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Role of Urotensin-2 in 5-Fluorouracil-Related Arterial Vasoconstriction in Cancer Patients

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Keywords

5-Fluorouracil · Cardiotoxicity · Urotensin 2 · Vasoconstriction

Summary

Background: The aim of this study was to identify the possible relationship of 5-fluorouracil (5-FU)-related arterial vasoconstriction with urotensin-2 (UT-2), which has a high potential as an endogenic vasoconstrictor. **Methods:** We assigned the patients to 1 of 3 groups. Patients in group 1 received a bolus of 5-FU, those in group 2 a continuous infusion (CI) of 5-FU, and those in group 3 no 5-FU, which was also a control group. Pre- and post-treatment UT-2 levels and brachial arterial diameters were measured and recorded in all patients. **Results:** 132 patients were included in the study. Pre- and post-treatment brachial artery diameters were similar in all groups: in group 1 (3.28 ± 0.52 vs. 3.25 ± 0.44 mm, p = 0.740), in group 2 (3.57 ± 0.47 vs. 3.46 ± 0.45 mm, p = 0.441) and in the control group (3.51 ± 0.52 vs. 3.25 ± 0.44 mm, p = 0.818). Pre- and post-treatment UT-2 levels were significantly different in each group: in group 1 (39.5 ± 30.9 vs. 56.7 ± 27.1 ng/ml, p = 0.0001), in group 2 (37.7 ± 33.7 vs. 62.5 ± 37.7 ng/ml, p = 0.0001) and in the control group (52.9 ± 40.2 vs. 60.8 ± 40.7 ng/ml, p = 0.006). **Conclusion:** Our findings suggest that UT-2 has a high potential as a vasoconstrictor agent in our bodies and its level increases through a bolus or CI 5-FU. Increased UT-2 levels are likely to play a role in 5-FU-related cardiac toxicity pathogenesis.

Introduction

5-Fluorouracil (5-FU) is administered widely as a single agent or in multidrug regimens for various types of malignancies, including gastrointestinal, breast, and head and neck cancers [1]. Although the drug is well known for its adverse effects involving the bone marrow, skin, mucous membranes, intestinal tract, and the central nervous system, its cardiotoxicity is less familiar to clinicians. Evidence for an adverse cardiovascular event related to 5-FU was first described by Roth et al. in 1975 [2]. Since then there has been an increasing number of case reports and small series with 5-FU-induced cardiac events [3].

The pathophysiology of 5-FU-associated adverse cardiac events is unclear, and so far conclusions have been based on clinical studies and case reports rather than on solid experimental evidence. While clinical and electrocardiographic features suggest that myocardial ischemia is the main etiological factor, possibly induced by coronary vasospasm, histomorphological and biochemical studies indicate a more direct drug-mediated cytotoxic action. The overall incidence of fluorouracil cardiotoxicity is estimated as 1.2–18%. Patients may present with angina-like chest pain, cardiac arrhythmias or myocardial infarction. There is no unequivocally effective prophylaxis or treatment for this syndrome once fluorouracil administration is discontinued. The symptoms are usually reversible, although fatal events have been described. The overall mortality rate has been estimated as 2.2–13.3%. There is a high risk of relapse when patients are re-exposed to this drug following previous cardiac events [4].

Urotensin 2 (UT-2) is an 11-amino acid cyclic peptide originally isolated from the goby fish. UT-2 is generally agreed to be the most potent endogenous vasoconstrictor discovered to date. Some of its

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physiological mechanisms are similar to other potent mediators, such as endothelin-1. UT-2 also has a wide range of actions in other systems, such as in the proliferation of vascular smooth muscle cells, fibroblasts, and cancer cells. Elevated plasma levels of UT-2 and increased expression levels of UT-2 and UT-2 receptor have been demonstrated in numerous disease conditions, including hypertension, atherosclerosis, heart failure, pulmonary hypertension, diabetes, renal failure, and metabolic syndrome. Some reports even suggest that UT-2 may be a marker of disease activity. As such, the UT receptor is emerging as a promising target for therapeutic intervention [5]. However, as the mechanisms of cardiotoxicity associated with 5-FU have not yet been completely identified, at present there are no prophylactic options for preventing this cardiotoxicity.

In this study, we investigated a possible relationship between arterial vasoconstriction due to 5-FU and UT-2, known to be the most potential endogenic vasoconstrictor agent in our body. If a significant relationship is identified, UT-2 receptor blockers could potentially be used to prevent 5-FU-related cardiotoxicity.

