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Increased Uptake of 5-FU in Experimental Liver Tumours by Simultaneous Infusion of Norepinephrine

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Abstract—*The effect of the simultaneous administration of norepinephrine and 5-fluorouracil (5-FU) on the uptake of radiolabelled 5-FU by liver tumours was studied in rats. Three different concentrations of 5-FU were used (15, 1.5 and 0.15 μg/g body weight). The drugs were infused over a 30 min period via the hepatic artery, following cannulation of the gastroduodenal artery. The radioactivity in liver tumour, normal liver, lungs and intestines was estimated by liquid scintillation counting. At all concentrations tested, an increased uptake of radioactive 5-FU was found in the tumour when norepinephrine was infused. Tumour/liver ratios also increased significantly in all these cases. No significant differences were noted between norepinephrine infused and control animals in the radioactivity in normal liver, lungs and intestines. The effects noted were possibly due to changes in blood flow within the liver, but the possibility of a direct effect of norepinephrine on DNA metabolism is discussed.*

INTRODUCTION

SYSTEMIC chemotherapy with 5-FU has an overall response rate of 20% in the treatment of hepatic metastases from colorectal cancer. It has not been shown that any other drug or combination of drugs have any advantages over 5-FU alone [1]. Regional administration of a cytotoxic drug via the hepatic artery will result in a higher drug concentration in the liver tumour, compared to systemic administration. Such a treatment modality used alone or in combination with hepatic artery ligation has been claimed to give favourable results in several studies [2–5]. Any beneficial effect seems however to be transient. In addition it is difficult to predict the response in an individual patient [6]. The systemic toxicity is small, but side-effects in the biliary tree limit the dose of the drug which may be used [7].

It has been shown that tumour vessels, being immature, are deficient in both smooth muscles and adrenergic innervation [8, 9]. They are thus much less responsive to vasoactive drugs than normal hepatic blood vessels. The administration of vasoconstricting agents should therefore constrict vessels in the liver parenchyma but leave the tumour vessels relatively unaffected, resulting in a different distribution pattern of blood flow between the tumour and normal liver. If adrenergic stimulation of liver blood vessels is combined with cytotoxic drug infusion via the hepatic artery, an increased concentration of the drug would be expected in the tumour, allowing an increased uptake by the tumour cells and a reduced uptake in normal liver parenchyma.

We have studied the combination of adrenergic stimulation and cytotoxin administration in an experimental tumour model. Norepinephrine was chosen as the adrenergic drug because it is a full α-receptor agonist and a low potency β-receptor agonist, has few side effects and a short half-life *in vivo*.

5-FU was used because, despite its low activity, it has a tolerable therapeutic index in the treatment of liver metastases in colorectal cancer [10].

MATERIALS AND METHODS

The experiments were carried out on 32 male inbred Wistar rats, weighing 250–350 g (Anticimex, Södertälje, Sweden). The animals were housed in cages of three and maintained on standard laboratory pellets and tap water *ad libitum*.

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The animals were anaesthetized by chloral hydrate 5% intraperitoneally (0.5 ml/100 g body weight). At laparotomy 0.1 ml of a tumour cell suspension containing 10^6 viable tumour cells was injected under the capsule of the left lateral liver lobe. The original tumour was a colonic adenocarcinoma induced by *N*-methyl-*N*-nitrosoguanidine and serially transplanted under the renal capsule. The tumour cells used in the present study had passed through about 25 passages.

The experiments were performed 12 days after tumour innoculation. Anaesthesia was induced with ether in combination with intramuscular injection of Ketamine (100 mg/kg body weight) and atropine (0.25 mg/kg body weight). A tracheostomy was performed and the animal connected to a small animal positive pressure ventilator. Anaesthesia was maintained with a mixture of oxygen/nitrous oxide, 30/70%, at a frequency of 85 breaths/min and a tidal volume of 400 ml/min. A positive end expiratory pressure of 1 cm H_2O was used to maintain better oxygenation. Arterial blood gases were examined every 15 min.

