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Original Paper

Targeted Cancer Chemotherapy for VX2 Tumour Implanted in the Colon with Lipiodol as a Carrier

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In this study, we examined the possibility of targeting drug delivery to tumours by dissolving the cytotoxic drug in a lipid fluid that is selectively deposited in tumours. Rabbits bearing VX2 tumour 10–20 mm in diameter in the large bowel received arterial injections of 0.2 ml of mitomycin C (MMC) dissolved in Lipiodol (MMC/Lipiodol), and the antitumour activity and adverse effects were examined. One week after treatment complete necrosis of the tumour was observed in 8 of 10 rabbits that received MMC/Lipiodol (3 mg/ml) without severe adverse effects on the surrounding caecum. In comparison 3/12 control animals that received MMC in saline and Lipiodol also showed complete necrosis. 6 of 7 rabbits killed eight weeks after the injection of MMC/Lipiodol were cured, with no viable tumour cells and with a normal appearance of the surrounding large bowel. In conclusion, MMC dissolved in Lipiodol may be adaptable for the treatment of colon cancer and may achieve antitumour activity without severe adverse effects. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: targeted chemotherapy, VX2 tumour, Lipiodol ultrafluid, mitomycin C
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INTRODUCTION

CHEMOTHERAPY HAS been used in the treatment of advanced unresectable or metastatic colon cancer, but the response rate is still low, with severe adverse effects. Even patients who respond relapse in a short period and the results are far from curative. It is thought that targeting of the anticancer agents to the tumour, which could achieve a long-lasting, selective high concentration of the drugs in the tumour, may improve results.

We found that a lipid lymphographic contrast medium, Lipiodol Ultrafluid (Lipiodol; Guerbet, Aulnay-Sous-Bois, France), was selectively deposited in hepatocellular carcinoma for more than 3 months and for more than 3 weeks in metastatic liver cancer and other solid malignant tumours when administered arterially. By using this characteristic of Lipiodol, targeted cancer chemotherapy with Lipiodol as a carrier was developed in our clinic [1–3]. These agents, termed oily anticancer agents, have the following properties: anticancer agents dissolve in Lipiodol, are stable in it and diffuse out from Lipiodol to the surrounding tissues very slowly. Each oily anticancer agent has a homogeneous colour and does not separate into the anticancer drug and Lipiodol, even if it is stored for 1 year. These oily anticancer agents have completely different antitumour activities and adverse effects compared with simple mixtures of Lipiodol and anticancer agents, as determined by pumping methods [3–5].

In this study, one oily anticancer agent, mitomycin C (MMC) dissolved in Lipiodol (MMC/Lipiodol), was prepared and its anticancer activity against VX2 tumour implanted in the large bowel of rabbits was examined. We were especially interested in the possibility of complete necrosis of the tumour without damage to the surrounding normal caecum.

MATERIALS AND METHODS

Drug

The oily anticancer agent, MMC/Lipiodol, was prepared by first dissolving MMC in acetone and then dissolving this mixture in Lipiodol that contained lecithin at five times the weight of MMC. The solvent was then removed. The endpoint for removal of the solvent was determined by the volume and smell of the product and the volume of the solvent removed. MMC/Lipiodol was clear and dark violet in

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colour. When MMC/Lipiodol was mixed with serum, transfer of MMC from Lipiodol to serum continued for 96 h [3].

Experimental animals and anaesthesia

We used a total of 54 male and female New Zealand White rabbits. The mean body weight was 1.6 kg. General anaesthesia was induced by intravenous injection of pentobarbital sodium (30 mg/kg) during both the laparotomy for tumour inoculation and the arterial injection of the drugs.

Tumour inoculation

Transplantable anaplastic VX2 tumour that had originated from a spontaneously transformed Shope papilloma, which was first described by Rous and Beard [6], was used. A 1.5 mm section of the VX2 tumour was inserted into the submucosal layer of the caecum. Three weeks later, the animals underwent laparotomy and rabbits with tumours measuring 10–20 mm in diameter were used for the experiments.