Patients and Methods

In our study, group 1 comprised patients receiving a bolus 5-FU treatment, group 2 those receiving a continue infusion (CI) 5-FU treatment and group 3 those not receiving 5-FU in their treatment, thus representing a control group. Group 1 patients were mostly breast cancer patients who received FEC (fluorouracil, epirubicin, cyclophosphamide) or FAC (fluorouracil, doxorubicin, cyclophosphamide) therapy. Group 2 patients had gastrointestinal system cancer and received FOLFOX 6 with or without monoclonal antibody (bevacizumab), FOLFIRI with or without monoclonal antibody or cisplatin with CI 5-FU. Group 3 patients were on therapies that did not include 5-FU (carboplatin +paclitaxel, epirubicin + cyclophosphamide, doxorubicin + cyclophosphamide). There were some cardiotoxic drugs administered to the control group, such as anthracycline, cyclophosphamide and platin derivatives; however, none resulted in clinically significant coronary vasospasm.

Pretreatment evaluation in the 3 groups comprised a detailed medical history, physical examination, determination of the vital functions, and laboratory investigations, including complete blood cell count, and liver and renal function tests. Patients with a history of cardiovascular disease (previous myocardial infarction and/or angina pectoris) and those using any medication that might affect UT-2 levels (calcium channel blockers, beta-blockers, nitrates, angiotensin-converting enzyme (ACE) inhibitors, or angiotensin receptor blockers) were excluded.

UT-2, complete blood cell counts, and hepatic and renal function tests were analyzed in blood samples drawn before and just after chemotherapy. Brachial artery diameters were measured in all patients by the same radiologist using B-mode imaging with a 12-MHz linear-array transducer (General Electric Logic 9, Milwaukee, USA). Scans of the brachial artery were performed 3–5 cm proximal to the arteria ulnaris. These diameters were calculated immediately before and after the first day of treatment with 5-FU.

Whole blood collected in ethylendiamine tetra-acetic acid (EDTA) tubes was assayed for UT-2, prior to (basal value) and immediately after the end of administration of the cytostatic drugs. Tubes were immediately placed on ice and centrifuged at 1,600 × g for 10 min at 4°C and plasma was stored at −80°C until the time of analysis. Plasma UT-2 levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Eastbiopharm, China), according to the manufacturer's instructions, and results were expressed in ng/ml. A 12-lead electrocardiography was performed for all patients in the study before and after chemotherapy.

Informed written consent was obtained from each subject included in the study, and the study protocol was approved by the Bezmialem Vakif University local ethical committee.

Statistical Analyses

All data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL, USA) software. A Wilcoxon test was used for comparison of non-categorical variables. A Chi-square test was used for comparison of categorical variables for all of the groups. Repeated measure analysis of variance was used in this study to identify the differences between the 3 treatment groups. A p value of ≤ 0.05 was considered significant.

Results

A total of 132 patients were included in this study. Of these, 42 patients received a bolus 5-FU (group 1), 51 patients received CI 5-FU (group 2) and the remaining 39 patients formed the control group (group 3) and were treated with 5-FU-free regimens in the same hospital unit. The median age of the 38 female and 4 male patients in group 1 was 52 years (range 24–86), the median age of the 23 female and 28 male patients in group 2 was 56 years (range 24–86) and that of the 13 female and 19 male patients in the control group was 54 years (range 24–86).

In group 1, 38 patients had breast cancer, 1 patient had gastric cancer, 1 esophagus cancer, 1 colon cancer and 1 hepatocarcinoma. In group 2, 19 patients had colon cancer, 9 pancreatic cancer, 9 gastric cancer, 12 rectum cancer, and 2 cholangiocarcinoma. In group 3, 17 patients had lung cancer, 11 breast cancer, 3 endometrium cancer, 2 cholangiocarcinoma and 6 other types of malignancies. In group 1, 13 patients received adjuvant, 25 neoadjuvant, and 4 metastatic disease therapy. In group 2, 21 patients received adjuvant, 3 neoadjuvant, and 27 metastatic disease therapy. In group 3, 10 patients received adjuvant, 5 neoadjuvant, and 24 metastatic disease therapy. In group 1, 31 patients received an FEC chemotherapy regimen, and 2 a bolus FUFA regimen. In group 2, 7 patients received a FOLFIRINOX regimen and 44 a FOLFOX (± bevacizumab) regimen. In group 3, 13 patients received carboplatin-paclitaxel, 6 received cisplatin-gemcitabine, 6 received EC, 3 received AC, 2 received cisplatin-etoposide, 2 received gemcitabine and 7 received other chemotherapy regimens. The patient groups, and clinical and laboratory features are shown in table 1.

