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SHORT COMMUNICATION

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Serum protein binding of lerisetron, a novel specific $5HT_3$ antagonist, in patients with cancer

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Abstract The aim of this study was, (1) to characterize the serum protein binding of lerisetron, a new 5-hydroxytryptamine (5-HT_3) receptor antagonist under investigation as an antiemetic agent, and (2) to measure the percentage of unbound lerisetron in cancer patients. The binding parameters were determined in human serum albumin (HSA), α_1-acid glycoprotein (AAG) and in pooled serum from six healthy volunteers. Concentrations of lerisetron ranging from 50 ng/ml to 2 μg/ml were used. The serum protein binding of ^{14}C-lerisetron (2 μg/ml) was determined by ultrafiltration in three groups of individuals. Group I comprised healthy subjects ($n = 11$), group II comprised cancer patients undergoing radiotherapy ($n = 9$), and group III comprised cancer patients receiving chemotherapy ($n = 18$). The unbound concentration of lerisetron was measured in all samples by liquid scintillation counting. Concentrations of both AAG and HSA were also measured in all serum samples. The drug was extensively bound in pooled serum, involving a nonsaturated process. In HSA, lerisetron was also highly bound (4.04 ± 0.8% unbound) and the protein binding was essentially unchanged within the studied concentration range of lerisetron. The extent of binding to AAG was high but significantly lower than in serum and in HSA and was also independent of lerisetron concentration. The unbound lerisetron was significantly decreased in group II cancer patients when compared with group I subjects (2.38 ± 0.64% vs 3.70 ± 0.70%; $P < 0.001$). No significant changes in lerisetron binding were observed in group III patients. HSA was diminished in both groups of patients and AAG was only significantly increased in group II. Unbound lerisetron was correlated with AAG in group II and with HSA in group III.

Key words Lerisetron · Serum protein binding · Cancer disease

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Introduction

Severe nausea and vomiting are common and distressing side effects associated with chemotherapy for malignant disease. In many cases the frequency and severity of the nausea and vomiting suffered are such that patients will refuse further chemotherapy. The cytostatic-induced emesis is due to activation of 5-hydroxytryptamine (5-HT_3) receptors [4, 16] and therefore the use of appropriate doses of selective and potent 5-HT_3 receptor antagonists could prevent this side effect [14].

However, important differences in pharmacokinetics-pharmacodynamics (pk-pd) seem to exist amongst these compounds which may have an impact on their clinical applications [5]. Particularly, protein binding is a pharmacokinetic parameter which can be very different among drugs even though they may belong to the same group [6, 22]. Although binding can alter both drug distribution and elimination, influencing the effect of the drug [3, 8], very few studies have dealt with this aspect of 5-HT_3 antagonists [17].

Lerisetron is a novel potent and selective 5-HT_3 receptor antagonist. This compound is being evaluated in humans for its antiemetic activity, for both the prevention and treatment of nausea and vomiting induced by chemo- and radiotherapy in cancer patients [13]. Research is underway to determine some of lerisetron's pharmacokinetic parameters (unpublished data) but, to date, no reports exist on the protein binding characteristics of lerisetron.

Several proteins may be involved in the binding of drugs, mainly human serum albumin (HSA) and α_1-acid glycoprotein (AAG). The levels of HSA are decreased

and AAG concentrations are increased in physiopathological situations including cancer [24, 25]. Alterations of serum protein binding in cancer patients are a very common occurrence and may have various causes [1, 18], for example, a diminished HSA content and an increased AAG content. Under these conditions, drugs which bind to HSA or to AAG could have their protein binding altered, as has been previously reported for several drugs [2, 10]. Additionally, the dynamic nature of the illness and its therapy, i.e. chemo- or radiotherapy can also affect protein binding of drugs [10].

Because the free drug concentration is generally that which is best related to pharmacological effects, changes in the binding of drugs to serum protein could result in suboptimal therapy [9, 19] or toxic effects [21], even when the total drug concentration is within the therapeutic range. Knowledge of the protein binding behaviour of a new drug could help establish correct dosage regimens in patients [8, 23].

The present study was designed to identify and evaluate the factors affecting lerisetron binding to human blood proteins in cancer disease. The work consisted of three parts: part 1 was intended to quantify protein binding or lerisetron in human serum and to assess its dependence on concentration; part 2 was aimed at identifying the serum proteins to which lerisetron binds; and part 3 was a comparison of protein binding among cancer patients and healthy volunteers.