Drug administration

All the drugs tested were injected over 20 sec as a bolus into the ileocaecal artery by use of a special fine needle (outer diameter 0.21 mm, inner diameter 0.12 mm) connected to a 10 cm long polyethylene tube. While the drugs were injected, the peripheral ileocaecal artery was clamped by bulldog forceps, as shown in Figure 1. After the injection, blood flow in the ileocaecal artery was confirmed as sustained.

Test for antitumour activity

Five groups of rabbits were given different drug preparations. Rabbits in group 1 ($n = 8$) underwent a sham operation, with puncture of the ileocaecal artery, but no drug administration. Group 2 ($n = 16$) received 0.2 ml of Lipiodol. Group 3 ($n = 7$) received 0.2 ml of MMC dissolved in a physiological saline solution (3 mg/ml). Group 4 ($n = 12$) received a total of 0.4 ml of the mixture of 0.2 ml of Lipiodol and 0.2 ml of MMC dissolved in physiological saline (3 mg/ml). Group 5 ($n = 17$) received 0.2 ml of MMC/Lipiodol (3 mg/ml).

All animals in groups 1, 3 and 4 were killed 7 days after arterial injection of the drugs. 6 animals in group 2 were killed 15 min ($n = 2$), 1 day ($n = 2$), and 3 days ($n = 2$) after arterial drug injection for the evaluation of Lipiodol retention. The other 10 animals in group 2 were killed 7 days after the injection. 10 animals in group 5 were killed at 7 days and the remaining 7 animals were killed 8 weeks after arterial injection of the drug. The caecum was removed and fixed in 20% formalin.

Radiographic studies of the removed specimens including the tumour were performed for all groups; low-kVp (kilovolt peak) radiographs (X-rays) were obtained with a Softex apparatus (Japan Softex, Osaka, Japan).

The antitumour effects of the drugs on VX2 carcinoma were evaluated by assessment of histological findings and changes in tumour size. Changes in tumour size were analysed by Student's *t*-test. Data were expressed as the mean ± standard deviation (S.D.), and a calculated P value of less than 0.05 was regarded as significant.

The adverse effect of each drug was evaluated by analysis of macroscopic and microscopic findings in the area of the caecum surrounding the tumour.

RESULTS

Selective retention of Lipiodol in VX2 tumour of the caecum: radiographic analysis

Lipiodol was retained both in the tumour and in the normal caecum, as seen on the X-rays taken 15 min after the injection of Lipiodol. On the X-rays taken 1 day after the injection, some retention in the normal caecum was observed, but 3 days after the injection almost all the Lipiodol has been cleared from normal caecum.

X-rays taken 7 days after the injection of Lipiodol with or without MMC revealed that Lipiodol was selectively retained in the VX2 tumours of the caecum (Figure 2). Selective retention of Lipiodol was also observed in lymph nodes with metastases. In group 2 (Lipiodol alone), retention of Lipiodol in the tumour appeared to be slight because of tumour enlargement in 1 week. In 6 of 7 rabbits that were killed 8 weeks after the injection in group 5, necrotic tumours were clearly observed as well-defined high-density areas (Figure 3a).

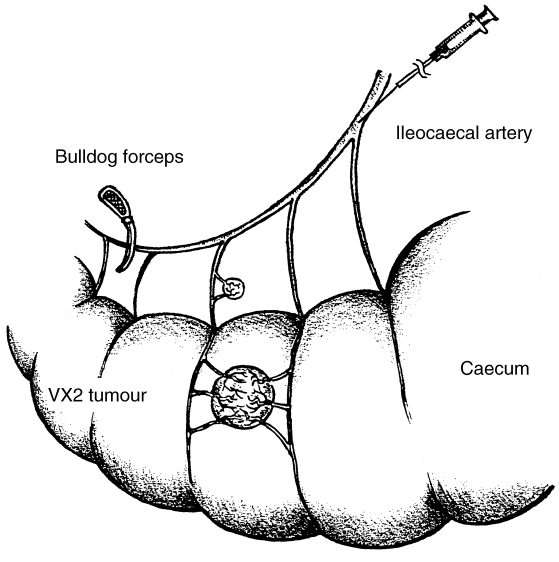

Figure 1. Schema of arterial administration of drug.

