), and morphological information content is low because of the poor signal-to-noise ratio (S/N), especially in a small animal. The purpose of the present study was to confirm the diagnostic potential of contrast-enhanced MRI (T1 weighted image) using Gadodiamide Injection in VX2 rabbit carcinoma of the brain, lung, ovary, bone and muscle. Gadodiamide Injection (Omniscan®, Daiichi Pharmaceutical Co., Ltd. and Nycomed AS, Norway) is a nonionic, paramagnetic gadolinium chelate developed as an MRI contrast agent, for which safety studies have shown high tolerance [6, 12, 16], and effective enhancement of signal intensity in T1 weighted images. Therefore, contrast-enhanced MRI with gadodiamide is expected to be useful for the diagnosis of tumors in various organs.

## MATERIALS AND METHODS

**Animals:** Twenty-six New Zealand White (NZW) rabbits of either sex purchased from Oriental Yeast (Oriental Yeast, Saitama, Japan) were used in the investigations. These

animals were housed in raised mesh-bottom cages in a ventilated room with controlled temperature ( $23 \pm 2^\circ\text{C}$ ) and relative humidity ( $55 \pm 15\%$ ) and with a 12-hr light/dark cycle. They were allowed free access to commercial laboratory chows (RC: Oriental Yeast, Saitama, Japan) and water. Before an experiment, the animals were fasted for at least 18 hr. All animals were anesthetized intravenously with pentobarbital sodium (30 mg/kg), and were then placed in the prone position inside the scan system.

**MRI units:** Images were obtained by a 1.5 T Gyroscan S15 (Philips Medical Systems, Netherlands) or a 2.0 T RS-200 (Siemens-Asahi Medical Systems, Tokyo, Japan). Radio frequency pulses were at 64 MHz with the 1.5 T MRI unit, and at 85 MHz with the 2.0 T MRI unit. Both MRI units incorporate a super-conductive magnet. A human head coil was used as a radio frequency coil in the 1.5 T unit, and a custom-made solenoid coil in the 2.0 T unit. In this study, all images were obtained by T1 weighted imaging. When a routine T2 weighted image was used in small animals including rats, rabbits and kittens, an adequate S/N for a diagnosis was often not obtained. In order to get better images from T2 weighted imaging, averaging and a long scan time were required. In addition, a customized radio frequency coil for each small animal was needed [20, 21]. Moreover, for long SE (T2 weighted) images in the abdominal organs, it was difficult to detect abnormal tissue because of the respiratory motion artifact, and the relaxation time of abdominal organs is short. As gadolinium produced shortening of T1 relaxation time, T1 weighted images were chosen for our study [11, 17].

\* CORRESPONDENCE TO: YAMADA, K., DVM, Clinical Radiology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080, Japan.

**Contrast Agent:** Gadodiamide Injection was provided by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), and contained of gadolinium (III) diethylenetriaminepentaacetic acid bis-methylamide (GdDTPA-BMA) at a concentration of 500 mmol/l and the sodium calcium complex of the same ligand, known as caldium sodium, (CaNaDTPA-BMA) at a concentration of 25 nmol/l. This contrast agent reduces T1 relaxation rates; the relaxivity in water at 10 MHz and at 37°C is 4.6 (mmol/l)<sup>-1</sup>sec<sup>-1</sup>. The viscosity is 2.8 cp at 20°C and 1.9 cp at 37°C [6, 12, 16]. In this study, Gadodiamide Injection was administered intravenously at a dose of 0.1 mmol/kg.

**Animal models:** The VX2 rabbit squamous cell carcinoma (VX2, Funabashi Farm, Chiba, Japan) was maintained via serial transplantations into the hind limb musculature of NZW rabbits. The carcinoma cell line is a solid carcinoma whose histology and growth characteristics have been widely studied [1–3, 10, 15, 18].

**Brain tumor:** Male NZW rabbits (2.2–2.7 kg, n=6) received injections into the cerebral cortex with biocultured VX2 (2 × 10<sup>4</sup> cells/animal) and then examined from day 12 to day 14 after inoculation (n=5).

**Lung tumor:** Male NZW rabbits (3.0–3.4 kg, n=6) were injected intravenously with VX2 (1 × 10<sup>4</sup> cells/animal) and then scanned 2 weeks after inoculation (n=2). In this case, no respiratory gate was performed, so a short TR (200 msec) was selected to preclude the respiratory motion artifacts.