The femoral artery was exposed and cannulated with a polyethylene catheter (0.7 mm Portex, Hythe, Kent, U.K.). The catheter was gently advanced to the aorta and connected to a transducer (Siemens-Elema, Sweden), for continuous recording of arterial blood pressure (BP). The abdomen was opened via a midline incision. Under an operating microscope (Zeiss OPMI 65, at 25–40 × magnification), the gastroduodenal artery was carefully dissected and cannulated by a polyethylene catheter (800/110/100/100 Portex, Hythe, Kent, U.K.), so that the tip of the catheter did not enter the hepatic artery. The various solutions tested were infused into the gastroduodenal artery, at a rate of 1 ml/h for 30 min, using a Harvard syringe pump.

Norepinephrine was obtained from Sigma, Chemicon, Sweden. It was kept at a concentration of 10^{-1}M at −70°C, and diluted just before use. [^{3}H]6-5FU in aqueous solution, containing 1 μCi/ml, was obtained from Amersham, U.K. 5-FU (25 mg/ml) was obtained from F. Hoffmann-La Roche, Basel, Switzerland. Norepinephrine was infused at a concentration of 10^{-3} M (320 μg/ml).

Immediately after the infusion the animals were sacrificed by exsanguination and blood collected for estimation of bilirubin, alkaline phosphate (ALP), alanine-aminotransferase (ALAT) and aspartate-aminotransferase (ASAT). All tests were performed by the routine laboratory methods, in the Department of Clinical Chemistry, Lund University, Sweden.

Liver and tumour biopsies were taken for histological examination. Two biopsies of approx. 100 mg were obtained from each of the middle and left lateral liver lobes. The tumour was then carefully dissected free of surrounding normal liver, measured, weighed, cut into small pieces and divided into three samples of approxim. 100 mg. Equal biopsies were obtained from right and left lung, duodenum, terminal ileum and sigmoid colon.

One ml of a tissue solubilizer (Soluene-350, Packard) was added to each of the tissue samples and they were digested 36 h. 250 μl of hydrogen peroxide was then added to bleach the solution and the samples were allowed to stand for 24 h to allow excess oxygen to escape. After that, 15 ml of liquid scintillation fluid (Insta-Gel, Packard) was added to each tissue sample and they were counted in a liquid scintillation counter (Packard, Tri-Carb, 460 CD). Quench correction was achieved by the method of external standard quench correction. The concentration of radioactivity was expressed as dpm/mg of wet tissue.

Experimental design

Three experimental groups were designed and six different combinations of drugs were examined:

Experiment 1. Group 1a: ($n = 6$) infusion of 15 μg/g body weight of 5-FU (labeled with 200 μCi [^{3}H]-6-5FU) and norepinephrine. Group 1b: ($n = 6$) infusion of 15 μg/g body weight of 5-FU (labeled with 200 μCi [^{3}H]-6-5FU).

Experiment 2. Group 2a: ($n = 5$) infusion of 1.5 μg/g body weight of 5-FU (labeled with 20 μCi of [^{3}H]6-6FU) and norepinephrine. Group 2b: ($n = 5$) infusion of 1.5 μg/g body weight of 5-FU (labeled with 20 μCi [^{3}H]6-5FU).

Experiment 3. Group 3a: ($n = 5$) infusion of 0.15 μg/g body weight of 5-FU (labeled with 2 μCi of [^{3}H]-6-5FU) and norepinephrine. Group 3b: ($n = 5$) infusion of 0.15 μg/g body weight of 5-FU (labeled with 2 μCi of [^{3}H]-6-5FU).

In vitro experiments in the pharmacological laboratory of our hospital had shown that no significant degradation of norepinephrine takes place after incubation with 5-FU (less than 5% after 24 h incubation at room temperature or after 48 h at + 4°C).

