rin by about 40 min. In an experimental tumour model in rats, 5-FU administered 40 min before leucovorin was more efficient than simultaneous administration in reducing hepatic tumour growth. Administration of leucovorin 1 h before 5-FU induced no synergism [9].

Leucovorin dose was correlated with toxicity in our study. The higher leucovorin dose caused toxicity that required either treatment interruption or dose reductions in all patients. Because of the limited number of patients it is not known whether this sequential approach with 5-FU preceeding leucovorin at 200 mg causes more toxicity than protocols with leucovorin preceeding 5-FU. In the patients with a 5-FU starting dose of 600 mg/m^2 plus the lower leucovorin dose, subjective toxicity of grade 2 or 3 (usually stomatitis, conjunctivitis and diarrhoea) was frequent. Since the treatment was palliative, toxicity should be low. Therefore, it was felt justified to lower the 5-FU dose to 500 mg/m^2. We think that 5-FU 500 mg/m^2 followed by leucovorin 50 mg, as a weekly schedule or given every other week on 2 consecutive days, has acceptable toxicity considering that it is palliative with probably no major survival benefit.

A palliative treatment should not only have acceptable toxicity but also antitumour activity. In our study, the objective response findings and the proportion of patients subjectively improved were about the same as those reported after combinations of 5-FU and leucovorin [1, 2, 4] or sequential methotrexate/5-FU/leucovorin [3]. Whether there are differences in antitumour activity between the 5-FU doses cannot be judged from our study because of the limited number of patients and the absence of random selection.

This sequential 5-FU/leucovorin schedule requires further clinical evaluation since the combination has clinical activity with acceptable toxicity. We are testing two daily doses every second week against sequential methotrexate, 5-FU and leucovorin and 5-FU in a multicentre phase III trial.

1. Erlichman C, Fine S, Wong A, Elhakim T. A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* 1988, **6**, 469–475.
2. Petrelli N, Douglass HO, Herrera L, *et al.* The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: A prospective randomized phase III trial. *J Clin Oncol* 1989, **7**, 1419–1426.
3. Nordic Gastrointestinal Tumor Adjuvant Therapy Group. Superiority of sequential methotrexate, fluorouracil, and leucovorin to flourouracil alone in advanced symptomatic colorectal carcinoma: A randomized trial. *J Clin Oncol* 1989, 7, 1437–1446.
4. Valone FH, Friedman MA, Wittlinger PS, *et al.* Treatment of patients with advanced colorectal carcinomas with fluorouracil alone, high dose leucovorin plus fluorouracil, or sequential methotrexate, fluorouracil, and leucovorin: A randomized trial of the Northern California Oncology Group. *J Clin Oncol* 1989, 7, 1427–1436.
5. Hines JD, Zakem MH, Adelstein DJ, Rustum YM. Treatment of advanced-stage colorectal adenocarcinoma with fluorouracil and high dose leucovorin calcium: A pilot study. *J Clin Oncol* 1988, **6**, 142–146.
6. Mortimer J, Anderson I. Managing the toxicities unique to high dose leucovorin (CF) and fluorouracil (FU). *Proc ASCO* 1989, **8**, 98.
7. Spears CP, Gustavsson BG, Berne M, Frösing R, Bernstein L, Hayes AA. Mechanisms of innate resistance of thymidylate synthase inhibition after 5-fluorouracil. *Cancer Res* 1988, **48**, 5894–5900.
8. Spears CP, Gustavsson BG, Frösing R. Folinic acid modulation of fluorouracil: Kinetics of bolus administration. *Invest New Drugs* 1989, 7, 27–36.
9. Carlsson G, Spears CP, Hafström LO, Gustavsson BG. Sequential 5-fluorouracil and leucovorin in experimental hepatic metastases of rat colon carcinoma. *Eur J Cancer Clin Oncol* (accepted for publication).

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Biodegradable Emboli and Antibody Targetting of Colorectal and Gastric Hepatic Metastases: A Pilot Study

William H. Allum, Alan J. Jewkes, Elsie Lanchbury, Steven Darby, Fiona Macdonald and Terry O'Brien

The effect of degradable starch microspheres (DSM) on the passage of a low molecular weight marker through the liver of patients with metastases was compared with the passage of an anti-carcinoembryonic antigen monoclonal antibody. In all six patients studied DSM reduced the passage of the marker into the systemic circulation. In three patients who received labelled whole antibody, DSM had no effect. In two of three who received antibody fragments a similar delay to the low molecular weight marker was observed. This delay is likely to be a result of the smaller size of the fragments and may represent accumulation within the extravascular space.