**Ovarian tumor:** VX2 (1 × 10<sup>6</sup> cells/animal) was injected into the ovaries of female NZW rabbits (2.1–2.6 kg, n=5), which were then examined 2 weeks after inoculation (n=5).

**Bone tumor:** Male NZW rabbits (1.7–2.0 kg, n=4) were injected intrafemorally with VX2 (1 × 10<sup>6</sup> cells/animal), and were examined 2 weeks after inoculation (n=4).

**Muscle tumor:** Male NZW rabbits (2.0–3.1 kg, n=5) were injected intramuscularly with VX2 (1 × 10<sup>7</sup> cells/animal) in the femoral muscles, and was scanned 4 weeks after inoculation (n=5).

**Pathology:** After completion of the imaging protocol, all animals were subjected to euthanasia by exsanguination under pentobarbital anesthesia, and each tumor-bearing site was examined macroscopically. Tissue samples were fixed in 10% buffered formalin, and embedded in paraffin wax. Thin sections 4 μm thick were prepared, stained with hematoxylin and eosin (H.E.) and examined histologically in order to characterize the lesion.

## RESULTS

**Brain carcinoma:** Although the implanted carcinoma was barely detectable in the pre-contrast image, it was significantly enhanced in post-contrast imaging (n=5). Histopathological examination confirmed the carcinoma (ca. 2 mm) (n=5) (Fig. 1.).

**Lung carcinoma:** The implanted carcinoma could hardly be detected in pre-contrast imaging, but enhanced areas were visible in the chest in the post-contrast images (n=2). Gross findings in the lungs included many scattered neoplastic

nodules (ca. 3 mm). Contrast-enhanced MRI easily detected these tiny nodules in a 5 mm slice section. Histopathologically, multiple sites of carcinoma were observed and confirmed (n=2) (Fig. 2.).

**Ovarian carcinoma:** In pre-contrast images, it was difficult to distinguish the walls of the digestive tract from the carcinoma. In the post-contrast image, however, the signal intensity was increased in the ovarian carcinoma (n=2). The rabbit ovary was found to be enlarged (to 2 × 2 × 2 cm) on macroscopic examination. Histopathologically, an overall picture of carcinoma was confirmed (n=2) (Fig. 3.).

**Bone carcinoma:** In pre-contrast images, a low signal intensity area was seen, but there was no distinction between carcinoma and necrosis. The post-contrast, implanted carcinoma images were significantly enhanced so that differentiation between carcinoma and necrosis was possible (n=4); and macroscopic examination also allowed clear differentiation. Histopathologically, carcinoma was seen in the bone marrow cavity (n=4) (Fig. 4.).

**Muscle carcinoma:** In pre-contrast images, there was no delineation of the boundary lines of the carcinoma. In post-contrast enhancement of carcinoma implanted in muscle, clear and unambiguous discrimination between muscle, carcinoma and necrosis was possible (n=5). Gross examination showed large carcinoma (ca. 7 cm) with central necrosis. Histopathologically, carcinoma with an area of encapsulation around the periphery was seen, and the center was found to have undergone ischemic necrosis (n=5) (Fig. 5.).

## DISCUSSION

We have investigated the diagnostic potential of contrast-enhanced MRI using Gadodiamide Injection in five experimental rabbit models of carcinoma. The signal intensity was found to be increased in the respective lesions of all models after gadodiamide was administered.

In the brain carcinoma model, the blood brain barrier (BBB) was apparently disrupted by proliferation of the carcinoma, thereby permitting the transfer of contrast agent into the cerebral tissue [2, 3, 5, 7, 17]. In our study, gadodiamide shortened the carcinoma tissue relaxation time, and consequently enhanced it.

In the lung carcinoma model, no clear image was obtained in the pre-contrast images, showing that MRI cannot provide an image of the lung because of the strong susceptibility of oxygen and respiratory motion artifacts [14]. However, a relatively clear image was obtained in post-contrast imaging. The contrast agent is transported via the peripheral blood flow, and diffuses into a carcinoma. In scanning the chest, the images were noisy because of respiratory motion artifacts, and hence enhanced T1 weighted images are superior to T2 weighted images because of the short repetition time. In addition, images of the lung have a low S/N due to the large volume of air present. Therefore, T1 weighted contrast-enhanced MRI has a clear advantage over

