Octreotide, the reticuloendothelial system, and experimental liver tumour

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Abstract

The inhibitory effect of octreotide on the growth of liver tumour is probably mediated (at least in part) by stimulation of the hepatic reticuloendothelial system (RES) activity. This study therefore investigated the effect of octreotide on the hepatic and splenic RES (assessed by the uptake of technetium 99m labelled albumin colloid, ^{99m}Tc-AC) in normal and tumour bearing rats and in animals treated with gadolinium chloride. The effects of gadolinium chloride and octreotide alone or in combination on the growth of liver tumour were also studied. Octreotide significantly stimulates both hepatic and splenic uptake of ^{99m}Tc-AC in normal rats and tumour bearing rats. In controls, the uptake of ^{99m}Tc-AC was significantly reduced by gadolinium chloride and was not changed by octreotide. RES blockade with gadolinium chloride significantly increased ($p < 0.001$) tumour growth compared with controls (hepatic replacement 42%; 95% confidence intervals (CI), 27.6 to 56.4 v 16.7%, 95% CI, 11.1 to 21.3%) whereas octreotide significantly inhibited ($p < 0.001$) the percentage hepatic replacement by tumour (0.7%; 95% CI, 0 to 2.3 v 16.7%; 95% CI, 11.1 to 21.3). This study highlights the importance of the RES in the development of liver tumour. Furthermore, octreotide inhibited the growth of liver tumour in rats with RES blockade, albeit to a lesser degree than in normal animals. These findings suggest that octreotide inhibits the growth of hepatic tumour by mechanisms other than stimulation of RES activity.

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Keywords: octreotide, reticuloendothelial system, liver tumour.

The reticuloendothelial system (RES) or mononuclear phagocytic system is made up of macrophages and their precursors and is distributed throughout the body. The cells of the RES possess a number of common functions including a role in tumour cell destruction.¹ The fixed macrophages of the liver, the Kupffer cells account for up to 80-90% of the body's RES capacity. It is perhaps somewhat surprising therefore, given the high hepatic concentration of macrophages, that the liver is such a common site for tumour metastases. There is experimental evidence, however, to suggest that RES function is depressed by

operative and experimental trauma² and that an impairment of Kupffer cell activity is associated with increased tumour growth in a number of experimental animals.²⁻⁴ Indeed, at least in rats the presence of hepatic tumour in itself is associated with a decrease in hepatic RES activity.⁵ Stimulation of hepatic RES activity therefore may be of benefit in the treatment of liver metastases.

Octreotide, an analogue of somatostatin, has a considerable inhibitory effect on the growth of hepatic tumour in animal models of liver metastases.^{5,6} Somatostatin analogue RC-160 inhibits the growth of liver metastases of human colon cancer cell lines in nude mice.⁷ The precise mechanism of action of somatostatin analogues is not clear but it may be a direct receptor mediated growth inhibition, or an indirect inhibition of the production and release of trophic factors thought to stimulate tumour growth.⁸ Another possible mechanism is the stimulation of RES activity⁷ and indeed octreotide is a potent stimulator of hepatic and splenic RES activity in experimental animals and in patients with cirrhosis.⁹⁻¹¹ These findings suggest that the effect of octreotide on the growth and development of liver tumour may be mediated at least in part through its stimulatory effects on the RES. The aim of this study was to investigate the effects of octreotide on hepatic and splenic RES activity and the growth of liver tumour in rats with or without manipulation of Kupffer cell activity by gadolinium chloride.

Methods

Measurement of RES activity

Hepatic and splenic RES activity were measured by the clearance of intravenously given radiolabelled technetium 99m labelled albumin colloid (^{99m}Tc-AC). In brief, the rats were anaesthetised with intraperitoneal sodium pentobarbitone, 6 mg/kg (Sagatal, RMB Animal Health Ltd, Dagenham, UK), the left femoral vein exposed at the groin, and ligated distally with a silk (4-0) ligature. Through a venotomy, the femoral vein was cannulated with a 2 Fr silastic tubing (Portex, London). The tubing was connected to a 1 ml syringe and the vein flushed with 0.2 ml of heparinised saline (1000 units sodium heparin in 1 l 0.9% saline). Each rat received an intravenous bolus of 2.5 MBq ^{99m}Tc-AC (in 0.2 ml) followed by 0.2 ml of heparinised saline to flush the line. Twenty minutes after the administration of ^{99m}Tc-AC the animals were bled, killed with an intravenous overdose of

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pentobarbitone (euthatal 200 mg, RMB Animal Health, UK), and the liver and spleen excised and weighed.