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INTRODUCTION

ALTHOUGH TUMOURS have been localised by radiolabelled polyclonal [1] and monoclonal antibodies [2, 3], the proportion of the labelled agent that is accumulated is small. In the context of tumour treatment Vaughan *et al.* [4] estimated that at the reported levels of uptake a lethal whole body dose would be reached before a tumoricidal dose could be delivered. Antibody uptake therefore needs to be increased by a factor of at least 10 before targetted therapy can be developed.

Techniques to increase antibody uptake have included regional arterial perfusion. Therapeutic responses have been reported [5, 6] although systemic toxicity has been significant. However, combining antibody with biodegradable microspheres (DSM) has produced a therapeutic effect with less toxicity, reflecting a transient delay in organ perfusion and enhancement of local tissue penetration [7].

In this study the combination of DSM and labelled antibody has been administered to patients with colorectal and gastric hepatic metastases to evaluate patterns of antibody activity further.

PATIENTS AND METHODS

Preparation and administration of monoclonal antibody

Monoclonal antibody L11-285-14 was raised against carcinoembryonic antigen (CEA) extracted from colorectal liver metastases. This antibody has been extensively characterised and its reactivity evaluated in normal and malignant tissue [8–10]. In our hands, immunohistochemistry has demonstrated detectable CEA expression in 84% of colorectal hepatic metastases. Specific tumour localisation has been demonstrated in animals bearing colorectal cancer xenografts [11] and in patients with gastrointestinal cancer [3].

Intact antibody and $F(ab')_2$ fragments were labelled with ^{131}I by the chloramine T method to a mean specific activity of 8.7×10^4 Bq/μg [12]. Retention of anti-CEA activity after labelling was assessed by ELISA [13]. Patients received an intrahepatic bolus of labelled antibody in 10 ml normal saline following administration of DSM. Approximately 200 μg intact antibody or 300 μg fragments were administered.

Patients

Six patients with liver metastases following colorectal (five cases) and gastric (one case) resection were studied. Informed consent was obtained before entry. The proportion of liver involvement as determined by enhanced computerised tomography was 30–55%. In addition to histological confirmation, the expression of CEA was measured by indirect immunoperoxidase staining of needle biopsy specimens from the metastases. Initial assessment included chest X-ray and full haematological and biochemical profiles. Patients were tested for possible hypersensitivity to the murine monoclonal antibody and prepared for infusion of the labelled preparation as described [3].

Transfemoral selective catheterisation of the hepatic artery was done with catheter placement just distal to the origin of the gastroduodenal artery. Selective arteriography demonstrated the hepatic artery anatomy and the specific arterial supply to metastases. Where appropriate selective superior mesenteric catheterisation was used to study aberrant hepatic arteries.

Embolisation and titration of DSM

Since tumour vascularity is very variable because of heterogeneity of newly formed capillaries, the amount of DSM required to alter blood flow is also variable. Furthermore, occlusion of the principal vessel can produce arteriovenous shunting, which could reduce uptake. Therefore, before infusing labelled antibody, the effect of a standard dose of DSM on the passage into the systemic circulation of a low molecular weight marker was assessed. In addition the extent of arteriovenous shunting was established.

^{99m}Tc-labelled methylene diphosphonate (MDP) was used as the marker. Flow through the liver was estimated from the radioactivity detected in the mediastinal great vessels by an externally placed scintillation detector. This was connected via a single-channel analyser to a counter from which the count rate was continuously transferred to a computer ('Spherex Monitoring System', Pharmacia). The effect of a standard dose (300 mg) of DSM (mean diameter 40–45 μm) on the passage of MDP was recorded as a decrease in the passing fraction.

The extent of arteriovenous shunting was estimated before and after infusion of DSM with ^{99m}Tc-labelled macro-aggregated albumin (MAA). In the presence of shunts MAA can be detected in lung capillary beds.