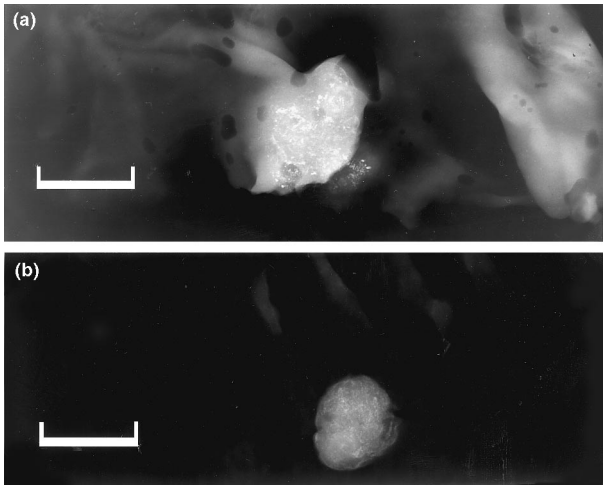

Figure 2. X-rays of resected specimens 7 days after arterial injection of the drugs showing selective retention of Lipiodol. Bar represents 10 mm. (a) Mitomycin C (MMC) physiological saline plus Lipiodol (group 4). (b) MMC/Lipiodol (group 5).

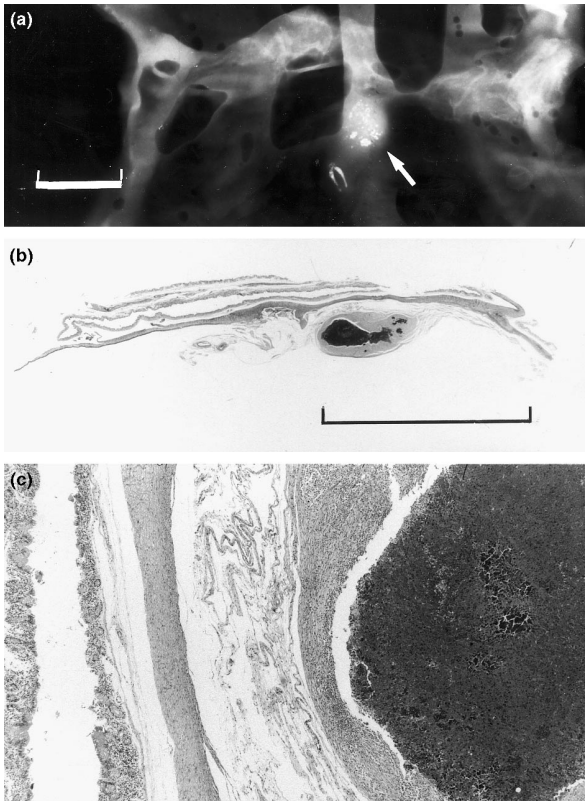

Figure 3. Resected specimen from a rabbit killed 8 weeks after arterial injection (group 5) showing that the tumour was necrotic and the animal was cured. (a) X-ray showing selective retention (arrow) in the shrunken tumour. Bar represents 10 mm. (b) Macroscopic findings. A necrotic mass is localised in the serosa of the caecum. Bar represents 10 mm. (c) Microscopic findings. The necrotic lesion is completely surrounded by a thick fibrous wall with infiltration of lymphocytes and macrophages. No viable tumour cells were seen in the specimen. Original magnification ×25.

Antitumour activity

Changes in tumour size (Table 1). In group 1 (sham operation), all VX2 tumours had more than doubled in size within a week (mean fold increase 2.19 ± 1.32). In group 2 (Lipiodol alone), group 3 (MMC/physiological saline) and group 4 (MMC/physiological saline plus Lipiodol), all tumours increased in size except in 3 rabbits in group 4 in which there was slight shrinkage. However, the relative increase in tumour size was significantly less in groups 2 and 3 compared with group 1. In group 5 (MMC/Lipiodol), all tumours shrank in 1 week by a mean of −41 ± 16%, which was a significant reduction compared with groups 2 and 4.

In 6 of 7 rabbits in group 5 killed 8 weeks after arterial drug injection, tumours shrank to 5 mm in diameter or less (Figure 3b). The other tumour enlarged to 40 mm in diameter.