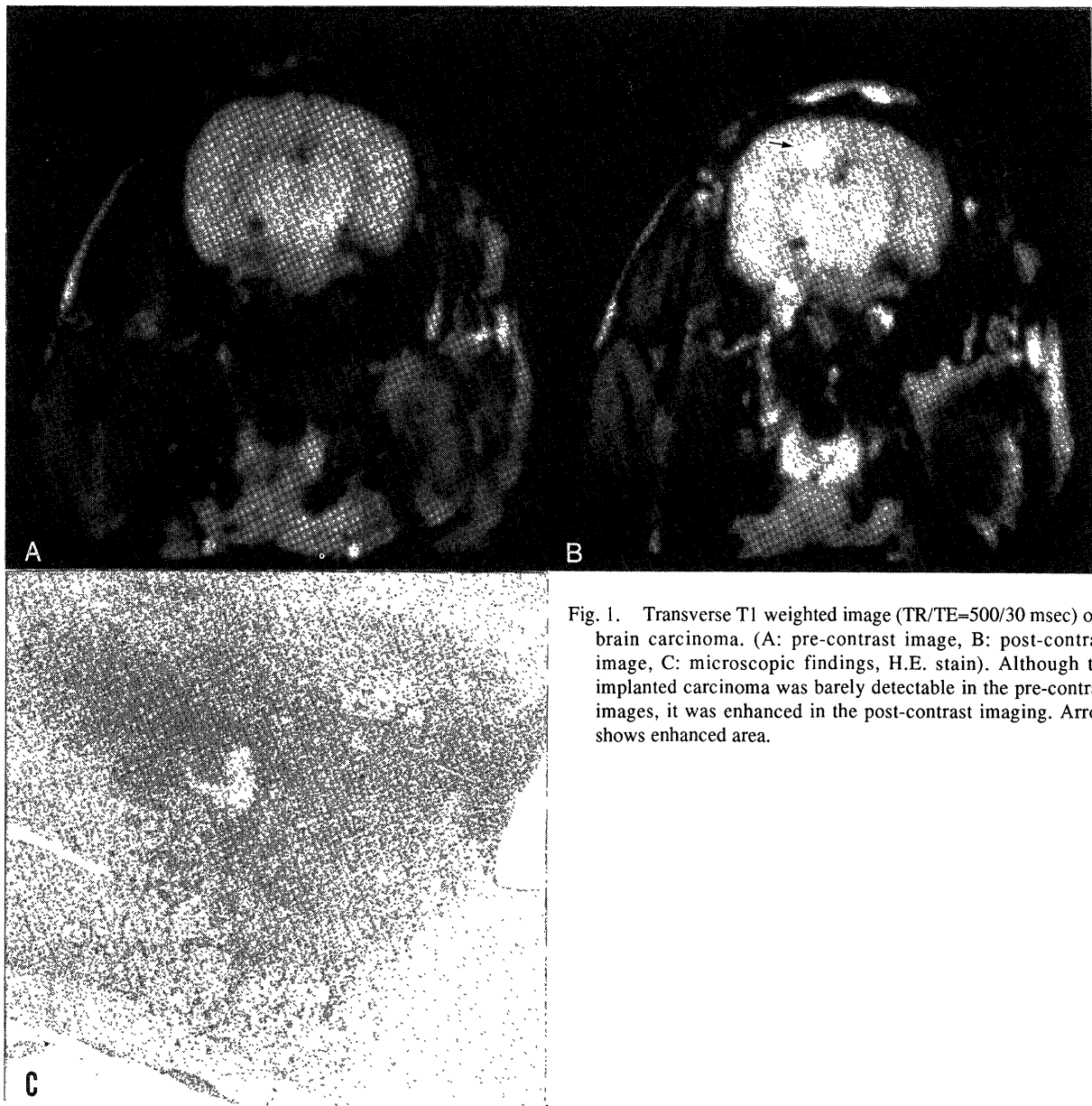


Fig. 1. Transverse T1 weighted image (TR/TE=500/30 msec) of a brain carcinoma. (A: pre-contrast image, B: post-contrast image, C: microscopic findings, H.E. stain). Although the implanted carcinoma was barely detectable in the pre-contrast images, it was enhanced in the post-contrast imaging. Arrow shows enhanced area.

the plain T2 weighted image, particularly with respect to obtaining a better S/N.

