The Effect of 5-Fluorouracil and Cisplatin on CA 125, CA 15-3 and CA 19-9 Laboratory Results

5- FLOROURASİL VE SİSPLATİNİN CA 125, CA 15-3 VE CA 19-9 LABORATUVAR SONUÇLARINA ETKİSİ

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Abstract

Objective: In this study, we aimed to investigate the interference of 5-Fluorouracil (5-FU), cisplatin and their combination on laboratory results of CA 125, CA 15-3 and CA 19-9.

Material and Methods: Blood samples were collected from ten volunteer donors who had not taken any medications. Each of these 10 blood samples were divided into four aliquots to form the control, 5-FU, cisplatin and 5-FU + cisplatin combination groups. Adequate amount of 5-FU, cisplatin and 5-FU + cisplatin combination, in accordance with maximum therapeutic plasma concentration, were added to aliquots. Serum CA 125 was determined by immunometric method, and CA 15-3 and CA 19-9 were determined by chemiluminescent immunometric method in each group.

Results: Compared to control groups, we observed that 5-FU and cisplatin had positive interference effect on CA 125 and CA 19-9 laboratory results ($p< 0.05$), whereas no interference of these drugs was observed on CA 15-3 laboratory results ($p> 0.05$).

Conclusion: We concluded that to obtain accurate laboratory results of tumor markers, interference of chemotherapeutic agents that were used to treat these patients should be considered.

Key Words: Fluorourasil, sisplatin, tümör belirteçleri

Özet

Amaç: Bu çalışmada 5-Florourasil (5-FU), sisplatin ve bunların kombinasyonunun CA 125, CA 15-3 ve CA 19-9 laboratuvar sonuçlarını bozup bozmadığını araştırmayı amaçladık.

Gereç ve Yöntemler: Herhangi bir ilaç almayan 10 gönüllüden kan örnekleri alındı. Alınan 10 örnekten her biri kontrol, 5-FU, sisplatin ve 5-FU + sisplatin olmak üzere 4 ayrı gruba bölündü. En yüksek terapötik plazma konsantrasyonuna göre yeterli miktarda 5-FU, sisplatin ve 5-FU + sisplatin kombinasyonu ilgili tüplere eklendi. Serum CA 125 düzeyi immünometrik yöntemle ve CA 15-3 ile CA 19-9 düzeyleri de kemilüminesans immünometrik yöntemle ölçüldü.

Bulgular: Kontrol grubu ile karşılaştırıldığında 5-FU ve sisplatinin CA 125 ve CA 19-9 laboratuvar sonuçları üzerinde pozitif bozucu etki yaptığı halde ($p< 0.05$), CA 15-3 laboratuvar sonuçlarını etkilemediği gözlendi ($p> 0.05$).

Sonuç: Tümör belirteçlerin laboratuvar sonuçlarının doğru elde edilebilmesi için, hastaların tedavilerinde kullanılan kemoterapötik ajanların bozucu etkisinin dikkate alınması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: Fluorouracil, cisplatin, tumor markers

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Early detection of cancer offers the best chance for cure. The goal is to diagnose cancer when a tumor is still small enough to be completely removed surgically. Unfortunately, most cancers do not produce symptoms until the tumors are either too large to be removed surgically or until cancerous cells have already spread to other tissues. Tumor markers are useful in evaluating the progression of disease status after initial therapy and in monitoring subsequent treatment modalities. An ideal tumor marker should be both specific for a given type of cancer and sensitive enough to detect small tumors during screening. However, most known tumor markers are neither specific nor sensitive enough for such purposes.[1]

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Biological and technical advances have led to significantly increased research and development of cancer biomarkers. Carbohydrate-related tumor markers represent a new generation of clinically useful tumor markers. They tend to be more specific than naturally secreted markers, such as enzymes and hormones. Carbohydrate markers are high-molecular-weight mucins (CA 125 and CA 15-3) or blood group antigens (CA 19-9). CA 125, CA 15-3 and CA 19-9 are important tumor markers for ovarian, breast and pancreatic cancers respectively.[1]

The determination of tumor markers requires special attention to the analysis and expression of the results. Extensive effort has been made to standardize preanalytical, analytical, and postanalytical methodology for cancer biomarkers.[2] Interference of assays for clinical analytes by exogenous and endogenous substances is a common problem in clinical laboratories. The major exogenous sources of interference are drugs prescribed for patients.[3] 5-FU, cisplatin and many other chemotherapeutic agents and their combinations are used to treat ovarian, breast and pancreatic cancers.[4] In this study we aimed to investigate the interference of 5-FU, cisplatin and their combination on laboratory results of CA 125, CA 15-3 and CA 19-9.