Microscopic examination

Microscopic examination of specimens (Table 2) removed 1 week after arterial drug injection revealed tumour necrosis in less than 10% of all tumours of group 1 animals (sham operation, Figures 4a, and 5a). In group 2 (Lipiodol alone), less than 50% of the tumour was necrotic in 8 of 10 rabbits, and 60% of the tumour was necrotic in the remaining 2 rabbits (Figures 4b and 5b). In all tumours of group 3 animals (MMC/physiological saline), less than 50% of the tumour was necrotic (Figures 4b and 5b). In group 4 (MMC/physiological saline plus Lipiodol), necrosis of less than 50% of the tumour was observed in 6 rabbits and of more than 50% was observed in 3 rabbits; complete necrosis of the tumour was present in 3 of 12 rabbits (Figures 4d, and 5d–f). In group 5 (MMC/Lipiodol), necrosis of more than 50% of the tumour was observed in two rabbits, and complete necrosis of the tumour was seen in 8 of 10 (Figure 4c, Figure 5g–h); Table 2).

In 6 of 7 rabbits of group 5 killed 8 weeks after arterial drug injection, complete necrosis of tumour surrounded by fibrous tissue was observed (Figure 3c). The other tumour increased to a size of 40 mm in diameter and contained viable tumour cells.

Adverse effects

No pathological findings in the caecum surrounding the tumours where the drugs were infused were observed in groups 2 and 3 (Figure 4b, c). A thick wall of the caecum surrounding the tumour was observed in 6 of 12 rabbits in group 4 and in 9 of 10 rabbits in group, 5. In group 4, ulcers in the caecum surrounding the tumour where the drug was infused were observed in 4 rabbits, and 3 of these rabbits also had extended necrosis of the muscle layers (Figure 4d). A thick caecum wall was not observed in any of the 7 rabbits in group 5 killed 8 weeks after arterial drug injection, and no pathological finding was present; the caecum appeared normal (Figure 3b,c).

DISCUSSION

The differences in retention of Lipiodol by normal and tumour tissues may be explained by differences in their

Table 1. *Changes in tumour size in each group (*1 *week after treatment)*

Group	No. of animals	Tumour size (mm^2)*		Relative change in tumour size†
		At administration	1 week later	
1. Sham operation	8	162 ± 55	480 ± 143	2.19 ± 1.32
2. Lipiodol alone	10	158 ± 72	274 ± 114	0.82 ± 0.64‡
3. MMC/saline (3 mg/ml)	7	168 ± 42	257 ± 70	0.58 ± 0.47§
4. MMC/saline (3 mg/ml) plus Lipiodol	12	158 ± 58	206 ± 85	0.31 ± 0.62‖
5. MMC/Lipiodol (3 mg/ml)	10	167 ± 61	95 ± 34	−0.41 ± 0.16¶,**

*Mean ± standard deviation. †Relative change in tumour size = $\frac{\text{value 1 week after administration} - \text{value at administration}}{\text{value at administration}}$; ‡Group 1 versus group 2, $P < 0.001$; §group 1 versus group 3, $P < 0.009$; ‖group 2 versus group 4, NS; ¶group 4 versus group 5, $P < 0.002$; **group 2 versus group 5, $P < 0.0001$. Compared by student's *t*-test. NS not, significant. MMC, Mitomycin C.