In group 1, the pre-chemotherapy brachial artery diameter was 3.28 ± 0.52 mm, and that after chemotherapy was 3.25 ± 0.44 mm (p = 0.740). In group 2, the pre-chemotherapy diameter was 3.57 ± 0.47 mm, and after chemotherapy 3.46 ± 0.45 mm (p = 0.441), and in group 3, the pre-chemotherapy diameter was 3.51 ± 0.52 mm, and after chemotherapy 3.25 ± 0.44 mm (p = 0.818). Thus, there were no significant differences between brachial artery diameter measurements before and after chemotherapy. Brachial artery diameter differences before and after chemotherapy in all groups are shown in figure 1.

The mean UT-2 level detected in group 1 was 39.5 ± 30.9 ng/ml before chemotherapy and 56.7 ± 27.1 ng/ml after chemotherapy (p = 0.0001); in group 2, 37.7 ± 33.7 ng/ml before chemotherapy and 62.5 ± 37.7 ng/ml after chemotherapy (p = 0.0001); and in

Table 1. Patient groups and demographical features

	Group 1	Group 2	Group 3
Patients, n	42	51	39
Age, years (range)	52.6 (24–68)	56.1 (21–71)	54 (29–75)
Sex			
Female	38	23	20
Male	4	28	19
Diagnosis (n)	breast cancer (38)	pancreatic cancer (9)	lung cancer (17)
	gastric cancer (1)	gastric cancer (9)	breast cancer (11)
	esophageal cancer (1)	colon cancer (19)	endometrial cancer (3)
	colon cancer (1)	rectal cancer (12)	cholangiocarcinoma (2)
	hepatocarcinoma (1)	cholangiocarcinoma (2)	other (6)
Treatment (n)	adjuvant (13)	adjuvant (21)	adjuvant (10)
	neoadjuvant (25)	neoadjuvant (3)	neoadjuvant (5)
	metastatic (4)	metastatic (27)	metastatic (24)
Chemotherapy type (n)			
	FEC (38)	FOLFIRINOX (7)	carboplatin-paclitaxel (13)
	FUFA (4)	FOLFOX (±bevacizumab) (44)	cisplatin-gemcitabine (6)
			EC (6)
			AC (3)
			cisplatin-etoposide (2)
			gemcitabine (2)
			others (7)
BSA, m^2	1.76 ± 0.19	1.77 ± 0.2	1.73 ± 0.19
BMI, kg/m^2	26 ± 3.5	26.2 ± 4.1	25.4 ± 0.35
Albumin, g/dl	3.9 ± 0.47	3.9 ± 0.5	4.2 ± 0.32
Hb, g/dl	11.4 ± 1.36	11.5 ± 1.5	12.2 ± 1.27
Ca, g/dl	9.5 ± 0.56	9.4 ± 0.6	9.7 ± 0.45
Smoker, n			
Yes	11	16	10
No	31	36	29

FEC = fluorouracil, epirubicin, cyclophosphamide, EC = epirubicin, cyclophosphamide, AC = doxorubicin, cyclophosphamide, BSA = body surface area, BMI = body mass index.

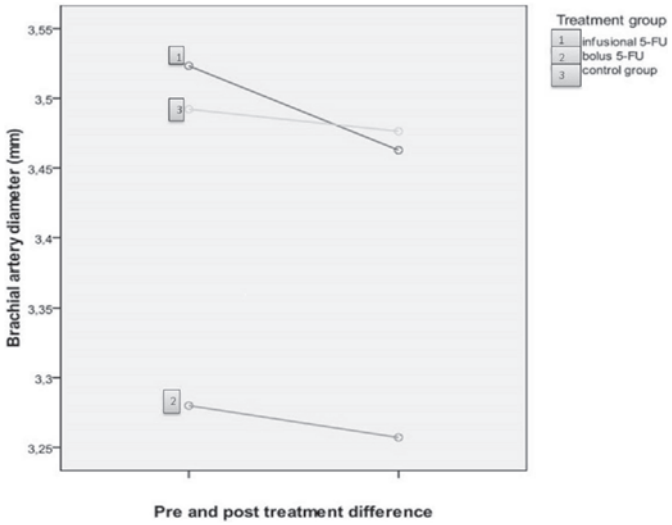

Fig. 1. Brachial artery diameter differences before and after chemotherapy in each group. 5-FU = 5-fluorouracil.