Preliminary experiments had shown that Wistar-FU rats could survive infusion of norepinephrine as used in this study for over 1 h without significant cardiac or respiratory problems. The results of the experiments were statistically analysed by Wilcoxon's rank sum test.

RESULTS

All animals survived the infusions. At the time of the experiments the mean tumour size was 10 × 8 mm and the mean tumour weight was 0.8 ± 0.08 g (mean ± S.E.).

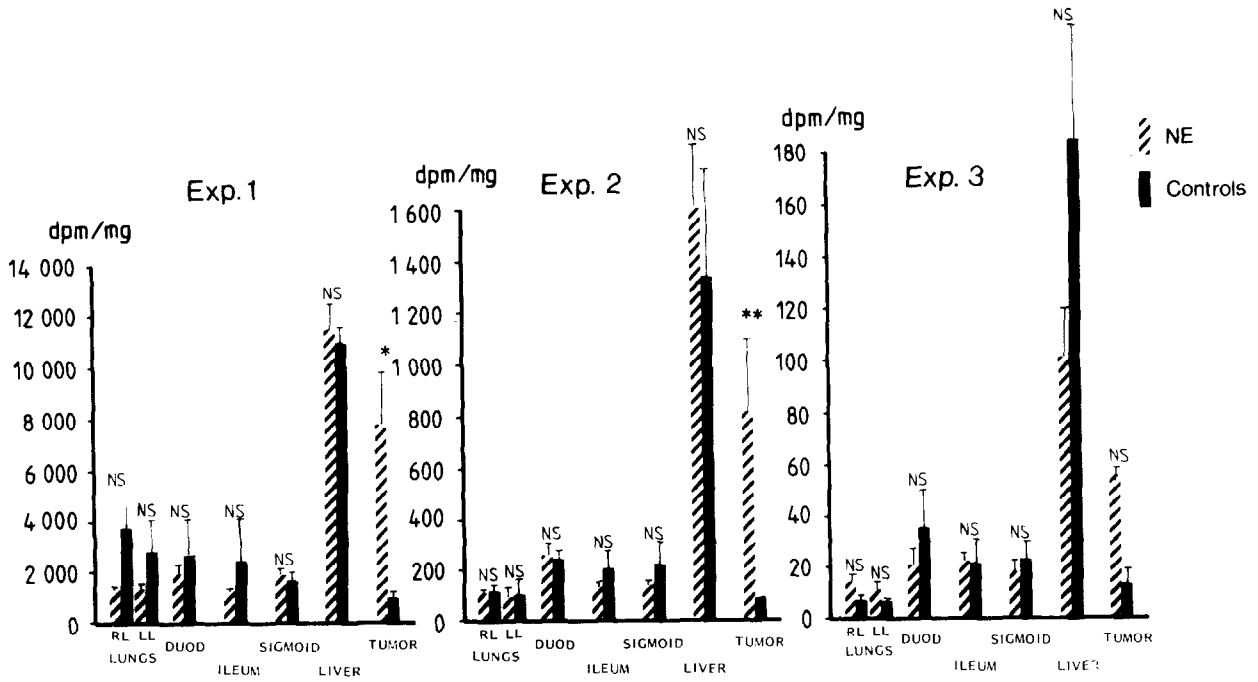

Fig. 1. Organ uptake of radioactivity in animals with norepinephrine infusion (shaded) and in controls (solid). Values are expressed as dpm/mg *of wet tissue and given as mean ± S.E. Statistical analyses were by Wilcoxon Rank Sum tests. Levels of significance are indicated as: NS (non-significant),* $^*P \leq 0.05$ *and* $^{**}P \leq 0.01$. *Experiment* 1. 200 μCi [^{3}H]6-5*FU* + 15 μg/g *of body weight 5-FU with or without NE infusion (Groups 1a and 1b, respectively). Experiment* 2: 20 μCi[^{3}H]6-5*FU* + 1.5 μg/g *of body weight 5-FU with or without NE infusion (Groups 2a and 2b, respectively). Experiment* 3. 2 μCi[^{3}H]6-5*FU* + 0.15 μg/g *of body weight 5-FU with or without NE infusion (Groups 3a and 3b, respectively).*