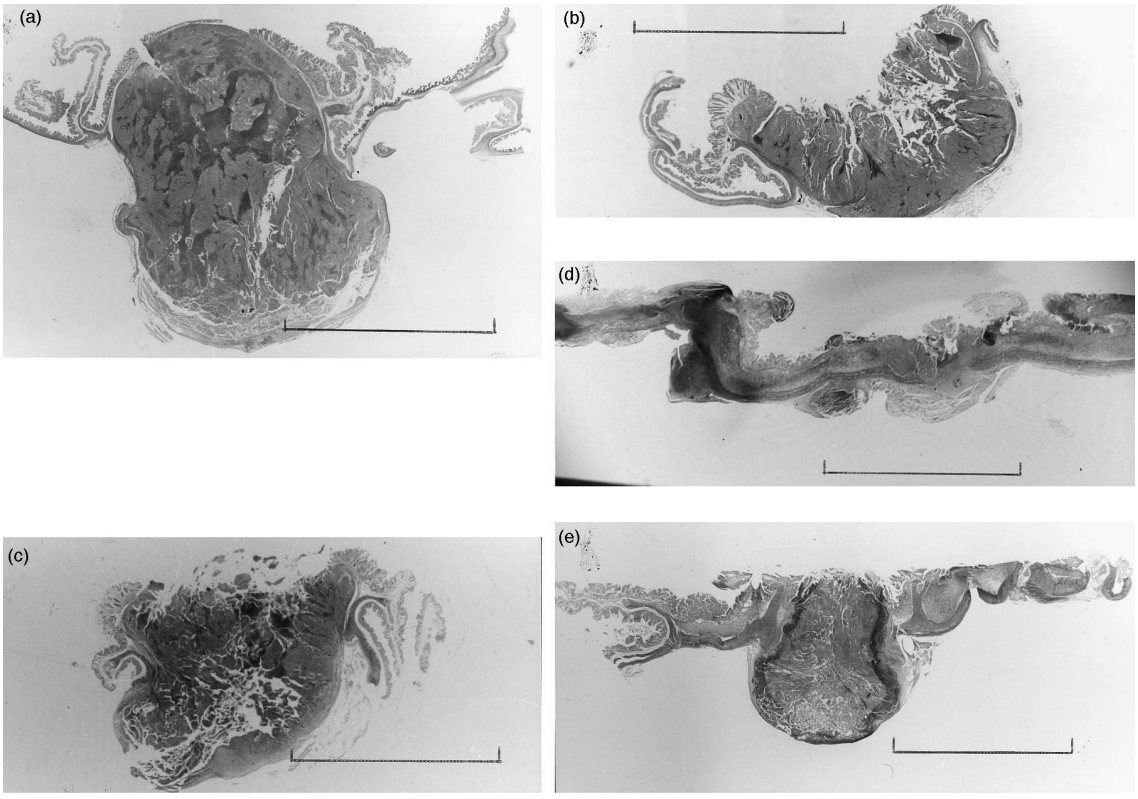

Figure 4. Low power appearance of resected tumours 7 days after arterial drug injection. (a) Group 1, the VX2 tumour was solid up to approximately 10 mm in diameter, thereafter showing central necrosis. However, the tumour periphery was always viable, so histological examinations concentrated on these areas; (b) group 2, (c) group 3, all tumours in these two groups showed invasion of the muscularis propria into the submucosa; (d) group 4, tumours showed severe inflammation with necrosis throughout all layers of the caecum; (e) group 5, tumours showed distinct necrosis, but had spread from the serosa to the mucosa of the caecum. Bar represents 10 mm.

vascular structure and mode of blood flow. In tumour vessels, neovasculature consists of one layer of endothelium and lacks a muscle layer and innervation, so it is dilated and cannot constrict. The mechanism of selective retention of Lipiodol in a tumour is not yet fully understood, but Lipiodol does remain selectively in tumours after a few days of arterial administration.

Slight suppression of enlargement of VX2 tumour in rabbits treated with Lipiodol alone may be caused by blood flow disturbance in the neovasculature, but there was little antitumour activity as shown by histological assessment. The results in rabbits treated with MMC/physiological saline were the same as those treated with Lipiodol alone. In this group, a high concentration of MMC could be achieved, but the duration of its retention is thought to be too short to kill the tumour. The antitumour activity of MMC/physiological saline plus Lipiodol was increased compared with that of MMC/physiological saline or Lipiodol alone, but complete necrosis of the tumour was observed in only 3 of 12 rabbits. In addition, in this group, ulceration of the normal caecum accompanied by necrosis of the muscle layers was present. In addition, in this group viable cancer cells remained in spite of necrosis of the caecum wall. Thus, there was a relatively high concentration of MMC, with MMC/saline plus Lipiodol, not only in the tumour but also in the normal surrounding caecum, so lacking selective toxicity for the tumour.