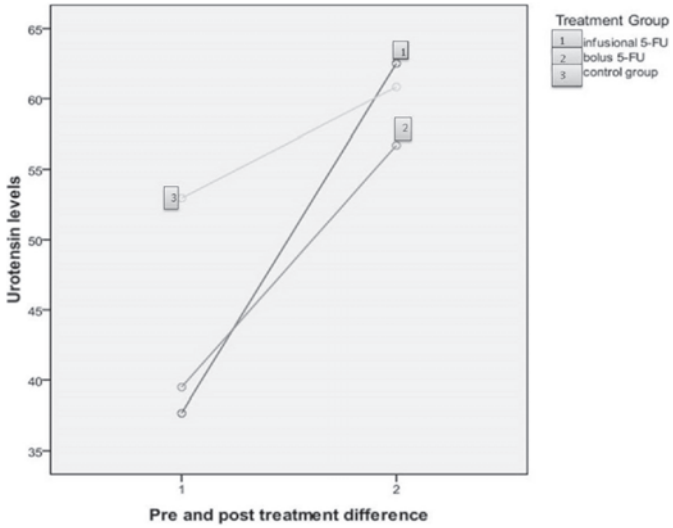

Fig. 2. Differences in urotensin-2 levels before and after chemotherapy in each group.

group 3, 52.9 ± 40.2 ng/ml before chemotherapy and 60.8 ± 40.7 ng/ml after chemotherapy (p = 0.006). The post-chemotherapy UT-2 levels were increased in each group compared to the pre-chemotherapy levels; however, the increments detected in groups 1 and 2 were more significant. Brachial artery diameters and UT-2 levels before and after chemotherapy are shown in table 2. UT-2 level differences before and after chemotherapy in all groups are shown in figure 2.

Table 2. Brachial artery diameters and urotensin levels before and after chemotherapy

	Group 1	Group 2	Group 3
Brachial artery diameter, mm			
Before chemotherapy	3.28 ± 0.52	3.57 ± 0.47	3.51 ± 0.52
After chemotherapy	3.25 ± 0.44	3.46 ± 0.45	3.47 ± 0.51
p value	0.740	0.441	0.818
Urotensin-2 levels, ng/ml			
Before chemotherapy	39.5 ± 30.9	37.7 ± 33.7	52.9 ± 40.2
After chemotherapy	56.7 ± 27.1	62.5 ± 37.7	60.8 ± 40.7
p value	0.0001	0.0001	0.006

Discussion

The precise pathogenesis of 5-FU-associated cardiotoxicity has yet to be clearly elucidated. Coronary vasospasm has historically been accepted as the main contributor to this clinical entity. Ultrasound and angiographic studies demonstrate both coronary artery and brachial artery vasospasm following 5-FU infusion [6–8]. 5-FU has also been shown to induce the vasoconstriction of vascular smooth muscle cells in vitro via the activation of protein kinase C, which resolves with administration of protein kinase inhibitors [9]. Taken together, these data support the theory of coronary artery vasospasm being an important contributor to 5-FU-induced cardiotoxicity. In all studies to date, 5-FU-related cardiotoxicity has been explained in relation to coronary vasospasm: however, the pathophysiology of this vasospasm has not yet been clearly identified.

In the present study, comparing groups 1 and 2, before administration of either the bolus and CI 5-FU there was no significant difference in the values of either UT-2 or brachial artery diameters; there was also no significant difference in these values after 5-FU administration between these groups.

One of our previous studies searched for a relationship between 5-FU-related cardiotoxicity and angiotensin II levels [10]. In that study, we evaluated the effect of 5-FU administration on the diameter of the brachial artery and the levels of angiotensin II. We found that 5-FU-associated vasoconstriction was not dependent on angiotensin II levels, and suggested that the prophylactic administration of ACE inhibitors cannot prevent cardiotoxicity in these patients [10].

