In Vitro Evidence That Myocardial Ischemia Resulting from 5-Fluorouracil Chemotherapy Is Due to Protein Kinase C-mediated Vasconstriction of Vascular Smooth Muscle

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ABSTRACT

5-Fluorouracil (5-FU) is a commonly employed chemotherapeutic agent. Among the various toxicities associated with 5-FU, cardiovascular toxicity, consisting principally of acute myocardial ischemia and/or myocardial infarction, has been reported in up to 8.5% of patients treated with this drug. While 5-FU-induced coronary vasospasm has been considered as a potential basis for such clinical toxicity, this hypothesis remains unsubstantiated by laboratory investigation. Accordingly, the present study was designed to investigate the hypothesis that 5-FU induces reversible vasocnstriction of vascular smooth muscle and to study the cellular mechanisms of such vasomotor alterations.

To investigate the effects of 5-FU on the vasoreactivity of vascular smooth muscle, 479 exposures were performed in 105 rings of aorta freshly isolated from 23 New Zealand white rabbits. Vasocnstriction was documented in 20 of 86 (23%) rings exposed to 5-FU at 7 x 10^{-6} M, 45 of 83 (54%) rings exposed to 5-FU at 7 x 10^{-5} M, and 41 of 49 (84%) rings exposed to 5-FU at 7 x 10^{-4} M. In each case, 5-FU-induced vasocnstriction was endothelium independent.

Pretreatment of rings with 10^{-9} M staurosporine, a protein kinase C (PK-C) inhibitor, reduced 5-FU-induced vasocnstriction from 25.0 ± 6.5 to 2.5 ± 1.7 mg; staurosporine at a concentration of 10^{-8} M abolished 5-FU-induced vasocnstriction. Pretreatment of rings with 10^{-7} M phorbol-12,13-dibutyrate, an activator of PK-C, increased the magnitude of 5-FU-induced vasocnstriction 23-fold, from 49.7 ± 11.1 mg before to 1163.6 ± 276.4 mg after phorbol-12,13-dibutyrate (P = 0.0002). Neomycin, an inhibitor of phosphoinositide turnover, did not alter the magnitude of 5-FU-induced vasocnstriction. Membrane receptor blockers, including the α-adrenergic receptor blocker phenolamine, the β-adrenergic receptor blocker propranolol, the H$_2$ receptor inhibitor diphenhydramine, the H$_3$ receptor inhibitor cimetidine, the Ca$^{2+}$ channel blockers verapamil and diltiazem, and the cyclooxygenase inhibitor indomethacin all failed to alter the magnitude of 5-FU-induced vasocnstriction. Finally, 5-FU-induced vasocnstriction was abolished by nitroglycerin.

These results indicate that (a) 5-FU causes direct, endothelium-independent vasocnstriction of vascular smooth muscle in vitro, (b) this vasomotor response involves activation of PK-C, and (c) this response is independent of vasoactive cell membrane receptors, phosphoinositide turnover, or activation of the cyclooxygenase pathway. These findings suggest that the cardiovascular toxicity of 5-FU is due to PK-C-mediated vasocnstriction of vascular smooth muscle.

INTRODUCTION

5-FU is a chemotherapeutic agent commonly employed for treatment of gastrointestinal, breast, head and neck, and pulmonary malignancies. Clinical reports (1–6) have documented the fact that cardiovascular toxicity, consisting principally of acute myocardial ischemia, represents a potentially fatal (7) complication of 5-FU chemotherapy. A recent review by Gradishar et al. (8) disclosed that, among 277 patients treated with bolus or continuous infusion of 5-FU, the frequency of cardiovascular toxicity was 8.5%.

In selected cases (6), coronary angiography performed subsequent to the acute ischemic event demonstrated no evidence of coronary arterial narrowing by atherosclerotic plaque. As a result, coronary artery spasm has been suggested as a potential pathogenetic basis for these acute ischemic events provoked by exposure to 5-FU. This hypothesis, however, has not been previously substantiated by measurements of vascular reactivity following exposure to 5-FU. The present study was performed to investigate the hypothesis that 5-FU induces reversible vasocnstriction of vascular smooth muscle in vitro and to study the cellular mechanisms responsible for observed alterations in vasomotor tone.