In our ovarian carcinoma model, because the rabbit ovary during non-pregnancy is very small (5 mm diameter), it does not give an image. In the pre-contrast images, the bowel wall showed a low signal intensity and an enlarged ovary also elicited a low signal intensity. After administration of gadodiamide, although the signal intensity of both organs was enhanced to the same extent, the bowel wall is very thin compared with the size of the ovary, therefore an enlarged ovary was easily identified.

In the bone carcinoma model, in pre-contrast images, normal bone marrow showed a high signal intensity because of the fatty cells, whereas necrotic bone marrow displayed a low signal intensity because of the presence of fluid. In

post-contrast images, both the carcinoma and normal bone marrow exhibited a high signal intensity. Areas of low signal intensity in pre-contrast imaging and high signal intensity in post-contrast imaging were found to be due to the presence of the carcinoma. The contrast agent diffused into viable carcinoma via the circulation, resulting in clear differentiation between viable carcinoma and necrosis. Marked enhancement due to invasion of the area by surgery is also visible in Fig. 4.

In the muscle carcinoma model, since the vessel fenestration in muscle is reported to be much greater than in brain, but less than in other organs [4], disruption of the capillary endothelial tight junctions by proliferation of carcinoma cells was the most likely reason for the passage of gadodiamide into the carcinoma interstices, similar to