The effect of DSM on labelled whole antibody or antibody fragments was assessed in a similar way to that for MDP. A reference injection of the labelled preparation was initially given. After a few minutes the background activity had equilibrated and 300 mg DSM was administered with a further bolus of labelled antibody. It was possible to determine the effect of DSM after allowing for the background activity by subtraction of the plateau level of the reference injection. The monitoring equipment was maintained in position for as long as possible to observe changes in systemic activity following the degradation of DSM.

Gamma camera imaging

Gamma camera imaging was done at 24 h after antibody administration in all patients on a 'CGR Gamma Tome 9000' camera. Two patients who received antibody fragments were also imaged in the first few hours after antibody administration. Subtraction scanning was performed with ^{99m}Tc-labelled pertechnetate and human serum albumin to estimate the background blood pool activity. The scans were further analysed to allow for statistical fluctuation in the counts [14].

RESULTS

Effect of standard dose of DSM on labelled MDP

All six patients showed a reduction in the passing fraction of marker substance MDP when coinjected with a standard dose of DSM (Table 1). The mean fall in passing fraction for all six patients was 70.2 (S.D. 11.0)%. The passing fraction of ^{99m}Tc-labelled MDP administered with DSM was compared with the average of two reference doses of MDP. Arteriovenous shunting was suggested in patients 1 and 6. However, the large reduction in passing fraction observed with DSM administration and the greater value for the pre-DSM level of MAA suggested that the detector may have been registering MAA lodged in the liver.

All patients had nausea for a short time after microsphere administration. Two also complained of upper abdominal pain.

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W.H. Allum, A.J. Jewkes and F. Macdonald are at the Department of Surgery, E. Lanchbury is at the Department of Nuclear Medicine, and S. Darby is at the Department of Radiology, Queen Elizabeth Hospital, Birmingham; and T. O'Brien is at Questec Biomedical Research, London, U.K.

Table 1. Effect of DSM on passing fraction of MDP on arteriovenous shunting of MAA

Patient	Control MDP (%) (injection 1/2)	DSM + MDP (% control)	Control MAA (% control)	DSM + MAA (% control)
1	103/97	51	36	31
2	96/104	77	19	13
3	101/99	64	4	11
4	101/99	73	4	12
5	99/101	81	26	30
6	103/97	75	50	39

Pharmacokinetics of whole antibody and fragments

The stability of labelled antibody and fragments in the systemic circulation was measured by recording activity at 2 and 3 min after intrahepatic arterial infusion. These data were assessed to ensure that labelled antibody could be used as its own reference. For the three patients receiving intact antibodies the mean percentage difference in the activity was 1.8% compared with 2.5% for the three receiving fragments. This minimal difference was considered to indicate that antibody was remaining in the systemic circulation and not accumulating in tissue. Thus radioactivity accumulated in the systemic pool from the reference injection could be subtracted to enable individual analysis of subsequent injections.

The first pass effect of intrahepatic arterial injection of $F(ab')_2$ fragments was assessed in two patients. In both, the passage from the hepatic to the systemic circulation was compared with an intravenous injection of the labelled preparation. In both patients the passing fractions were similar irrespective of the origin of the injection, suggesting that there was no regional uptake by the liver during the first pass through the hepatic vasculature.

DSM effect on whole antibody and antibody fragments

The effect of DSM on labelled antibody infusion is shown for intact antibody and for antibody fragments in Fig. 1. Whole antibody appeared rapidly in the systemic circulation with control values reached at 150 and 135 s in patients 2 and 5, and to 96% of control in patient 1 by 480 s.

In contrast, in two of the three receiving $F(ab')_2$ fragments, the passing fraction curves plateaued at systemic levels considerably below control. At 9 min the passing fractions for patients 4 and 3 were, respectively, 79% and 74% of control. In patient 3 the passing fraction plateau value was unchanged for 20 min after co-administration of DSM and fragments, a time when most of the DSM would be degraded. In patient 6 similar findings to those for whole antibodies were observed, suggesting that DSM had no effect.

Gamma camera imaging

It was not possible to delineate specific areas of increased uptake within the liver irrespective of the timing of the scans. The high background level of circulating ^{131}I-labelled antibody produced intense activity in the liver as part of the blood pool. This precluded satisfactory external detection of the localised areas of slightly higher activity which would be expected from the results obtained with the monitoring equipment even after subtraction of the background pool activity produced by ^{99m}Tc-labelled human serum albumin.