Histological examination showed poorly differentiated adenocarcinoma with areas of necrosis and infiltration of neutrophils. No histological alterations were seen in the non-tumour-bearing liver parenchyma of either NE perfused or control animals. Infusion of NE resulted in a significant increase in blood pressure in all animals with an average of 54 ± 4 mmHg. There was no increase in BP in control animals. No significant cardiovascular side effects were noted.

Bilirubin, ALP, ASAT and ALAT were similar in animals with or without NE infusion. There was no significant difference in pulmonary uptake of radioactivity between NE infused animals and controls (Fig. 1). The radioactivity of the right and the left lung was statistically the same, indicating equal distribution of the drug.

There was no significant difference between NE infused and control animals in the radioactivity of the three parts of intestine examined.

No statistically significant difference in radioactivity in the liver was found between the NE infused and control animals, although there was a non-significant tendency to a decreased hepatic uptake after NE infusion, in the groups receiving the lower dose of 5-FU.

Concerning the tumour tissue radioactivity there was a significant increase in experiment 1, in the NE treated (Group 1a), compared with control animals (Group 1b). The same holds true in animals of experiment 2. Tumours in the NE treated group

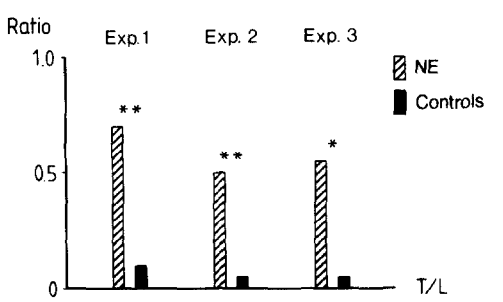

Fig. 2. Tumour/liver ratio of radioactivity in norepinephrine infused animals (shaded) and controls (solid). Statistical analyses were by Wilcoxon Rank Sum Tests. Levels of significance are indicated as: $^*P \leq 0.005$ *and* $^{**}P \leq 0.01$. *Experiment* 1: 200 μCi [^{3}H]6-5*FU* + 15 μg/g *of body weight 5-FU, with or without NE (Groups 1a and 1b, respectively). Experiment* 2: 20 μCi [^{3}H]6-5*FU* + 1.5 μg/g *of body weight 5-FU, with or without NE (Groups 2a and 2b, respectively). Experiment* 3: 2 μCi [^{3}H]6-5*FU* + 0.15 μg/g *of body weight 5-FU, with or without NE (Groups 3a and 3b, respectively).*

(Group 2a) demonstrated significantly higher radioactivity than controls (Group 2b). In experiment 3, the mean values of the NE infused animals were higher than the controls, but this difference did not reach significance ($P > 0.01$).

Norepinephrine infused animals exhibited an increase in tumour to liver ratio of radioactivity compared with the controls at all concentrations used (Fig. 2). This consisted mainly of an increase in tumour radioactivity. The ratios were almost identical at the three concentrations of 5-FU used in both NE infused animals and controls.

DISCUSSION

Administration of cytotoxic drugs via the hepatic artery is often used in palliation of unresectable liver tumours. The amount of drug infused has been limited by generalized cytotoxic side effects, as well as local toxic effects on the non-tumourous liver [11]. A high incidence of late biliary sclerosis has been recently reported after liver perfusion with Floxuridine [7]. Several ways to selectively increase the concentration of the drug delivered to the tumour have been tried. By infusing an oil-based contrast medium, Lipoidol, in combination with a cytostatic drug into the hepatic artery, a longer and more selective concentration of the drug in the liver tumour was achieved, with prolonged survival of the experimental animals [12]. By coupling daunomycin to monoclonal antibodies directed towards alpha fetoprotein, it has been possible to attack specific target, rat hepatoma cells. This has resulted in increased specific activity against cancer cells and also prolonged survival [13].