MATERIALS AND METHODS

Preparation of Isolated Vascular Rings. Following premedication with xylazine, male New Zealand white rabbits weighing 3–3.5 kg were anesthetized with a mixture of ketamine HCl and acepromazine. The thoracic aorta was exposed, ligated at both ends, dissected from the mediastinum, and immediately immersed in Krebs bicarbonate buffer solution (in mM: NaCl, 118; KCl, 4.8; CaCl$_2$, 2.5; MgSO$_4$, 1.2; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 24; glucose, 110) to which 0.03 mM Na$_2$EDTA had been added to prevent oxidation. Adherent perivascular connective tissue was gently dissected away. Aortic rings 3 mm in length, 3.0–3.5 mm in diameter, 0.20–0.25 mm in wall thickness, and free of side branches were prepared by performing a series of transverse cuts in an isolated segment of aorta. A total of 105 rings were harvested in this fashion from a total of 23 rabbits. In certain cases, specific care was taken not to disrupt the aortic endothelium; in other cases, the aortic endothelium was purposely disrupted by gently rubbing the intimal surface with a cotton swab.

Organ Chamber Experiments. Each vascular ring was then mounted isometrically by placing two 24-gauge stainless steel hooks through the lumen and closing them in a “coat-hanger” shape; the lower one was attached to the bottom of the organ bath, and the upper one was connected to a Grass FTO3 force transducer (Quincy, MA). The transducers were connected to a Grass 7P122D preamplifier, DC amplifier, and model 7 polygraph to allow continuous recording of isometric tension. The 30-ml organ bath contained Krebs bicarbonate buffer. The bath was maintained at 37°C and was continuously gassed with 95% O$_2$/5% CO$_2$. The pH (pH 7.4) and the pO$_2$ (>500 mm Hg) were controlled. Throughout the preparation and mounting procedure, the aortic rings were kept wet with Krebs buffer and care was taken not to abrade or disturb the endothelial surface of the nondenuded rings. When drugs were added to the baths, the additional volume was always <2%. The vascular rings were preloaded with 3.5 g and allowed to equilibrate for 1 h; this preload was based on prior experiments in which optimal basal ring tension was determined by the isometric response to KC1 (60 mM). Isometric tension was then recorded continuously.

Pharmacological agents and inhibitors were added to the organ bath in a cumulative fashion in 0.3–1 log unit increments. After each drug addition, time...
was allowed for the ring to attain a new steady state of vasomotor tone. Each ring was tested for response to 5-FU and washed with buffer 3 times until the vascular tone returned to base-line.

For experiments designed to determine the vasomotor response to various concentrations of 5-FU, a relatively large sample size was employed for each of three concentrations (see Table I). In contrast, for experiments designed to investigate the mechanistic properties of the effect of 5-FU on vascular smooth muscle, a relatively small sample size, typically <10 muscular rings/experiment, was arbitrarily employed.

**Pharmacological Assessment of Endothelial Integrity.** Selected specimens were precontracted with 10^{-4} M 5-HT (Sigma). ACh was then added to the bath in incremental concentrations from 10^{-6} to 10^{-2} M to determine the preservation of functional endothelial integrity, when indicated. Relaxation of the ring in response to ACh at concentrations between 10^{-6} and 10^{-2} M was interpreted as indicating preservation of functional endothelial integrity. The buffer was then exchanged with normal buffer and the samples were allowed to re-equilibrate for 45 min prior to exposure to other vasoactive compounds.

**Measurement of 5-FU Dose-Response Curve**. 5-FU in concentrations varying from 7 \times 10^{-3} to 7 \times 10^{-3} M (0.25 g/5 ml/vial; Roche) was introduced into the bath; this range of concentrations was selected to include concentrations corresponding to therapeutic levels of 5-FU (9). A concentration of 7 \times 10^{-4} M corresponds to 0.1 mg/ml, a peak plasma concentration achieved after injection of 500 mg/m^2 5-FU to an adult patient weighing 70 kg and having a blood volume of 5 liters (9, 23). The resulting effect on the vasomotor reactivity of each vascular ring was then recorded and normalized as a percentage of the maximum response to 10^{-4} M 5-HT recorded in the same ring. In those cases in which specimens were exposed to pharmacological pretreatment, the magnitude of vasoconstriction was reported as an absolute amount (mg). Dose-response curves were then constructed using these normalized values. In addition to the magnitude of vasomotor response, each recording was also analyzed according to previously employed methods (10) for the following characteristics of each response, as a function of drug concentration: time to onset of response, time to peak alteration in tension, and recovery time (time required to return to base-line tone). To evaluate the potential role of the fluorinated impurities previously suggested to be responsible for certain cases of 5-FU-related cardiovascular toxicity (11), the Sigma preparation of 5-FU, which contains no fluorinated impurities, was used for a selected series of experiments.