The radioactivity of the liver, spleen, and blood was counted in a Phillips well gamma-counter (Pye Unicam, Cambridge). The splenic and hepatic RES activity was expressed in terms of the ratio of the uptake of ^{99m}Tc-AC by the liver or spleen:residual ^{99m}Tc-AC in the blood sample.

$$\text{Liver: blood} = \frac{\text{cpm/g liver}}{\text{cpm/ml blood}}$$

$$\text{Spleen: blood} = \frac{\text{cpm/g spleen}}{\text{cpm/ml blood}}$$

Effects of octreotide and gadolinium chloride on RES activity in normal rats

Twenty male Wistar rats were randomly allocated to receive either octreotide 2 µg subcutaneously twice daily or an equivalent volume of 0.9% saline as a control. At the end of a two week treatment period hepatic and splenic RES activity were measured by the method already described. A further 60 male Wistar rats were anaesthetised and injected with an intravenous bolus injection of 5 mg/kg gadolinium chloride 99.9% (ICN Flow, High Wycombe, Bucks). The rats were then randomly assigned to receive either octreotide (2 µg subcutaneously twice daily in 0.2 ml) or 0.9% (0.2 ml) saline as a control. Ten rats from each group had RES activity measured at 72 hours, one week, and two weeks after the start of treatment.

Induction of hepatic tumour

Liver tumours were induced in rats by intraportal inoculation of tumorigenic cells. The HSN cell line is derived from a fibrosarcoma and produces discreet hypovascular liver

Figure 1: RES activity shown as the hepatic and splenic uptake of ^{99m}Tc-AC 20 minutes after an intravenous bolus in rats treated with saline (control) or octreotide for two weeks. Blocks represent median values and bars the range of values.

TABLE I Hepatic and splenic uptake of ^{99m}Tc-AC in control (saline) and octreotide treated normal rats

Uptake of ^{99m} Tc-AC	Liver		Spleen	
	Control	Octreotide	Control	Octreotide
Median	4.9	8.9†	10.4	20.8*
Range	3.3-7.7	5.4-14.4	4.7-19.9	12.5-32
95% CI	4 to 5.8	8 to 11.7	5 to 14.6	13.5 to 32

*p<0.002; †p<0.001; Mann-Whitney U test.

tumours in hooded Lister rats. The K12-Tr cell line is derived from a carcinogen induced rat colon tumour and produces a moderately to poorly differentiated adenocarcinoma when injected into BDIX rats. In brief hooded Lister or BDIX rats were anaesthetised with intraperitoneal sodium pentobarbitone. Through a midline abdominal incision the portal vein was exposed and tumour cells injected into the portal vein, which was then compressed to prevent bleeding. Male hooded Lister rats²⁰ received an intraportal inoculation of 1×10⁶ HSN tumour cells and 20 male BDIX rats received an intraportal inoculation of 1×10⁷ K12-Tr cells. After recovery from the procedure the rats were allocated to receive either octreotide 2 µg twice daily or 0.9% saline as a control for four weeks. At the end of the treatment period hepatic and splenic RES activity was measured as described.

Gadolinium chloride, octreotide, and tumour growth

Twenty male BDIX rats were anaesthetised with immobilon and received an intravenous dose of gadolinium chloride (5 mg/kg), through the femoral vein. A control group of rats received an intravenous injection of 0.9% saline. All animals then immediately had a laparotomy to facilitate injection of 1×10⁷ K12-Tr cells intraportally as previously described. The animals were allowed to recover from the anaesthetic and 18 hours later 10 rats from each group were given either, octreotide 2 µg subcutaneously twice daily or an equivalent volume (0.2 ml) of 0.9% saline as a control. Treatment was continued for four weeks, after which the rats were killed and the liver removed, fixed, sliced at 4 mm intervals, and blocked. A 1 µm section was taken from every 4 mm slice, mounted on a glass microscope slide, and stained with haematoxylin and eosin. The % hepatic replacement (%HR) of liver by tumour was calculated using an image analyser. In brief the outline of liver and tumour were traced using the image analyser and the area for each calculated. Between 10 and 16 sections were processed for each liver. The %HR for each liver was calculated using the formula:

$$\% \text{ Hepatic replacement} = \frac{\text{Area of tumour}}{\text{Area of liver}} \times 100$$

Statistical analysis

The statistical significance of any difference between treatments was evaluated using the

Figure 2: Hepatic uptake of ^{99m}Tc-AC in rats 3, 7, and 14 days after an intravenous injection of gadolinium chloride (5 mg/kg), followed by treatment with 0.2 ml of saline (control) or 2 µg of octreotide twice daily. Results from normal rats (taken from Fig 1) are included for comparison.

Mann-Whitney U test and the Kruskal-Wallis test for analysis of variance (ANOVA).