Material and Methods

Blood samples were collected from ten volunteer donors who had not taken any medications. The subjects fasted overnight (12 h) in order to avoid effects of turbidity interference. Hemolysed, lipemic and icteric specimens were excluded. Each of these samples was immediately centrifuged at 3000 rpm for 10 minutes at room temperature and the serum was divided into four aliquots to form the control, 5-FU, cisplatin and 5-FU + cisplatin combination groups. Adequate amount of 5-FU (600 μg/L), cisplatin (450 μg/mL) and 5-FU + cisplatin (600 μg/L 5-FU + 450 μg/mL cisplatin) combination, in accordance with the optimum therapeutic plasma concentration, were added to the aliquots.[5-7]

Serum CA 125 was determined by immunometric method in each group on an automatic hormone analyzer (Immulite, DPC, Los Angeles, USA). We used a murine monoclonal antibody for the capture and a rabbit polyclonal antibody for the detection of CA 125 antigen. Reference range was 2.6-18.0 U/mL. Total and within-run CVs of the method were 5.0% and 3.1% respectively. Analytical sensitivity of the method was 1.0 U/mL and specificity was over 99%. The cross reactivity with CA 15-3 and CEA was 0.41% and 0.05% respectively.

Serum CA 15-3 was determined by a two-step sequential chemiluminescent immunometric method in each group on an automatic hormone analyzer (Immulite, DPC, Los Angeles, USA). Reference range was 9.0-51.0 U/mL. Total and within-run CVs of the method were 7.7% and 6.5% respectively. Analytical sensitivity of the method was 1.0 U/mL and specificity was over 99%. Cross reactivity with CA 125, CA 19-9 and CEA was not detected.

Serum CA 19-9 was determined by a solid-phase, two-site chemiluminescent immunometric method in each group on an automatic hormone analyzer (Immulite, DPC, Los Angeles, USA). Reference range was 0.0-33.0 U/mL. Total and within-run CVs of the method were 5.0% and 4.8% respectively. Analytical sensitivity of the method was 2.0 U/mL and specificity was over 99%. Cross reactivity with CA 15-3 and CEA was 0.57% and 0.34% respectively.

Statistical analysis

All experimental data were expressed as the mean ± SD. The significance of difference among all groups was analyzed using analysis of variance for repeated measurements and if the F value was significant, differences between means were then analyzed using the post-hoc (Bonferroni procedure) test.[8] Values of $p < 0.05$ were considered statistically significant.

Results

The interference effect of 5-FU and cisplatin on laboratory results of CA 125, CA 15-3 and CA 19-9 are summarized in Table 1. As shown in table I, in comparison to control group we observed that 5-FU and Cisplatin had positive interference effect on CA 125 and CA 19-9 laboratory results ($p <$

Table 1. Interference effect of 5-FU, cisplatin and their combination on CA 15-3, CA 125 and CA 19-9 laboratory results.

Parameters	Control (n= 10)	5-FU (n= 10)	Cisplatin (n= 10)	5-FU+Cisplatin (n= 10)
CA 15-3 (U/mL)	17.3 ± 6.5	20.8 ± 8.3	20.2 ± 8.5	19.1 ± 8.2
CA 125 (U/mL)	9.5 ± 4.2	12.6 ± 6.3[a]	12.7 ± 6.4[a]	12.1 ± 6.2[a]
CA 19-9 (U/mL)	9.2 ± 4.3	13.3 ± 4.5[b]	12.4 ± 4.0[b]	11.3 ± 3.4[b]

$p < 0.05$ was considered statistically significant **a, b** statistically significant in comparison to control group.

0.05), whereas no interference of these drugs was observed on CA 15-3 laboratory results ($p > 0.05$) (Figure 1).

Addition of 5-FU, cisplatin and 5-FU + cisplatin to serum increased CA 19-9 and CA 125 concentrations significantly ($p < 0.05$). However, addition of 5-FU, cisplatin and 5-FU + cisplatin to serum increased CA 15-3 concentrations but the difference between groups were not statistically significant ($p > 0.05$).