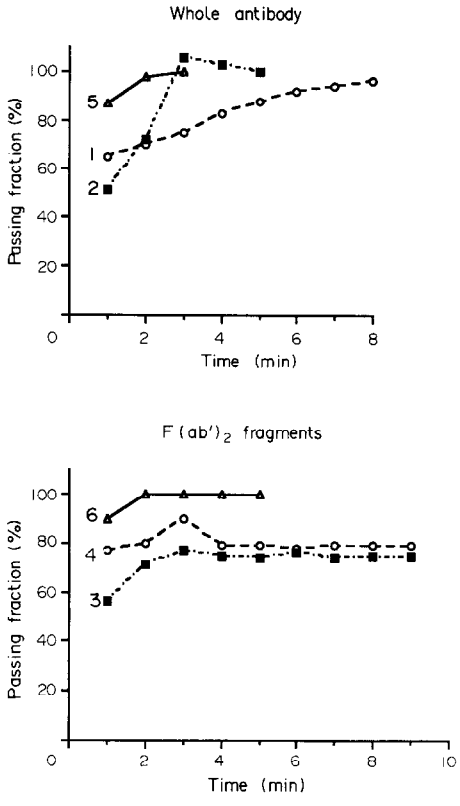

Fig. 1. Percentage passing fraction of whole antibody or $F(ab')_2$ fragments after co-administration of DSM. Patient's number shown.

DISCUSSION

Microspheres increase the targetting of a variety of substances when co-injected intra-arterially [7, 15, 16]. Most microspheres have been low molecular weight substances that can rapidly diffuse from the vascular to the tissue compartment. The clear reduction in the passing fraction of ^{99m}Tc-MDP in our group of patients suggested that this low molecular weight marker has similarly passed into the hepatic extravascular space. The parallel effects observed with antibody fragments also suggested that they have passed into the extravascular space. The lack of any effect by DSM on the passage of whole antibody may be related to larger molecular weight. Starkhammer and Hakansson [16] have demonstrated that large molecules are rapidly cleared by the liver despite partial hepatic blood flow blockade by DSM. There may be a molecular size cut-off in the hepatic capillary bed such that large molecules are unable to pass into tissue spaces. The absence of any reduction in passing fraction in one of the patients receiving $F(ab')_2$ fragments would contradict this hypothesis. However, because antibody administration occurred 24 h after hepatic arterial catheterisation, catheter displacement may have impaired adequate perfusion. In addition this patient was one of the two in whom arteriovenous shunting may have occurred.

Our preliminary findings in a small group of patients would need to be confirmed in a larger series before any conclusions could be made. Supportive evidence would have implications for targetted therapy. However, the specificity of the effect would need to be established. Tumour vasculature is leaky and studies have described non-specific accumulation of carrier substances [17]. The prolonged delay in the clearance of antibody fragments observed in one of our patients would not only imply retention but also accumulation by the tumour. However, should

a non-specific antibody produce a similar effect, the need for specific antibody fragments would be negated.

The monitoring system we used cannot assess such an effect. More specific external monitoring of regions of interest by gamma camera could provide some evidence of the uptake of antibody. This was not possible in our study because of high background activity. Alternatively, targetted biopsy of metastases and normal liver would allow measurement of tissue radioactivity.

Further studies are therefore required to confirm these early results and also to assess any effect on tumour uptake. The need for tissue confirmation would suggest that a reproducible hepatic metastases model would be worthwhile. We are evaluating such a model together with the effect of labelled antibody on metastatic growth.