**Evaluation of Cellular Mechanisms Responsible for 5-FU-induced Vasospasm**. To study the potential involvement of membrane receptors in 5-FU-induced vasomotor reactivity, rings were exposed to 5-FU before and after treatment with the following membrane receptor blockers: the \(beta\)-adrenergic receptor blocker pranopanol (10^{-4} M), the \(alpha\)-adrenergic receptor blocker phentolamine (10^{-5} M), the H1 receptor antagonist diphenhydramine (10^{-5} M), and the H2 receptor inhibitor cimetidine (10^{-5} M). The effect of calcium channel blockade was evaluated using diltiazem and verapamil (10^{-5} M). Finally, rings were also pretreated with the cyclooxygenase inhibitor indomethacin (5 \times 10^{-6} M) to evaluate possible involvement of arachidonic acid metabolism.

Pharmacological signal transduction pathways in vascular smooth muscle involving hydrolysis of PIP2 to inositol-1,4,5-trisphosphate and diacylglycerol, with subsequent activation of PK-C, have been well documented (12, 13). To test whether 5-FU-induced vasoactivity is mediated through one of these pathways, vascular rings were exposed to 5-FU before and after treatment with the following: neomycin, an inhibitor of PIP2 metabolism (14), at a concentration of 10^{-4} M for 10 min; PDBU; an activator of PK-C, at a concentration of 10^{-9} to 10^{-3} M for 50 min; and PK-C inhibitors staurosporine and H-9, at concentrations of 10^{-4} to 10^{-3} M.

**Evaluation of Other Chemotherapeutic Agents.** Several other antineoplastic agents were evaluated as controls. These included the alkylating agents cyclophosphamide (2-20 mg) and nitrogen mustard (4 \times 10^{-4} M). The antimebolitides methotrexate (folic acid analogue, 10 mg) and 6-thioguanine (purine analogue, 10 mg), and the antibiotic doxorubicin (Adriamycin, 2 \times 10^{-3} to 2 \times 10^{-4} M). Leucovorin (1-10 mg), frequently administered in conjunction with 5-FU as an “enhancing” agent for folic acid-depleted neoplastic cells, was also studied.

**Evaluation of the Vasoreactivity of 5-FU-related Compounds.** To determine the relationship between 5-FU-induced vasoreactivity and certain of the constituents of 5-FU, several chemically related compounds were also studied. These included uracil, a naturally occurring nucleotide, in concentrations of 10^{-6} to 10^{-3} M; 5-iodouracil at 10^{-5} to 10^{-2} M; fluoramide (fluorodeoxyuridine), a biologically active metabolite of 5-FU, in concentrations of 10^{-6} to 10^{-2} M; and cytarabine, another pyrimidine analogue, at 3 \times 10^{-4} M.

**Evaluation of the Response of 5-FU-constricted Arteries to Nitroglycerin.** To study the ability of nitroglycerin to reverse the vasoconstriction induced by 5-FU, vascular rings were first contracted with a high concentration of 5-FU (7 \times 10^{-4} M) and then exposed to 10^{-1} M nitroglycerin.

**Statistical Analysis.** All results are expressed as mean ± SE. Alterations in the magnitude of the vasomotor response to 5-FU and/or various pharmacological pretreatments were statistically analyzed using Student’s t-test. One-way analysis of variance with Scheffe’s test was used for comparing data among three groups. A P value of <0.05 was considered to indicate statistical significance.

**RESULTS**

A total of 479 exposures were performed in 105 vascular rings harvested from 23 rabbits.

**Vector, Magnitude, Time to Onset, and Duration of Vasomotor Response.** The prevalence of vasoconstriction produced by 5-FU correlated directly with the molar concentration of 5-FU in the bath buffer (Table I). In no case did 5-FU cause a reduction in vasomotor tone. Furthermore, at concentrations of \leq 10^{-4} M 5-FU caused no or negligible vasoconstriction. A representative response to 5-FU is shown in Fig. 1.