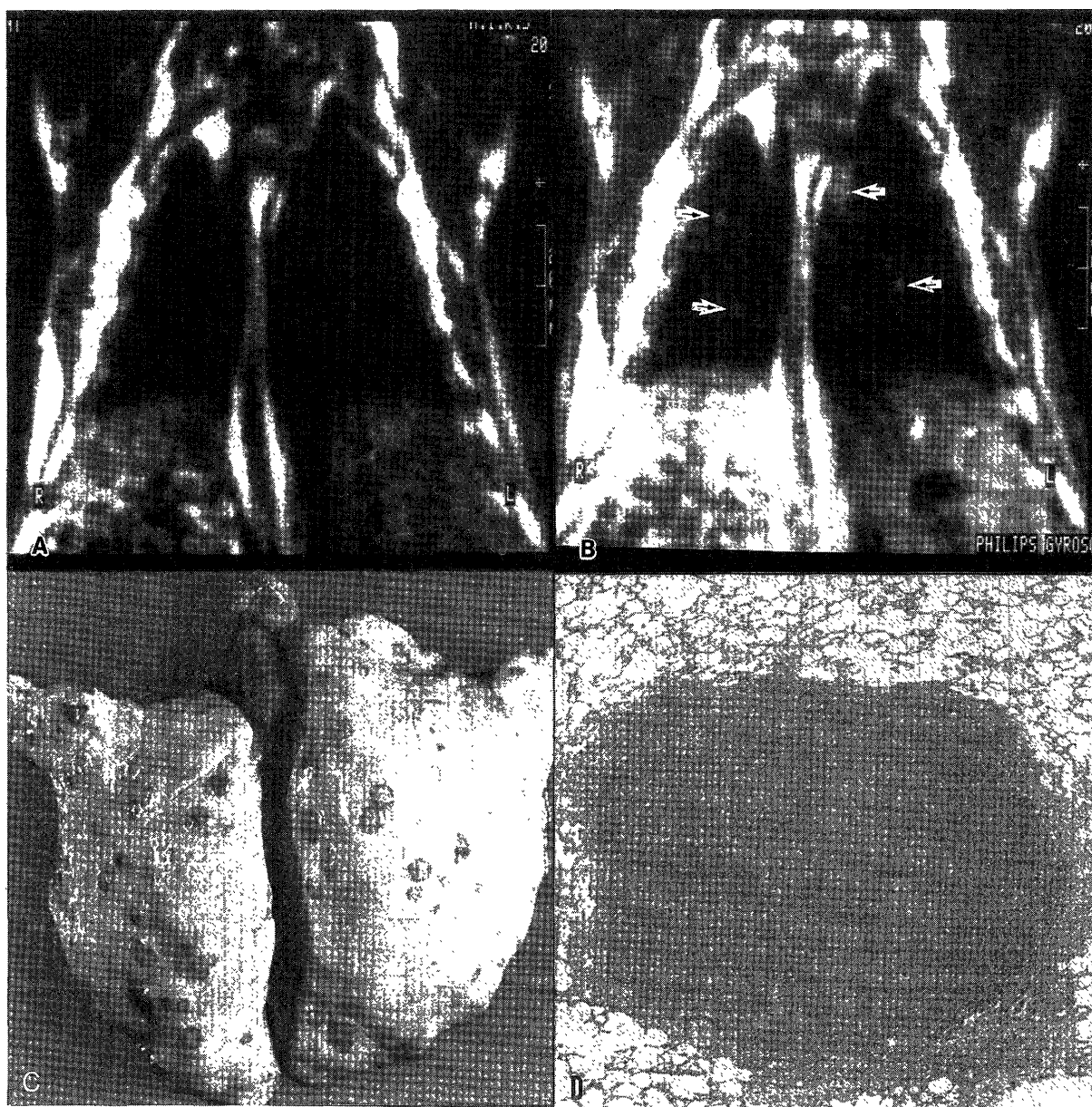


Fig. 2. Dorsal-plane T1 weighted image (TR/TE=200/20 msec) of a lung carcinoma model. (A: pre-contrast image, B: post-contrast image, C: gross findings, D: microscopic findings, H.E. stain). The implanted carcinoma was hardly visible in the pre-contrast image, but small enhanced areas in the chest were visible in the post-contrast image. Arrows show enhanced areas.

BBB disruption [3]. Choi *et al.* reported that enhanced T1 weighted images not only distinguished necrosis but also characterized different phases of necrosis in VX2 carcinoma [1], which is consistent with our results. Contrast-enhanced MRI is clearly useful for differentiating highly vascularized carcinoma from necrosis, and hence for evaluating carcinoma viability and selecting the most appropriate therapy [11].

Contrast-enhanced MRI shows different contrast effects, depending on the rabbit model, as these and other results show. Gadodiamide is distributed in the extracellular space after intravenous injection and tends to accumulate in tissues with rich vascularity or expanded interstitial spaces, and

particularly in tumors which have larger interstitial spaces and higher capillary permeability than normal tissues [11]. Contrast effects in the brain and muscle carcinoma models were related to differences in capillary permeability, and imaging of lung, ovary and bone carcinoma depends on differences in vascularization and the size of interstitial spaces [6]. Therefore, contrast-enhanced MRI with gadodiamide is useful not only for the detection of tumors but also for their qualitative assessment [1, 11].

We have exhaustively compared Gadodiamide Injection and Meglumine Gadopentetate (Magnevist®, Schering, Osaka, Japan) with regard to their main pharmacological characteristics in different species, and for imaging various