In group 5, enlargement of the tumour was completely suppressed and histologically, complete necrosis of the tumour was observed in 8 of 10 rabbits killed 7 days after arterial drug injection, without severe damage to the mucosa

Table 2. Microscopic findings in tumour specimens (1 week after treatment)

Group	No. of animals	No. of animals with grade 1–4 characteristics*			
		Grade 1	Grade 2	Grade 3	Grade 4
1. Sham operation	8	8	0	0	0
2. Lipiodol alone	10	7	1	2	0
3. MMC/saline (3 mg/ml)	7	5	2	0	0
4. MMC/saline (3 mg/ml) plus Lipiodol	12	3	3	3	3
5 MMC/Lipiodol (3 mg/ml)	10	0	0	2	8

*Grade 1, necrosis of the tumour was less than 10% of the tumour. Grade 2, necrosis of the tumour was between 10 and 50% of the tumour. Grade 3, necrosis of the tumour was more than 50% of the tumour. Grade 4, complete necrosis of the tumour.

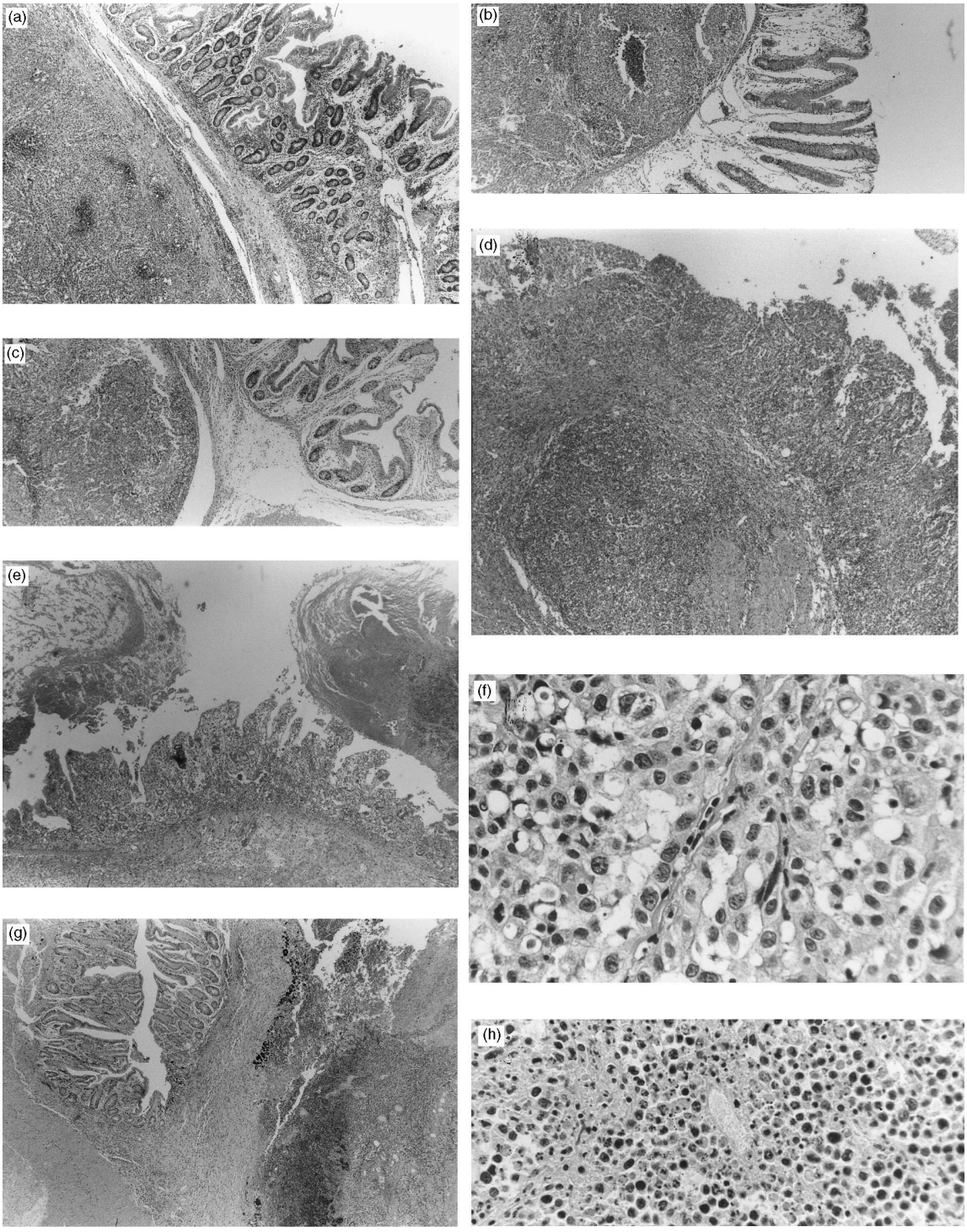

Figure 5. Microscopic appearance of resected tumours 7 days after arterial drug injection. (a) Group 1, (b) group 2, (c) group 3, all tumours in these three groups contained viable tumour cells, magnification ×25; (d) group 4, viable tumour cells which had proliferated in the submucosa of the caecum showed inflammatory and necrotic changes, with destruction of the mucosa and submucosa surrounding the tumour and ulcer formation (e), magnification ×25; (f) Higher magnification of (d) showed viable tumour cells arranged in sheet-like or solid nests. Inflammatory cells, including lymphocytes, were scattered among the tumour cells, magnification ×100; (g) group 5, tumour cells were necrotic and calcification was found at the periphery of the necrotic mass, magnification ×25; (h) higher magnification of (g) showed necrotic cells with nuclear shrinkage, fragmentation and lysis, magnification ×100.

and muscle layer. Small viable cancer nests were observed in the remaining 2 rabbits, possibly surviving because the distribution of the drug in the tumour is not uniform, so small areas in the tumour 'escape' the drug. This uneven drug distribution could be the result of the blood flow in the tumour at the moment of arterial injection or because the dose used was too small for the size of tumour. Clinically, it is commonly observed that there are some small areas in tumours

where no drug reaches after a single injection of these drugs, and thus, administration of several doses is recommended [4]. The enlarged tumour in a rabbit killed 8 weeks after drug injection may be explained by these small residual cancer nests that had grown in the intervening 7 weeks.