Results

Octreotide, gadolinium chloride, and RES activity

Two weeks treatment with octreotide significantly increased both hepatic and splenic uptake of ^{99m}Tc-AC in normal rats compared with control rats treated with saline (Fig 1, Table 1). In contrast a single intravenous dose of gadolinium chloride significantly reduced both hepatic and splenic uptake of ^{99m}Tc-AC compared with normal rats ($p < 0.01$

Figure 3: Splenic uptake of ^{99m}Tc-AC in rats 3, 7, and 14 days after an intravenous injection of gadolinium chloride (5 mg/kg), followed by treatment with 0.2 ml of saline (control) or 2 µg of octreotide twice daily. Results from normal rats (taken from Fig 1) are included for comparison.

Figure 4: RES activity shown as the hepatic and splenic uptake of ^{99m}Tc-AC in hooded Lister rats four weeks after intraportal injection of HSN cells and treatment with octreotide or saline (control). $n = 10$.

ANOVA). This inhibitory effect of gadolinium chloride on RES activity was seen at 72 hours, one week, and two weeks after injection. Administration of subcutaneous octreotide in gadolinium chloride treated rats had no significant effect on the uptake of ^{99m}Tc-AC by the liver or spleen at any of the time periods studied (Figs 2 and 3).

The presence of liver tumour in both hooded Lister and BDIX rats significantly reduced hepatic and splenic RES activity compared with normal rats (Figs 4 and 5). In rats with liver tumour, octreotide significantly increased hepatic and splenic uptake of ^{99m}Tc-AC compared with their appropriate controls (Tables II and III). However, the uptake of ^{99m}Tc-AC in tumour bearing rats receiving octreotide was not significantly different from normal rats.

Gadolinium chloride, octreotide, and hepatic tumour growth

Gadolinium chloride given before intraportal inoculation of tumorigenic cells significantly increased the %HR of the liver by tumour compared with controls (Fig 6, Table II). In contrast octreotide significantly decreased tumour growth compared with controls. Furthermore, octreotide significantly reduced the %HR of liver by tumour after intravenous gadolinium chloride but not to the extent as that seen without RES blockade.

Discussion

The results of this study clearly show that octreotide stimulates both hepatic and splenic RES activity, results in accord with previous findings.^{9,10} The mechanism by which octreotide stimulates RES activity is not known but may result from a direct effect of the analogue on the Kupffer cells of the liver and the fixed macrophages of the spleen.

Figure 5: RES activity shown as the hepatic and splenic uptake of $^{99m}\text{Tc-AC}$ in BDIX rats four weeks after intraportal injection of K12-Tr cells and treatment with octreotide or saline (control). $n=10$.

Alternatively octreotide may stimulate RES activity indirectly by modulation of cytokine activity.

The presence of liver tumour in rats resulted in a reduction in both hepatic and splenic RES activity. Treatment with octreotide restored both hepatic and splenic RES activity towards normal. Furthermore, in animals treated with octreotide, the %HR of liver by tumour was significantly less than in the control animals. It is possible that the higher RES activity in the octreotide treated groups reflects a reduction in tumour burden rather than a true stimulation of a depressed RES. The %HR of liver by tumour in the untreated rats in these studies, however, was around 15 to 20% and at this stage the actual amount of liver tissue is probably not reduced, the tumours growing by displacing normal liver parenchyma. The effects of octreotide on RES activity in rats with hepatic tumour have been investigated previously⁵ and the rapid increase in RES activity suggest that octreotide has a true stimulatory effect on hepatic and splenic RES, which is probably independent of tumour burden.

Hepatic RES activity is important in controlling tumour growth in a number of experimental models. Stimulation of Kupffer cell activity by a number of factors such as glucan, levamisole, and *Corynebacterium parvum* is associated with a decreased incidence of liver metastases and an inhibition of tumour growth

TABLE II Hepatic and splenic uptake of $^{99m}\text{Tc-AC}$ in hooded Lister rats four weeks after intraportal injection of HSN cells and treatment with saline (control) or octreotide

Uptake of $^{99m}\text{Tc-AC}$	Liver		Spleen	
	Control	Octreotide	Control	Octreotide
Median	1.8	3.7†	3.3	8.6*
Range	1.2–2.8	2.2–11.1	1.6–7.1	2–19.2
95% CI	2.2 to 5.6	1.3 to 2.8	1.9 to 5.2	3.1 to 16

* $p=0.017$; † $p<0.002$; Mann-Whitney U test.