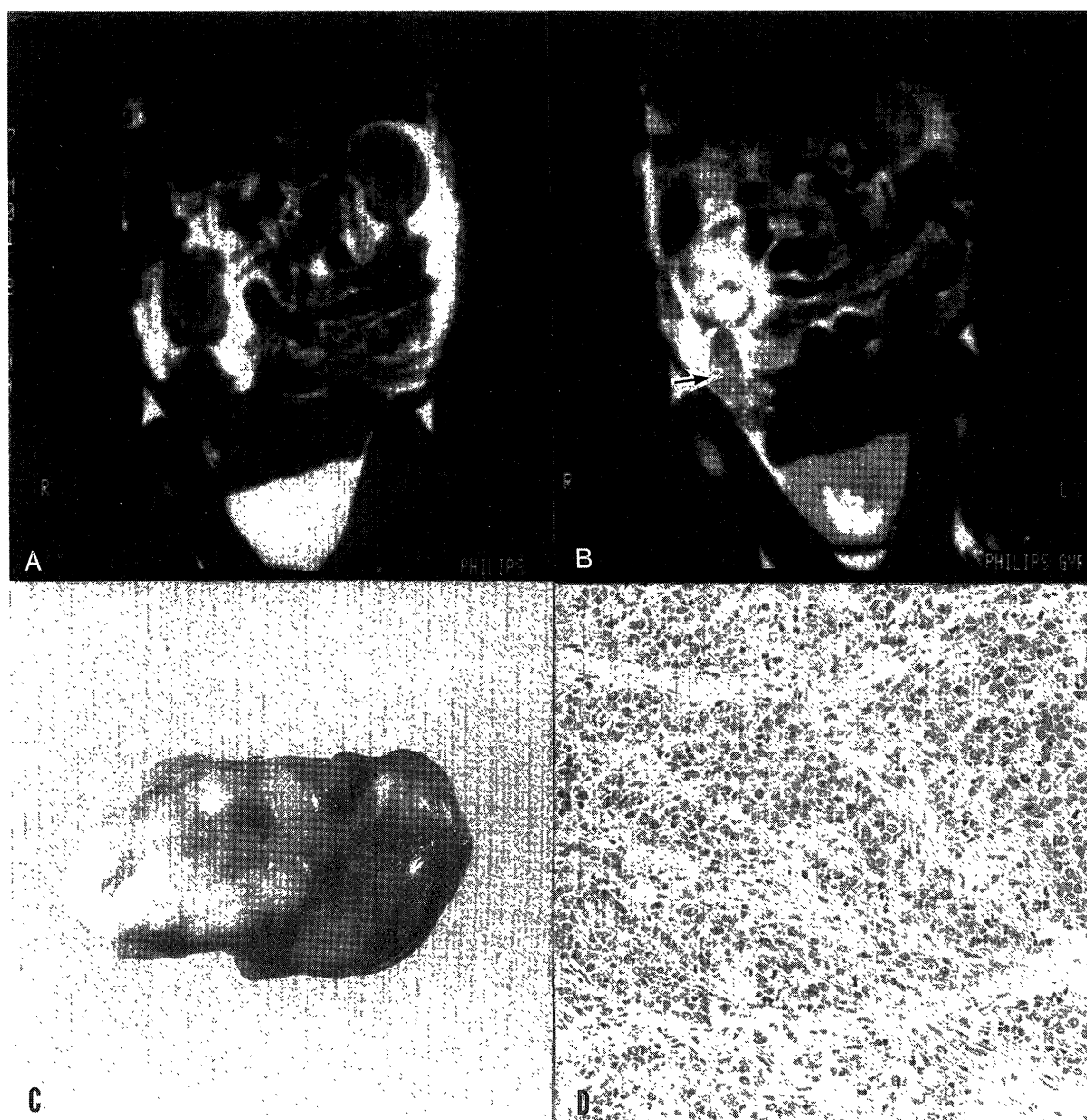


Fig. 3. Dorsal-plane T1 weighted image (TR/TE=370/20 msec) of ovarian carcinoma model. (A: pre-contrast image, B: post-contrast image, C: gross findings, D: microscopic findings, H.E. stain). In the pre-contrast image, it was difficult to distinguish the digestive tract from the carcinoma. In the post-contrast image, however, signal intensity was increased. Arrow shows enhanced area.

tumors. The contrast effects of Gadodiamide Injection were similar to those of Meglumine Gadopentetate, and in particular VX2 carcinoma showed negative enhancement in the liver, which is typical for metastatic tumors in the liver [18]. The tumor-liver signal difference and peripheral enhancement are due to differences in vascular perfusion. The liver has portal venous blood flow and more vasculature than a tumor [8, 10, 13, 18, 19].

Rabbit VX2 carcinoma is frequently used as a model of clinical carcinoma [1–3, 10, 15, 18, 19], but such models are always metastasis models, and there was a difference in the quality of the imaging between metastasis models and

primary carcinoma models. The difference was thought to be due to a difference in the degree of vascularization of the tumors. Strictly speaking, primary or spontaneous tumor should be used in future studies for evaluation for use in clinical diagnosis [7].

No significant side effects were observed during the imaging studies. The favorable characteristic of this contrast agent is that it shows substantially lower toxicity. In mice, the median lethal dose of Gadodiamide Injection ( $LD_{50}$  value: 34.4 mmol/kg, i.v.) was significantly higher than that of Meglumine Gadopentetate ( $LD_{50}$  value: 6.0 mmol/kg, i.v.) [6, 12, 16]. Gadodiamide was administered intravenously





Fig. 4. Transverse T1 weighted image (TR/TE=235/20 msec) of bone carcinoma model. (A: pre-contrast image, B: post-contrast image, C: gross findings, D: microscopic findings, H.E. stain). In the pre-contrast image, a low signal intensity area was seen, with no distinction between carcinoma and necrosis. In the post-contrast image, the implanted carcinoma was enhanced and distinction between carcinoma and necrosis was clear. Arrows show enhanced areas.

at a dose of 0.1 mmol/kg in this study. This is approximately 300 times less than the LD<sub>50</sub> value in mice. Our results suggest that contrast-enhanced MRI is safe, provided that care is taken with anesthesia during MRI examinations.

We conclude that contrast-enhanced MRI with gadodiamide is useful for the diagnosis of tumors and for monitoring the development of metastasis, as well as for monitoring response to therapy.