In the present study, however, UT-2 levels were increased after 5-FU administration in groups 1 and 2 in relation to the initial levels. The increases in these groups were significantly higher than that in the control group ($p = 0.019$). Although in our previous study [10] we were able to demonstrate constriction of the brachial artery diameter due to bolus 5-FU administration, in the present study brachial artery diameters did not significantly decreased in either group before and after 5-FU administration; only a very slight insignificant decrease was seen. The increase of UT-2 levels in the bolus and CI groups thus does not appear to be related to brachial artery diameter values; nevertheless, we consider that this slight decrease may indicate a possible relationship between 5-FU-related cardiac toxicity and UT-2 levels. It is possible that, due to measurement technique or other uncertain issues, this vasoconstriction were not clearly identified. In our previous study [10], significant constriction in brachial artery diameters was shown with bolus 5-FU administration. In addition, 5-FU-related vasoconstriction was shown in 2 other studies [8, 9]. Sudhoff et al. [8] and our previous study [10] showed brachial artery vasoconstriction related to 5-FU infusion. The 5-FU-induced vasoconstriction was short term, recurred with repeated 5-FU administration and was canceled by glycerol nitrate [8].

In the study by Sudhoff et al. [8], no patients had symptoms of cardiotoxicity, whereas in our previous study [10] 3 of the 31 patients treated with 5-FU developed chest pain. ECG abnormalities were documented in 5 of the 31 patients in our previous study [10]; ECG recordings were not performed by Sudhoff et al. [8]. In the present study, 4 patients in group 1 and only 1 patient in group 2 had angina pectoris without ECG abnormalities. Mosseri et al. [9] explored 5-FU-related vasoconstriction under in vitro conditions, using isolated aorta rings excised from rabbits. The proportion and size of vasoconstriction was directly correlated with the molar concentration of 5-FU. The size of vasoconstriction was similar for aorta rings with functionally protected endothelium and those with purposely damaged endothelium, demonstrating that an intact endothelium was not a necessary element for 5-FU-induced vasoconstriction [9]. 5-FU-induced vasoconstriction was eliminated by nitroglycerin, and 5-FU treatment did not affect acetylcholine-induced endothelium-related relaxation, which showed that 5-FU-induced vasoconstriction is not due to impairment of the endothelial relaxation system. Pretreatment with staurosporine, a protein kinase C (PK-C) inhibitor, reduced 5-FU-induced vasoconstriction, but phorbol-12,13-dibutyrate exposure, as an activator of PK-C, increased the magnitude of 5-FU-induced vasoconstriction 23-fold [9]. Neomycin, an inhibitor of phosphoinositide turnover, and the cyclo-oxygenase inhibitor indomethacin did not affect the 5-FU-related vasoconstriction [9]. The entire set of membrane receptor blockers was investigated in that study, including the α-adrenergic receptor blocker phentolamine, the β-adrenergic receptor blocker propranolol, the H1 receptor inhibitor diphenhydramine, the H2 receptor inhibitor cimetidine and the Ca^{2+} channel blockers verapamil and diltiazem, and did not change the degree of 5-FU-related vasoconstriction [9].

The reason we are considering a relationship between 5-FU-related vasospasm and UT-2 levels is because UT-2 is generally agreed to be the most potent endogenous vasoconstrictor discovered to date. Its physiological mechanisms are similar in some ways

to other potent mediators, such as endothelin-1. For example, both compounds elicit a strong vascular smooth muscle-dependent vasoconstriction via Ca^{2+} release. UT-2 also exerts a wide range of actions in other systems, such as the proliferation of vascular smooth muscle cells, fibroblasts, and cancer cells. It also: (1) enhances foam cell formation, chemotaxis of inflammatory cells, and inotropic and hypertrophic effects on heart muscle; (2) inhibits insulin release, and modulates glomerular filtration, and release of catecholamines; and (3) may help regulate food intake and the sleep cycle. Elevated plasma levels of UT-2 and UT-2 receptor expression have been demonstrated in numerous disease conditions, including hypertension, atherosclerosis, heart failure, pulmonary hypertension, diabetes, renal failure, and the metabolic syndrome. Some of these reports have suggested that UT-2 is a marker of disease activity. As such, the UT receptor is emerging as a promising target for therapeutic intervention.

Sudhoff et al. [8] showed that patients receiving 5-FU exhibited a trend towards an increase in big endothelin plasma levels; however, this finding was independent of whether or not patients developed 5-FU-induced contraction of the brachial arteries. It is therefore unlikely that big endothelin is an important mediator of 5-FU-induced vessel contraction.

In conclusion, our findings suggest that an increased UT-2 level has a high potential as a vasoconstrictor agent in our body and is related to bolus or CI 5-FU. An increased UT-2 level may play a role in 5-FU-related cardiac toxicity pathogenesis. It is clear that identifying the relationship between UT-2 and 5-FU-related cardiac toxicity requires more comprehensive studies.

Disclosure Statement

The authors declare that they have no conflict of interest.

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