The magnitude of vasoconstriction recorded was proportional to the concentration of 5-FU administered (Table 1; Fig. 2). At concentrations of 7 \times 10^{-5}, 7 \times 10^{-4}, and 7 \times 10^{-3} M, the magnitude of vasoconstriction recorded in response to 5-FU was 1.3 ± 0.3% (range, 0.1-4.3%), 4.0 ± 0.6% (0-25%), and 13.5 ± 3.6% (0-100%), respectively, of maximum constriction induced by 10^{-4} M 5-HT.

The onset of vasoconstriction varied from 5 to 30 s (12.0 ± 2.7 s) following administration of 5-FU. Peak vasoconstriction was recorded within 2-6 min (4.0 ± 0.9 min). For most (85 of 106, 80.2%) exposures, the increase in vasomotor tone persisted for 3-20 min following peak contraction (Table 1). A sustained increase in vasomotor tone persisting for 1–5 h was observed in 1 of 86 (0.9%), 2 of 83 (1.8%), and 18 of 49 (36.9%) of the rings exposed to 7 \times 10^{-5}, 7 \times 10^{-4}, and 7 \times 10^{-3} M 5-FU, respectively.

A parallel series of experiments was performed to investigate the possibility that fluorinated impurities, previously shown to be present in the clinical (Roche) preparation of 5-FU (11), could account for 5-FU-induced vasoconstriction. These experiments were performed using equimolar concentrations of the Sigma preparation of 5-FU, previously studied (11) to contain no such impurities, and the Roche preparation used above. A total of 10 rings were exposed to 7 \times 10^{-3} M concentrations of either the Sigma (5 rings) or Roche (5 rings) preparation of 5-FU. When vascular tone returned to base-line, the rings were challenged with 7 \times 10^{-5} M 5-HT. No statistically significant difference was observed in the magnitude (Sigma, mean = 88.7%, range = 33–154%; Roche, mean = 103.8%, range = 39–157%) or duration (Sigma, mean = 27.8 min; Roche, mean = 37 min) of vasoconstriction induced by the two preparations of 5-FU. The

**Table 1 Prevalence, magnitude, and duration of vasoconstriction induced by increased concentrations of 5-FU**

<table>
<thead>
<tr>
<th>[5-FU] (m)</th>
<th>Prevalence of vasoconstriction (%)</th>
<th>Magnitude of vasoconstriction (% of maximum vasoconstriction induced by 10^{-4} M 5-HT)</th>
<th>Duration of increased vasomotor tone (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 \times 10^{-5}</td>
<td>23 (23/66)</td>
<td>1.3 ± 0.3</td>
<td>6.89 ± 0.61</td>
</tr>
<tr>
<td>7 \times 10^{-4}</td>
<td>54 (45/93)^a</td>
<td>4.0 ± 0.6^a</td>
<td>8.16 ± 0.37^a</td>
</tr>
<tr>
<td>7 \times 10^{-3}</td>
<td>84 (4/49)^a</td>
<td>13.5 ± 3.6^a</td>
<td>13.2 ± 1.09^a</td>
</tr>
</tbody>
</table>

^a P < 0.01 versus 7 \times 10^{-5} M.

^b P < 0.01 versus 7 \times 10^{-4} M.

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S-FU-INDUCED VASOSPASM

Fig. 1. Representative recording of vasomotor response to 5-FU. Minimal vasoconstriction was observed in response to a concentration of $7 \times 10^{-5}$ M. At concentrations of $7 \times 10^{-4}$ M and $7 \times 10^{-3}$ M, 5-FU-induced vasoconstriction measuring 300 mg and 1200 mg, respectively.