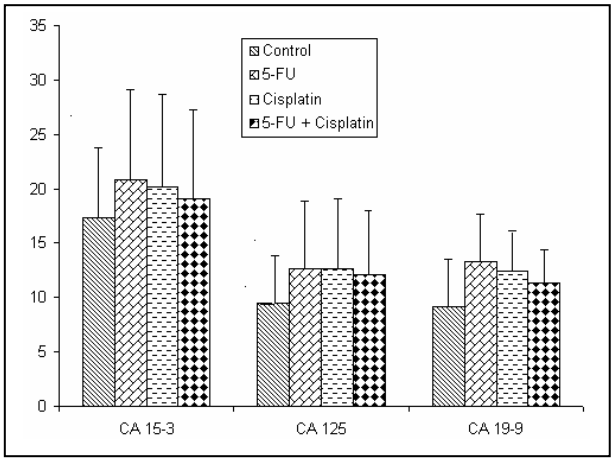

Figure 1. Interference effect of 5-FU, cisplatin and 5-FU + cisplatin combination on CA 15-3, CA 125 and CA 19-9 laboratory results.

Discussion

Analytical interference may be defined as the effect of a substance on any step in the determination of the concentration or catalytic activity of the analyte.[3] Hemolysis, lipemia, hyperbilirubinemia, and paraproteinemia are common endogenous interferents; whereas drugs and additives (materials that are added to the blood collection tubes for anticoagulation, inhibition of glycolysis, etc.) are common exogenous interferents.

Interference is a serious problem; especially anti-animal antibodies are known to interfere unpredictably with immunoassays. Heterophilic antibodies and human anti-mouse antibodies (HAMAs) are important sources of both positive and negative interference, particularly in two-site (sandwich) immunoassays.[9-12]

The data obtained in this study showed that addition of 5-FU and cisplatin had positive interference effects on CA 125 and CA 19-9 ($p < 0.05$) but not on CA 15-3 laboratory results ($p > 0.05$).

Any medication given to patients by any route (intravenous, oral, subcutaneous and so on) may interfere with analytical methods. Medications, designed to be biologically active and given in high pharmacological doses, have a high probability of reacting with analytes or reagents. Metabolites of drugs also may cause interferences and are as important as the parent drugs.[3] In a previous study, Ingen and co-workers showed that 18 widely used drugs (five-fold therapeutic concentration) and 11 cytotoxic drugs (one- or five-fold therapeutic concentration) did not affect the results of measurement of CA 125 by electrochemiluminescense immunoassay using commercial reagents (Elecsys 2010, Roche Diagnostic, Mannhaim, Germany).[13] Unfortunately they did not mention the names of the drugs that were used in their study.

5-FU is an antimetabolite and is biotransformed to 5-fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP), which inhibits thymidylate synthase and leads to 'thymineless death' cells. When adminis-

tered intravenously, 5-FU is widely distributed including the cerebrospinal fluid. Elimination is mainly by metabolism. 5-FU is used in bladder, breast, colon, head and neck, liver, and ovarian cancers.[4] In the present study, we found a significant interference effect of 5-FU on CA 125 and CA 19-9 laboratory results. In addition to this observation we can speculate that the metabolite of 5-FU, 5-FdUMP, may also interfere with these laboratory test results. To detect the interferences caused by the metabolites of drugs may be more difficult than to detect those of the parent compounds, because the concentrations of the metabolites are more unpredictable and the metabolites are often unknown.[3]

Cisplatin is an alkylating agent and forms reactive molecular species that alkylate nucleophilic groups on DNA bases, particularly the N-7 position of guanine. This leads to cross-linking of bases, abnormal base pairing, and DNA strand breakage. When used intravenously, the drug is distributed to most tissues and is eliminated by the kidney unchanged.[4] In the present study, like 5-FU, cisplatin also interfered with the results of CA 125 and CA 19-9 significantly.

5-FU and cisplatin may have formed complexes with CA 125 and CA 19-9 or any other biomolecules that cause interference. The interference mechanism of 5-FU and cisplatin on CA 125 and CA19-9 laboratory results needs further studies.

In conclusion, to consider the interference effect of chemotherapeutic agents may be an important factor to obtain accurate laboratory results of tumor markers in the diagnosis and follow-up of cancer patients.

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