Patients and methods

Subjects

Six healthy drug-free volunteers aged from 28 to 57 years participated in part 1 of this study. All were within 10% of their ideal weight and did not suffer a febrile illness during the 72 h before entering the study. All control subjects manifested normal renal and hepatic functions. The Ethics Committee of the Galdakao Hospital granted ethical approval for venepuncture of healthy subjects for the purpose of this study. The subjects involved in part 3 were divided into three groups. Group I comprised 11 healthy drug-free volunteers aged from 28 to 55 years as controls. The criteria for inclusion of this group were similar to those applied in part 1. Group II comprised 9 patients aged from 35 to 65 years with a histologically diagnosed tumour of various origins (prostate, oesophagus, lung, colon, breast). They were undergoing regular radiation therapy at the Hospital of Cruces (Vizcaya). Group III comprised 18 patients aged from 30 to 50 years with a histologically proved tumour of various types (tongue, lung, oesophagus, larynges) undergoing antineoplastic treatment. Owing to the state of their disease, they were frequently prescribed drugs including morphine, cimetidine, calcitonin, haloperidol and digoxin, none of which is associated with known protein binding displacement at therapeutic concentrations. In the 2 weeks prior to the study, none of these subjects was given other drugs that could interfere with the binding studies performed. Hospital Ethics Committee approval was obtained and all patients gave their informed consent.

Blood samples 10–20 ml were collected by venepuncture at between 9.00 and 10.30 a.m. into dry tubes for separation of serum. Serum was immediately separated by centrifugation. For part 1 of the study, three different serum samples were formed by pooling serum from subgroups of three or four individuals from a total of six individual donors.

Chemicals

Lerisetron (F-0930) was obtained from FAES, S.A. and ^{14}C-lerisetron was obtained from HRC (Huntingdon Life Sciences, Cambridgeshire, England, UK)and had a specific activity of 28.07 mCi/mmol. The radiochemical purity of the labelled lerisetron was 98% as determined by thin-layer chromatography. A mixture of appropriate amounts of radioactive and pure drug was used to prepare a stock solution in buffer, pH 7.4. HSA (fatty acid free) and AAG (purity 99%) were obtained from (Aldrich Quimica, Madrid). All proteins were dissolved in buffer (pH 7.4; 120 m*M* NaCl, 5 m*M* KCl, 2.5 m*M* $CaCl_2$, 1 m*M* $MgSo_4$, 20 m*M* TRIS) to obtain protein concentrations of 600 and 50 μM respectively (physiological serum concentrations).

Binding Experiments

Protein binding was determined by an ultrafiltration technique using Amicon Micropartition units [12]. All serum samples (900 μl) containing ^{14}C-lerisetron (10 μl) were incubated at 37 °C for 10 min and then were immediately transferred to the ultrafiltration device. These devices contain a membrane filter of controlled porosity with a cut-off molecular weight (10 000 Da), which retains plasma proteins and allows free drugs in solution to pass through. Centrifugation was performed at 3000 r.p.m. and 37 °C for 8 min. The binding of lerisetron to the membranes and devices was found to be less than 1%. Serum pH did not affect protein binding of lerisetron. After ultrafiltration, 100 μl aliquots of serum ultrafiltrate were added to 10 ml of liquid scintillation. The ^{14}C-lerisetron concentration was assayed in the original serum-drug solution (C_T) and in the ultrafiltrates (C_u) by scintillation.

Experimental protocols

In part 1 the protein binding in pooled serum of six healthy subjects was determined as described above. A wide range of lerisetron concentrations were used, between the limits of 50 ng/ml and 2 μg/ml. In part 2 (identification) 10 μl ^{14}C-lerisetron was added to a 600 μ*M* solution of HSA ($n = 3$) and to a 50 μ*M* solution of human AAG ($n = 3$) to give final concentrations ranging from 50 ng/ml to 2 μg/ml. The binding was measured in each sample as described above. In part 3 (comparison) the binding of lerisetron in group I subjects and cancer patients (groups II and III) was measured using ^{14}C-lerisetron at the single concentration of 2 μg/ml. Serum concentrations of AAG and HSA in all samples were measured by the radial immunodiffusion method [11].

Statistical analysis

The results are expressed as means ± standard deviation. The statistical analyses included Student's *t*-test to compare the unbound percentage and protein levels between healthy subjects and patients. A multivariate analysis was performed relating the unbound lerisetron and protein concentrations (AAG, HSA) measured in the same sample. This was followed by a linear regression derivation. The limit of statistical significance was $P < 0.05$.