Effect of Phosphoinositide Turnover and PK-C Activity on 5-FU-induced Vasoconstriction. The effect of the PK-C inhibitor staurosporine on 5-FU-induced vasoactivity was studied in 7 vascular rings. Four rings were exposed to 5-FU at $5 \times 10^{-5}$, $10^{-4}$, $5 \times 10^{-4}$, and $10^{-3}$ M, before and after pretreatment with $3 \times 10^{-9}$ M staurosporine (data not shown). In these 4 rings mean vasoconstriction in response to all indicated concentrations of 5-FU was $25.0 \pm 6.5$ mg ($0-80$ mg) before staurosporine and was reduced to $2.5 \pm 1.7$ mg ($0-20$ mg) after staurosporine pretreatment ($P < 0.01$). The remaining 3 rings were exposed to increasing concentrations of 5-FU before and after pretreatment with $10^{-9}$ M staurosporine; in these 3 rings, vasoconstriction in response to 5-FU before staurosporine was $26.2 \pm 8.4$ mg ($0-80$ mg), whereas vasoconstriction after $10^{-9}$ M staurosporine was blocked (Fig. 3). Fig. 4 demonstrates a representative tracing from one of these experiments. In two additional experiments, 5-FU-induced vasoconstriction was reversed to baseline by exposure to $10^{-9}$ M H-9 (data not shown).

The effect of PDBU, a specific activator of PK-C, was studied in 10 rings. These 10 rings were exposed to 5-FU before and after treatment with $10^{-7}$ M PDBU. Vasoconstriction in response to 5-FU was dramatically increased after treatment with PDBU at concentrations of

Fig. 2. Magnitude of 5-FU-induced vasoconstriction in relation to the concentration of 5-FU. The response observed at $7 \times 10^{-5}$ M significantly exceeded the response observed at $10^{-4}$ M and $10^{-3}$ M.

clinical (Roche) preparation of 5-FU was employed exclusively for all subsequent experiments.

Endothelial Integrity and 5-FU-induced Vasoconstriction. The effect of functional endothelial integrity on 5-FU-induced vasoconstriction was studied in 31 rings. Twenty rings were handled so as to preserve the endothelium intact, while 11 rings were purposely denuded. The functional status of the endothelium was tested in each ring by the response to $10^{-6}$ to $10^{-7}$ M ACh. The magnitude of vasoconstriction induced by $7 \times 10^{-4}$ M 5-FU was similar for the specimens with functionally preserved endothelium, compared to those in which the endothelium had been purposely disrupted ($P = \text{NS}$; data not shown).

In 10 additional rings, the effect of exposure to 5-FU on subsequent endothelial function was studied. Each ring was precontracted with $10^{-4}$ M 5-HT and then tested with ACh, at concentrations of $10^{-6}$ and $10^{-7}$ M, before and after exposure to $7 \times 10^{-5}$ M 5-FU for 60 min. The mean magnitude of ACh-induced relaxation was $12.0 \pm 1.7\%$ ($10.0$-$30.0\%$) of the response to 5-HT before and $12.9 \pm 3.0\%$ ($29.6$-$37.0\%$) after 5-FU treatment ($P = \text{NS}$). Thus, 5-FU-induced vasoconstriction is endothelium independent and 5-FU treatment does not alter the ACh-induced endothelium-dependent relaxation.
5-FU-induced vasospasm

**DISCUSSION**

**Cardiovascular Toxicity following 5-FU Administration.** Adverse cardiovascular events related to 5-FU chemotherapy were first described by Roth et al. in 1975 (1). Multiple reports of acute myocardial ischemia developing in close temporal relationship to administration of 5-FU have been published subsequently (1–8). Symptoms consisting of angina and/or dyspnea typically appear several hours after completion of bolus or continuous infusion of 5-FU (6, 7). Symptoms are generally accompanied by electrocardiographic changes, reappear with subsequent drug exposures, and respond to treatment with nitrates. In some patients, however, signs of myocardial ischemia progress to frank myocardial infarction, pulmonary edema, and/or ventricular arrhythmias and, on occasion, may be fatal (6, 7). More recently, prospective ambulatory monitoring has documented clinically silent ST-segment deviation suggestive of myocardial ischemia in 17 of 25 (78%) patients receiving 4–5 g/m² 5-FU by continuous infusion (7). Continuous infusion and higher doses have led to increased incidence of cardiovascular events (9).

**5-FU-INDUCED VASOSPASM**

**Effect of Other Chemotherapeutic Drugs.** A total of 25 aortic rings were studied with antineoplastic agents other than 5-FU. Cyclophosphamide, nitrogen mustard, methotrexate, 6-thioguanine, and doxorubicin (Adriamycin) in clinically relevant concentrations (10⁻⁷ to 10⁻⁵ M) each failed to alter vasoactivity in any of the rings studied (n = 5 for each drug). In these experiments, cyclophosphamide was used without prior activation; nitrogen mustard was used to represent a direct-acting alkylating agent. Likewise, leucovorin did not induce vasomotor alterations in any of 5 rings studied (data not shown).