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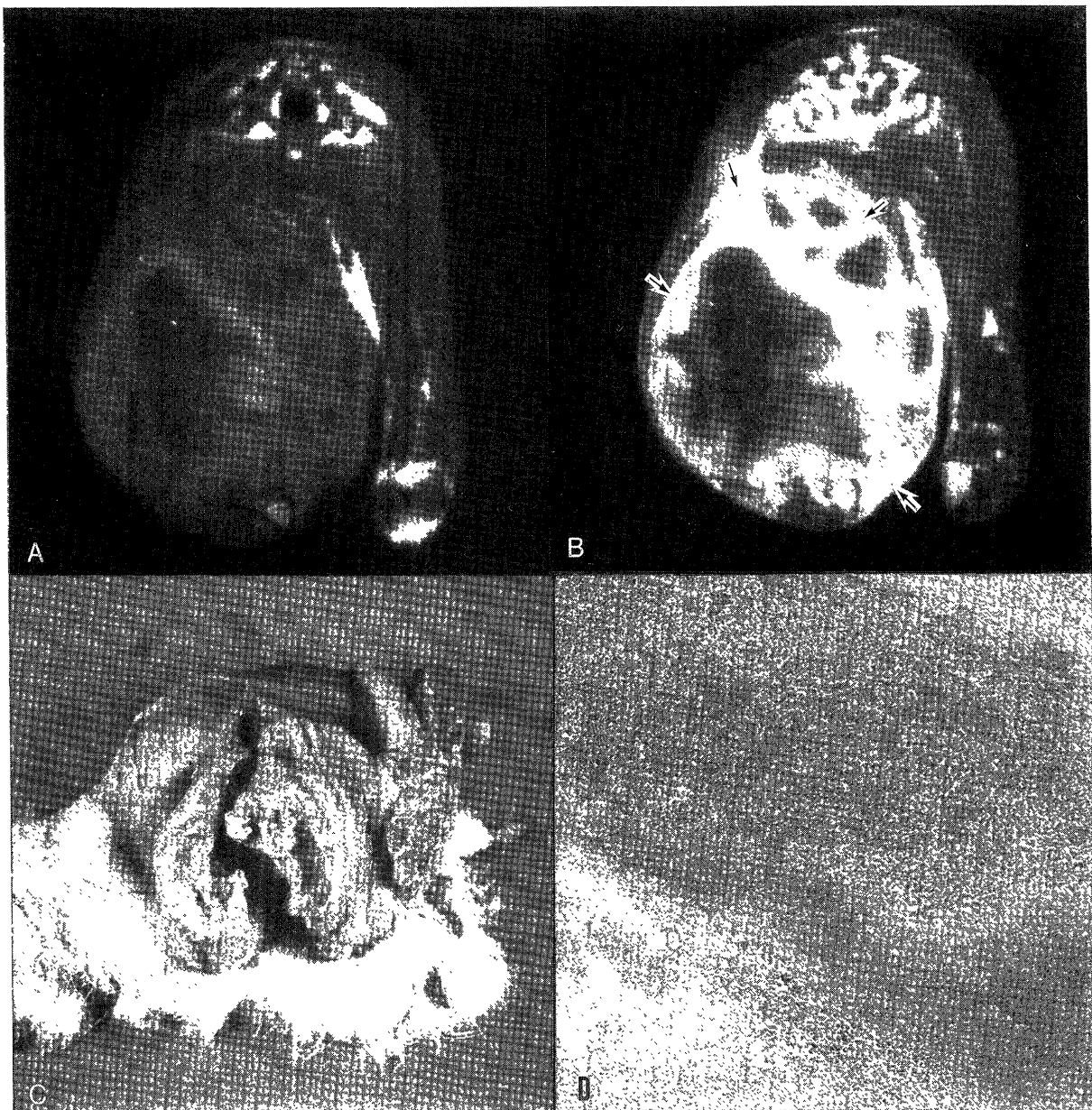


Fig. 5. Transverse T1 weighted image (TR/TE=492/15 msec) of muscle carcinoma model. (A: pre-contrast image, B: post-contrast image, C: gross findings, D: microscopic findings, H.E. stain). In the pre-contrast image, there was no delineation of the boundary of the carcinoma. In the post-contrast image, the carcinoma implanted in the muscle was enhanced and muscle, carcinoma and necrosis could be distinguished unambiguously. Arrows show enhanced areas.

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