Results

Part 1 Lerisetron was extensively bound in pooled serum. The percentage of unbound lerisetron was 3.70 ± 0.70 and it was independent of lerisetron concentrations ranging from 50 ng/ml to 2 μg/ml. The plot of protein bound lerisetron (C_B) against unbound lerisetron (C_U) for each concentration, across the range of concentrations, permits the determination of the binding characteristics. A linear plot indicates a nonsaturable

Table 1 Percentage of unbound lerisetron [$(C_u/C_T) \times 100$] and protein levels in serum from healthy (group I) and cancer patients (groups II and III). The results are expressed as means ± standard deviation and range

	Unbound lerisetron (%)	HSA (g/l)	AAG (g/l)
Group I (n = 11)	3.70 ± 0.70 (2.60–4.74)	47.4 ± 2.3 (39.8–59.5)	0.7 ± 0.1 (0.5–0.9)
Group II (n = 9)	2.38 ± 0.64* (1.25–3.47)	39.9 ± 8.4** (26.2–50.4)	1.7 ± 0.5* (0.8–2.3)
Group III (n = 18)	5.17 ± 2.3 (2.0–13.0)	36.8 ± 9.1*** (28.9–48.6)	0.9 ± 0.3 (0.5–1.6)

*$P < 0.001$; ** $P < 0.02$; ***$P < 0.005$

binding process and consequently the slope of the plot represents the affinity constant, nKP ($C_B = nKPC_U$; $r = 0.99$). The behaviour here corresponds to a binding system with a single class of nonsaturable binding sites [23]. The mean value of the binding constant (nK) found in serum was $6.5 \pm 0.5 \times 10^5\ M^{-1}$.

Part 2 (identification) Lerisetron was bound to both HSA and AAG. The mean percentage of unbound lerisetron (2 μg/ml) in commercially available HSA was 4.04 ± 0.80. The unbound percentage in AAG was 14.01 ± 2.0, significantly lower than in serum samples ($P < 0.001$) and in HSA solutions ($P < 0.001$). No changes over the studied concentrations of lerisetron in either protein solution were observed. The binding constant was $4.9 \pm 0.4 \times 10^5\ M^{-1}$ and $1.4 \pm 0.05 \times 10^5\ M^{-1}$ to HSA and AAG, respectively.

Part 3 (comparison) The results of this part of the study are shown in Table 1. Serum concentrations of AAG in group II cancer patients were significantly higher ($P < 0.001$) than in group I subjects or group III patients. In contrast, both groups of patients (groups II and III) had lowered serum concentrations of HSA. Lerisetron was more extensively bound in group II cancer patients than in group subjects. No changes were observed in lerisetron binding in group III patients. The percentage of unbound lerisetron in this group showed a high interindividual variability, ranging from 2.0 to 13.3, with a mean of 5.17 ± 2.3.

The multivariate analysis of unbound lerisetron against both HSA and AAG showed that AAG participated in drug binding in group II patients and HSA in group III patients. Figure 1 indicates that lerisetron binding was increased in group II patients in association with increased AAG concentrations ($r = 0.54$; $P < 0.05$). In patients from group III, a significant correlation was only observed with HSA levels (Fig. 2)

Discussion

Lerisetron is being evaluated for its antiemetic activity in humans but, until now, its protein binding properties have not been studied. Answering a series of questions regarding binding would allow better prediction of the pharmacokinetics, especially applicable to the design stage of clinical studies [8, 23]. The questions include: (1) whether the unbound fraction is constant over the expected concentration range, (2) whether the drug competes for binding sites of coadministered therapeutic agents, (3) which proteins are primarily involved in drug

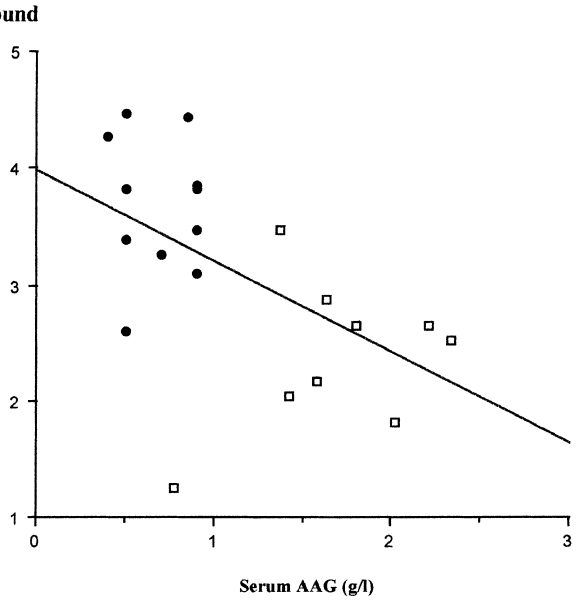

Fig. 1 Correlation between in vitro lerisetron binding (% unbound) and serum AAG levels (g/l) in 11 healthy subjects (●, Group I) and 9 cancer patients undergoing radiotherapy (□, group II) ($P < 0.05$; $r = 0.54$)