**Vasomotor Response to Structural Homologues of 5-FU.** Constituent requirements for 5-FU-induced vasoconstriction were studied using structural homologues of 5-FU. Uracil given to 5 rings induced no response at concentrations of ≤10⁻⁴ M and mild relaxation [decrease in tone of 33.0 ± 4.3 mg (25–50 mg)] at a concentration of 10⁻³ M. 5-Iodouracil given to the same rings after they had been washed 3 times with Krebs buffer induced no response at concentrations of ≤10⁻⁴ M and mild relaxation [decrease in tone of 24.2 ± 2.4 mg (20–30 mg), P < 0.05] at a concentration of 10⁻³ M. Neither fluorouracil (fluorodeoxyuridine) (n = 5) nor cytarabine at 3 × 10⁻⁴ M (n = 5) induced any vasomotor response, before or after exposure to PDBU.

**Response of 5-FU-Constricted Vascular Rings to Treatment with Nitroglycerin.** Three rings were exposed to 7 × 10⁻³ M 5-FU. The resulting magnitude of vasoconstriction was 253.3 ± 26.0 mg (210–300 mg). Subsequent exposure to nitroglycerin completely reversed the vasoconstriction induced by 5-FU and further relaxed the rings below their baseline tone (Fig. 6).

**Relation of Membrane Receptors, Ca²⁺ Channels, and Cyclooxygenase Pathway Products to 5-FU-induced Vasoconstriction.** Pretreatment of rings with the α-adrenergic receptor blocker phentolamine at 10⁻⁵ M (n = 5), the β-adrenergic receptor blocker propranolol at 10⁻⁶ M (n = 10), the H₁ inhibitor diphenhydramine at 10⁻⁵ M (n = 5), or the H₂ inhibitor cimetidine at 10⁻⁵ M (n = 5) had no effect on the magnitude of 5-FU-induced vasoconstriction. Ca²⁺ channel blockers verapamil (10⁻⁵ M) and diltiazem (10⁻⁵ M) likewise failed to alter the magnitude of 5-FU-induced vasoconstriction (n = 20). Finally, pretreatment with the cyclooxygenase inhibitor indomethacin (10⁻⁵ M) also did not alter the magnitude of 5-FU-induced vasoconstriction.

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been cited as possible factors predisposing patients to the development of 5-FU cardiovascular toxicity (15, 16).

Previous laboratory investigation of 5-FU cardiovascular toxicity has been infrequently reported. Suzuki et al. (17), using a rabbit model, administered 5-FU at 15 mg/kg (1.6 × 10^{-3} M) over 1 h and produced ischemic ST-T wave changes indicative of myocardial ischemia at 17 h; death due to ventricular fibrillation was observed at 24 h. All rabbits receiving a continuous infusion of 30 mg/kg (3.2 × 10^{-3} M) 5-FU died of ventricular fibrillation within 8 h following initiation of the infusion. Matsubara et al. (18) studied the effect of 5-FU given to open-chest guinea pigs. Electrocardiographic abnormalities indicative of myocardial ischemia appeared within 3 h following drug administration in seven of seven (100%) animals treated with 60 mg/kg 5-FU. appeared in four of nine (49%) treated with 30 mg/kg, and were not observed in animals treated with 10–20 mg/kg (1–2 × 10^{-3} M) 5-FU.

Previously Proposed Mechanisms of 5-FU-induced Vasoconstriction. The mechanisms responsible for 5-FU-induced cardiovascular toxicity have not been previously defined. Both coronary vasospasm (6) and autoimmune phenomena (5) have been previously proposed as pathogenetic bases. Other mechanisms previously suggested include biochemical alterations in vascular smooth muscle membranes (18), pulmonary hypertension secondary to pulmonary arterial vasoconstriction or changes in platelet aggregability (19), and radiotherapy-induced small-vessel thrombosis (20).

The absence of focal luminal diameter narrowing in two patients studied by coronary angiography following clinically evident 5-FU-induced myocardial ischemia (6) has been cited as evidence to support the concept that myocardial ischemia may be the result of transient reversible vasoconstriction, or spasm. Such coronary arterial spasm has been previously documented as the antecedent basis for total thrombotic occlusion of a previously patent coronary artery both in patients (21) and in experimental studies (22).