Although the same drugs and dosages were used, in groups 4 and 5, differences in antitumour activity were evident. The reasons for these differences are thought to depend on the achievement of targeting of the anticancer agent to the tumour in group 5.

The amount of anticancer agent selectively delivered to the tumour is the amount that diffuses out from Lipiodol while it is deposited selectively in tumour. Clinically, it takes at least 24 h for selective retention of Lipiodol in a tumour, and Lipiodol is retained for more than 2 months in hepatocellular carcinoma and 3–4 weeks in metastatic liver cancer, bronchogenic cancer, cancer of the kidney and colon cancer. Therefore, oily anticancer agents, with long-lasting release of anticancer agents from Lipiodol, can increase the amount of selectively delivered anticancer agents to the tumour. MMC release from MMC/Lipiodol (4 days) was the most rapid of the various oily anticancer agents tested [3]. Thus, the amount of anticancer agent selectively delivered using MMC/Lipiodol is likely to be the minimum compared with other oily anticancer agents. In spite of this property of MMC/Lipiodol, arterial injection therapy with MMC/Lipiodol achieved antitumour activity without severe damage to the surrounding normal tissues, indicating the importance of targeting anticancer agents to the tumour in the treatment of cancer.

1. Konno T, Maeda H, Iwai K, *et al.* Effect of arterial administration of high molecular weight anticancer agent SMANCS with lipid lymphographic agent on hepatoma. A preliminary report. *Eur J Cancer Clin Oncol* 1983, **19**, 1053–1065.
2. Konno T, Maeda H, Iwai K, *et al.* Selective targeting of anticancer drug and simultaneous image enhancement in solid tumor by arterially administered lipid contrast medium. *Cancer (Phil)* 1984, **54**, 2367–2374.
3. Konno T. Targeting cancer chemotherapeutic agents by use of Lipiodol contrast medium. *Cancer (Phil)* 1990, **66**, 1897–1903.
4. Konno T. Transcatheter arterial embolization with anticancer agents for hepatocellular carcinoma. In Kurihara M, ed. *Gastrointestinal Seminar* 58. Tokyo, Herusu Publishing, 1995, 163–176.
5. Group d'Etude et de Traitment du Carcinoma Hepatocellulaire. A comparison of Lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. *N Engl J Med* 1995, **332**, 1256–1261.
6. Rous P, Beard JW. The progression to carcinoma of virus-induced rabbit papillomas (Shope). *J Exp Med* 1935, **62**, 523–548.

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