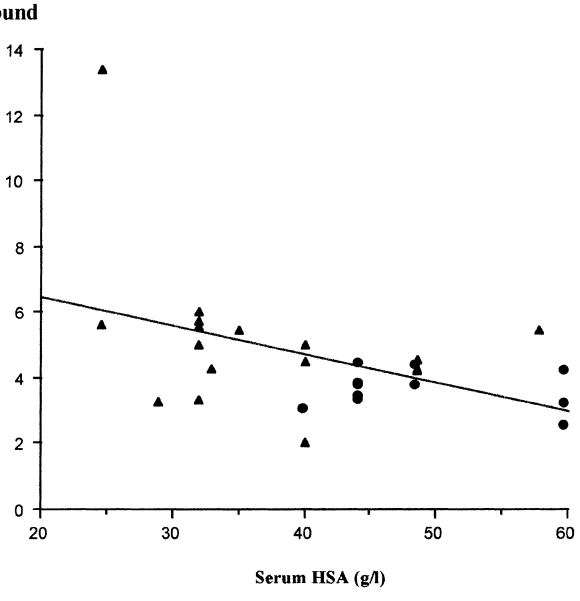

Fig. 2 Correlation between in vitro lerisetron binding (% unbound) and serum albumin level (g/l) in 11 healthy subjects (●, group I) and 18 cancer patients receiving chemotherapy (◆, group III) ($P < 0.05$; $r = 0.45$)

binding, and (4) what is the interindividual protein binding variability and the effect of disease on protein binding behaviour.

When lerisetron was added to serum from healthy volunteers, the percentage binding of unbound lerisetron showed to modification with increasing drug concentration (question 1). A linearity in the relationship between bound and unbound (free) drug concentrations was observed, indicating nonsaturable kinetics. The binding capacity (nK) indicates the strength of the drug-protein association and is an essential parameter for the assessment of the pharmacokinetic implications of drug-protein binding. For example, it can be used for prediction of C_U in various clinical situations using the patient's altered protein concentration value and it could be useful for calculating initial doses of the drug [23].

On the basis of our results on lerisetron binding and summarizing (answers to questions 1 and 2) we could predict that: (a) changes in protein concentration would have little effect on the percentage of unbound lerisetron (high value of nK); (b) the extent of binding in whole serum and isolated proteins is independent of lerisetron concentration (nonsaturated process) showing a large binding capacity. This could imply two specific advantages in clinical use: (1) the percentage of binding will remain constant over time after systemic lerisetron administration, and (2) a lack on interactions involving protein binding displacement could be expected.

Regarding questions 3 and 4, according to the present study, HSA and AAG are both involved in lerisetron binding. Serum binding of lerisetron in patients with cancer was increased only in those patients undergoing radiotherapy (group II); this group showed high levels of AAG and low levels of HSA. However, in the other cancer patient group (group III), protein binding was not different from that in healthy volunteers. The variability was higher in this last group, probably because of a higher variability in both protein levels. A lack of correlation of lerisetron protein binding with these proteins was observed when all patients were included, but when the groups were evaluated separately, a weak correlation was seen between unbound drug and AAG in group II and with HSA in group III. In summary, a weak association between the binding of lerisetron and serum protein levels, as well as a preference of lerisetron for AAG binding, was observed. This suggests that other proteins (e.g. lipoproteins) or endogenous substances (e.g. bilirubin) could be implicated. This has also been observed with other drugs such as etoposide [20] and anthracycline [7].

This is the first report of lerisetron protein binding in patients with cancer, and the study demonstrated that patients undergoing radiotherapy fell into a distinct group, in association with high levels of AAG. Importantly, the increased binding observed in this group should not be extrapolated directly to all patients with cancer, but only to those showing significant increase in AAG concentration. Because these changes in protein binding could yield a lower level of systemic exposure to unbound (active) drug at any given total drug concentration [3], such patients could exhibit decreased pharmacological effects from standard doses of lerisetron.

The repercussions of altered plasma protein concentration on lerisetron kinetics and dynamics are difficult to establish because they depend on pharmacokinetic data in humans (i.e. whether elimination is largely restricted to the unbound fraction or is unrestricted) and these have not been reported. However, preliminary results from an experimental study in tumour-bearing rats, with high AAG levels [15], show changes in both the kinetic (Vd $\downarrow$ and Cl $\downarrow$) and the dynamic (C_{50} $\uparrow$) parameters of lerisetron, suggesting that an increased dosage could be necessary in cancer patients showing high levels of AAG.

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