1. Goldenberg DM, De Land FH, Kim E, *et al.* Use of *radiolabelled* antibodies to carcinoembryonic antigen for the detection and localisation of diverse cancers by external photoscanning. *N Eng J Med* 1978, **298**, 1384–1388.
2. Mach J-P, Forni M, Ritschard J, *et al.* Use and limitations of radiolabelled anti-CEA antibodies and their fragments for photoscanning detection of human colorectal carcinomas. *Oncol Dev Biol Med* 1980, **1**, 49–69.
3. Allum WH, Macdonald F, Anderson P, Fielding JWL. Localisation of gastrointestinal cancer with a ^{131}I-labelled monoclonal antibody to CEA. *Br J Cancer* 1986, **53**, 203–210.
4. Vaughan ATM, Anderson P, Dykes PW, Chapman CE, Bradwell AR. Limitations to tumour killing using radiolabelled antibodies. *Br J Radiol* 1987, **60**, 567–572.
5. Order SE, Klein JL, Ettinger D, *et al.* Phase I–II study of radiolabelled antibody integrated into the treatment of primary hepatic malignancies. *Int J Radiat Oncol Biol Phys* 1980, **6**, 703–710.
6. Epenetos AA, Courtenay-Luck N, Pickering D, *et al.* Antibody guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor and blood group A antigen. *Br Med J* 1985, **290**, 1463–1466.
7. Epenetos AA, Courtenay-Luck N, Dhokia B, *et al.* Antibody-guided irradiation of hepatic metastases using intrahepatically administered radiolabelled anti-CEA antibodies with simultaneous and reversible hepatic blood flow stasis using biodegradable starch microspheres. *Nucl Med Commun* 1987, **8**, 1047–1058.
8. Crowson MC, Hockey MS, Newman J, *et al.* An immunohistochemical study of CEA expression and their metastases. *Br J Surg* 1984, **71**, 376.
9. Hockey MS, Stokes HJ, Thompson H, *et al.* Carcinoembryonic antigen (CEA) expression and heterogeneity in primary and autologous metastatic gastric tumours demonstrated by a monoclonal antibody. *Br J Cancer* 1984, **49**, 129–33.
10. Allum WH, Stokes HJ, Macdonald F, Fielding JWL. Demonstration of carcinoembryonic antigen (CEA) expression in normal, chronically inflamed, and malignant pancreatic tissue by immunohistochemistry. *J Clin Pathol* 1986, **39**, 610–614.
11. Macdonald F, Crowson MC, Allum WH, Life P, Fielding JWL. *In vivo* studies on the uptake of radiolabelled antibodies to colorectal and gastric carcinoma xenografts. *Cancer Immunol Immunother* 1986, **23**, 119–124.
12. Hunter WM, Greenwood FC. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 1962, **194**, 495–496.
13. Woodhouse CS, Ford CHJ, Newman CE. A semi-automated enzyme-linked immunosorbent assay (ELISA) to screen for hybridoma cultures producing antibody to carcinoembryonic antigen. *Prot Biol Fluids*, 1981, **29**, 641–644.
14. Chandler ST, Anderson P. A method for thresholding subtracted images in radiolabelled antibody imaging. *Br J Radiol* 1987, **60**, 881–886.
15. Ensminger WD, Gyves JW, Stetson P, Walker-Andrews S. Phase I study of hepatic arterial degradable starch microspheres and mitomycin. *Cancer Res* 1985, **45**, 4464–4467.
16. Starkhammer H, Hakansson L. Effect of starch microspheres on the passage of labelled erythrocytes and a low molecular weight marker through the liver. *Acta Oncol* 1987, **26**, 361–365.
17. Epenetos AA, Snook D, Durbin H, Johnson PM, Taylor-Papadimitriou J. Limitations of radiolabelled monoclonal antibodies for localisation of human neoplasms. *Cancer Res* 1986, **46**, 3183–3191.

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Correlation of Anti-emetic Efficacy and Plasma Levels of Ondansetron

Steven M. Grunberg, Susan Groshen, Daniel C. Robinson, Lani L. Stevenson and Polly E. Sanderson

Intravenous ondansetron was administered at doses from 0.01 to 0.48 mg/kg every 4 h for three doses to patients receiving cisplatin 60–120 mg/m^2 for the first time. Plasma samples were collected from 28 patients at baseline and at suitable times post-dose for pharmacokinetic analysis, and were assayed for ondansetron by high-pressure liquid chromatography. Plasma trough level was defined as the level before the third dose and 4 h area-under-the-curve (AUC4) was calculated with the linear trapezoidal method. Despite wide inter-patient variation, a correlation was seen between both trough level ($r = 0.737$, $P < 0.0001$) and AUC4 ($r = 0.903$, $P < 0.001$) related to dose. Trough level was also predictive of AUC4 ($r = 0.824$, $P < 0.0001$). Frequency of complete protection (no emetic episodes) was equivalent throughout the AUC4 range, suggesting anti-emetic activity even at low AUC4. However, a trend toward better protection against failure (5 or more episodes) was seen when higher values of AUC4 were achieved, suggesting more consistent anti-emetic activity at moderate to high AUC4.
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