Current Findings. The current trend toward more aggressive use of 5-FU in the treatment of a variety of malignancies, including its use in adjuvant therapy for patients with resected colorectal or breast cancers, and the paucity of previous laboratory investigation of 5-FU-induced cardiovascular toxicity led us to further define the effects of 5-FU on vascular smooth muscle reactivity. The in vitro experiments presented here demonstrate an endothelium-independent, direct, vasoconstrictor effect of 5-FU on vascular smooth muscle. The range of concentrations (5 × 10^{-3} to 1 × 10^{-3} M) over which 5-FU produced vasoconstriction in the isolated vascular ring model approximates therapeutic levels achieved in patients undergoing bolus chemotherapy with 5-FU (7, 9, 23). At higher concentrations of 5-FU, vasoconstriction was severe (Fig. 1), reaching 100% of maximal vasoconstriction observed in response to 5-HT. While the duration of 5-FU-induced vasoconstriction in most rings was <20 min, some rings, particularly those exposed to higher concentrations, demonstrated increased tone for up to 4 h.

Fluorinated impurities have been implicated as the causative agent in 5-FU-mediated cardiovascular toxicity. As many as six fluorinated compounds have been identified by nuclear magnetic resonance spectroscopy in the clinical preparation (Roche) of 5-FU. Work by Lemarié et al. (11) has suggested that fluorooacetate, resulting from the metabolic conversion of the impurity fluoracetaldehyde, constitutes the principal toxin. The Sigma preparation has been shown to contain no such impurities (11). The results of our experiments demonstrated direct vasoconstriction in response to both preparations. These findings thus indicate that fluorinated impurities, including fluorooacetate, are not responsible for 5-FU-induced vasoconstriction recorded in vitro.

Previous investigations have established the pivotal role of the enzyme PK-C in modulating a variety of intracellular events (24). In particular, Endo et al. (25) demonstrated the role of PK-C in both the phosphorylation of the 20-kDa light chain of gizzard myosin and the phosphorylation of intact myosin. PK-C has also been shown to phosphorylate smooth muscle myosin light chain kinase in vitro (26, 27). Thus, PK-C has been suggested to be a subcellular mediator of vascular smooth muscle tone.

The data from the current series of experiments suggest that 5-FU-induced vasoconstriction involves activation of PK-C. The tumor-promoting phorbol ester PDBU is structurally similar to diacylglycerol and has been previously shown to activate PK-C directly, both in vitro and in vivo (28, 29). In the present study, pretreatment with PDBU produced a 23-fold increase in the magnitude of 5-FU-induced vasoconstriction. Furthermore, staurosporine, a PK-C inhibitor (30), completely abolished 5-FU-induced vasoconstriction and a more specific PK-C inhibitor, H-9, reversed 5-FU-induced vasoconstriction to base-line.

Tonic vasoconstriction of vascular smooth muscle has been considered to be a function of PK-C activation (31, 32). This is consistent with the prolonged duration of increased vasomotor tone observed in some rings exposed to 5-FU. The primary molecular mechanism responsible for 5-FU-induced activation of PK-C requires further elucidation. Neomycin, a competitive inhibitor of the phosphodiesterase responsible for the metabolism of PIP_2 (14), failed to alter 5-FU-induced vasoconstriction; this suggests that 5-FU activates PK-C directly, rather than inducing breakdown of PIP_2, and that this response is most likely not a consequence of augmented intracellular Ca^{2+} concentrations.

The findings of this investigation thus suggest the following: (a) 5-FU causes direct endothelium-independent vasoconstriction of vascular smooth muscle in vitro; (b) this vasomotor response involves activation of PK-C; and (c) this response is independent of vascular smooth cell membrane receptors, phosphoinositide turnover, or activation of the cyclooxygenase pathway. The clinical implication of these findings is that the cardiovascular toxicity of 5-FU is due to PK-C-mediated constriction of vascular smooth muscle. Moreover, the finding that nitroglycerin, which induces vasodilatation via increased levels of cGMP, successfully reversed 5-FU-induced vasoconstriction confirms the suggestion that nitrates represent appropriate therapy for 5-FU-induced vasospasm observed in vivo.
ACKNOWLEDGMENTS

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REFERENCES

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Morris Mosseri, Howard J. Fingert, Lyuba Varticovski, et al.


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