TABLE III Hepatic and splenic uptake of $^{99m}\text{Tc-AC}$ in BDIX rats, four weeks after intraportal injection of K12-Tr cells and treatment with saline (control) or octreotide

Uptake of $^{99m}\text{Tc-AC}$	Liver		Spleen	
	Control	Octreotide	Control	Octreotide
Median	2.8	4.5†	5.1	9.2*
Range	1.2–4.7	2.9–8.6	1.4–12.4	4–14.7
95% CI	1.2 to 4.2	3 to 5.7	3.7 to 10.2	6.8 to 14.2

* $p=0.25$; † $p<0.005$; Mann-Whitney U test.

in the liver.^{12–16} Furthermore, levamisole has also been shown to reduce the incidence of recurrent cancer (including liver metastases) in patients with Dukes's B resected colon cancer when used in combination with 5-fluorouracil.¹⁷ The exact mechanism of action of levamisole in inhibiting tumour growth and development in humans is not known but it does have a stimulatory effect on hepatic RES activity in normal rats, which is comparable with that of glucan and zymosan but less potent than octreotide.¹⁰ Conversely, depression of Kupffer cell activity is associated with an increased incidence of liver metastases.¹⁸ Human Kupffer cells are cytotoxic and cytostatic against neoplastic cells in vitro,¹⁹ and more specifically against human colon cancer cell lines,²⁰ both when unstimulated and after stimulation by interferon alpha and lipopolysaccharide (which increases cytotoxicity). Kupffer cells isolated from patients with liver metastases derived from colorectal cancer have an increased capacity to bind tumour cells compared with Kupffer cells isolated from normal livers.²¹ It would therefore seem that RES activity and Kupffer cell function are important in the defence against hepatic tumour growth.

In this study, the rare earth metal gadolinium chloride depressed RES activity after a single intravenous dose and although there was some recovery of RES function, it remained significantly reduced for at least two weeks in

Figure 6: Effects of pre-treatment with intravenous gadolinium chloride (GAD) (or saline; control) on the %HR of liver by tumour in BDIX rats before intraportal injection of K12-Tr cells and followed by treatment with octreotide or saline. $n=10$.

TABLE IV Percentage hepatic replacement of liver by tumour in BDIX rats after intravenous gadolinium chloride or saline followed by tumour induction and treatment with either octreotide or saline for four weeks

Percentage hepatic replacement	Control		Gadolinium chloride	
	Control	Octreotide	Control	Octreotide
Median	16.7	0.7	42	11.2
Range	7–31.6	0–2.8	21.2–68	1.9–32.4
95% CI	11.1 to 21.3	0 to 2.3	27.6 to 56.4	8.3 to 15.6
Significance		p<0.0001	p<0.0001	p=0.149

p Values compared with controls using ANOVA.

normal rats. Octreotide had no stimulatory effect on RES activity after blockade induced by gadolinium chloride. Similarly gadolinium chloride blockade of the hepatic RES is also unaffected by a number of other RES stimulants such as zymosan, triolein, and oestradiol.^{22–24}

Administration of gadolinium chloride before tumour induction by intraportal injection of K12-Tr adenocarcinoma cells significantly increased the growth of tumour in the liver of the BDIX rat, presumably because of the RES blockade. Octreotide was effective in reducing the growth of liver tumour in rats with and without blockade of RES activity by gadolinium chloride. Our results suggest that octreotide is more effective in inhibiting the growth of liver tumour in the presence of a functioning RES (that is, without gadolinium chloride). Octreotide was still able to exert at least some inhibitory effect on hepatic tumour growth, however, when RES activity was reduced by administration of gadolinium chloride. Thus, although stimulation of hepatic RES activity by octreotide may be important in reducing tumour growth in the liver there are clearly other mechanisms operating.

A reduction in immune competence occurs with malignancy in general and after surgery for colorectal cancer.^{25–27} Kupffer cell function is also probably affected in this generalised immune depression. It seems probable that patients with gastrointestinal cancer have some dysfunction in RES activity, which is further compromised by the trauma of resectional surgery. This may allow exfoliated tumour cells to seed both in the liver or locally (anastomotic sites and peritoneal cavity), or both and may potentiate the growth of occult hepatic tumours. It is clear from this study that octreotide has a stimulatory effect on both hepatic and splenic RES activity in normal rats and rats with liver tumour. This action may be important in the inhibition of growth and development of liver tumour. Octreotide will stimulate hepatic RES activity in patients with cirrhosis and there is no reason to suggest that it will not have an effect on the RES system in patients with liver tumour either overt or occult. The mechanisms whereby octreotide exerts its effects on RES and Kupffer cell function warrant further investigation. Moreover, octreotide may stimulate the RES at a time

when it is depressed after resectional surgery for cancer and may therefore be useful as an adjuvant to surgery in these patients.

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