To use vasoactive agents combined with cytotoxic drug infusion, with the intention of directing blood flow towards the tumour, increasing drug concentration and prolonging drug exposure of cancer cells, has been advocated by several authors. Norepinephrine administered through the celiac artery with an anticancer drug improved symptoms of prolonged survival in patients with gastric cancer [14]. In rat tumours, angiotensin II, injected as a bolus injection with mitomycin C, resulted in a significant increase in tumour blood flow. When this regime was repeated, it prolonged survival and diminished the size of lymph node metastases [15]. Administration of epinephrine, preceeding mitomycin C infusion through the hepatic artery, significantly increased the ratio of mitomycin C concentration between liver tumour and normal liver parenchyma in rabbits [16].

In our study we examined the effect of NE on 5-FU distribution in normal liver and liver tumour in rats. As the intestine is often a site of 5-FU side effects, the concentrations of the drug in duodenum, terminal ileum and sigmoid were also studied.

In all three pairs of animal groups, representing different drug doses, there was a substantial increase in the tumour/liver ratio of radioactivity, indicating that the concentration of the 5-FU tumour is much higher than in the liver after NE infusion. In experiment 1 (groups 1a and 1b) and experiment 2 (groups 2a and 2b), this was the result of a significant increase in concentration in the tumour. In experiment 3 (groups 3a and 3b), although the increase in tumour radioactivity was not significantly greater in NE infused animals, when viewed with the relatively lower liver radioactivity, a significant change in the tumour/liver ratio is apparent.

A decrease in radioactivity was expected in normal liver tissue after NE infusion, secondary to decreased blood flow. However, uptake of radioactivity in normal liver was not decreased after NE infusion in experiments 1 and 2. An explanation could be saturation of a carrier transport mechanism of 5-FU infused through the cell membrane [17]. The amount of 5-FU infused (15 and 1.5 μg/g body weight), was possibly high enough to saturate this transport mechanism even when the liver blood flow was decreased by NE. When the smaller dose of 5-FU was infused in experiment 3 (0.15 μg/g body weight), this saturation level was not reached and there was a decrease in liver radioactivity in infused rats compared to controls.

No differences were detected in intestinal radioactivity in animals with or without NE infusion. An increased cytotoxic effect on intestine when NE is simultaneously infused is therefore unlikely.

The probable explanation for our findings is an altered distribution of blood flow inside the liver. Blood flow measurements in rats in our laboratory, using Tc and Cr labeled microspheres, have shown significant increase in the ratio between flow in tumour tissue and normal liver parenchyma when NE was injected intravenously [18].

Norepinephrine may also have some metabolic effects on 5-FU uptake. It has recently been shown that there exists a promotor effect of NE on DNA synthesis in primary cultures of rat hepatocytes [19]. This is probably exerted through α_1-adrenoreceptors, found in relatively high concentrations in the rat liver. Although the exposure time to NE in our experiment was short, only 30 min, a temporary stimulation of DNA synthesis in the tumour cells, mediated by adrenergic receptors, which may be present in the tumour, could result in an increase in 5-FU uptake by these cells, as 5-FU is a proliferation-dependent agent [20].

In conclusion, treatment of hepatic tumours by combining a vasoconstrictive agent with a cytotoxic drug seems promising. It seems that selectivity in tumour treatment could be achieved and higher concentrations of the anticancer drug could be reached inside the tumour. This would allow us, by reducing the total amount of administered drug, to minimize systemic and hepatic toxic side effects. Ongoing experiments in our laboratory are being designed to elucidate the details of the mechanisms of the phenomenon reported here.

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