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Short communication

Decrease in penbutolol protein binding as a consequence of treatment with some alkylating agents

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Abstract. The effect of in vitro treatment of serum with the alkylating agents carmustine (BCNU) and mechlorethamine on the protein binding of penbutolol, a basic agent mainly bound to alpha$_1$-acid glycoprotein (AAG), was investigated. The free fraction of penbutolol increased significantly ($P < 0.001$) after the treatment of serum with BCNU (5.27% ± 0.47%) and with mechlorethamine (5.23% ± 0.17%), being 1.98% ± 0.18% in serum not treated with BCNU or mechlorethamine. In addition, after incubation with BCNU (2 h), the free fraction of penbutolol continued increasing (10.96% ± 0.70% vs 5.27% ± 0.47% at time 0; $P < 0.001$), whereas it remained unchanged after incubation with mechlorethamine. Moreover, dialysis against saline for 24 h did not restore the free fraction of penbutolol, which increased after treatment with carmustine (9.05% ± 1.24% vs. 11.04% ± 1.55%, nondialyzed). We concluded that the treatment of cancer patients with alkylating agents could alter the serum proteins and modify their binding capacity, and this should be taken into account in the simultaneous treatment of these patients with other basic drugs like penbutolol, e.g., methadone.

Introduction

Qualitative changes in the albumin molecule and its influence on drug/protein-binding alterations have attracted a great deal of attention from investigators in recent years. These modifications in albumin's structure (in relation to its binding capacity) may be related to the presence of endogenous compounds, produced when certain pathological situations are developing (i.e., uremia) [10], or be associated with the presence of drugs or chemicals capable of releasing reactive radicals [4]. The carbamylation of albumin by the cyanate molecule could be a good example of the alteration of plasma proteins caused by endogenous compounds [6]; cyanate is spontaneously released in plasma when high urea concentrations are reached and can react with amino groups (mainly with lysine), producing carbamyl compounds that are responsible (at least in part) for the decreased binding capacity of acidic drugs to albumin [6].

Several anticancer agents, i.e., mechlorethamine, an alkylating agent, and carmustine (BCNU), an alkylating and carbamylating agent, are very active drugs that can react chemically with proteins to form covalent compounds [9]. Basic drugs (e.g., propranolol) show protein binding that is unpredictable because they bind not only to the albumin molecule but also to alpha$_1$-acid glycoprotein (AAG) and to other plasma proteins such as lipoproteins [8]. This multiple binding capacity explains why a decrease in or an alteration of one of them (e.g., albumin) cannot completely affect the protein binding of these drugs. Drugs used in cancer treatment also show binding to AAG (e.g., etoposide) [7].

Penbutolol is a basic beta-blocker that possesses the unusual characteristic of being fixed in plasma almost exclusively by AAG and not by albumin [2]. Thus, an experiment was designed using serum from healthy volunteers to investigate the effect of mechlorethamine or carmustine on the binding of penbutolol to AAG before and after the addition of and incubation with both drugs.

Materials and methods

Serum penbutolol binding study after the addition of mechlorethamine or carmustine. In the first study, blood was drawn from ten healthy, drug-free volunters (age range, 22–38 years; their weight was normal for their age and sex) by direct venipuncture, and 0.980-ml aliquots of the serum samples obtained were used immediately.

Mechlorethamine (Caryolysine, Delagrange Laboratories; 5 mg/ml) dissolved in triethyleneglycol was used, and 10 µl was added to each sample of serum to attain a final concentration of 50 µg/ml. BCNU was obtained from Bristol-Squibb Laboratories. A solution in ethanol (200 mg/ml) was prepared, and 10 µl was added to each

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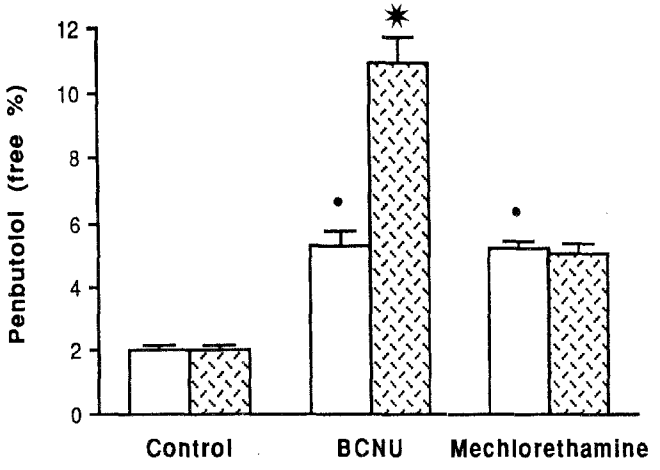

Fig. 1. Free fraction of penbutolol in control serum and in the presence of BCNU and mechlorethamine (mean ± SEM) as determined without (□) and after 2 h of incubation (▨). • $P < 0.001$ (comparison with control); * $P < 0.001$ (comparison before and after incubation)

sample to reach a final concentration of 4000 µg/ml. Either ethanol or triethyleneglycol (10 µl) was added to control serum samples in each case. Tritium-labeled penbutolol (kindly provided by Hoechst, Germany) had a specific activity of 155 Ci/g and was used as a methanol/water solution (40/60, v/v).

The serum protein binding of penbutolol was determined by ultrafiltration using an Amicon MPS-1 system. Before and after the addition of mechlorethamine or BCNU, 10 µl of the [^{3}H]-penbutolol solution was added to each sample of serum to attain a final concentration of 500 ng/ml (therapeutic range), and the pH was adjusted in serum samples before ultrafiltration when necessary. All samples (1 ml) were centrifuged at 1000 *g* at 20° C for 10 min, and 100 µl of the radioactive ultrafiltrate was used to measure the free penbutolol concentration in a Packard Tri-Carb 300 Scintillation Spectrometer after the addition of 10 ml of Econofluor to ultrafiltrates.

Additional serum samples (control, with mechlorethamine or BCNU) at the above-mentioned concentrations were incubated in a water bath at 37° C under continuous stirring for 2 h. Immediately after the removal of each sample from the bath, [^{3}H]-penbutolol was added and binding was determined as described above.

Serum penbutolol binding study after dialysis. In the second experiment, serum samples were treated with BCNU as described above and dialyzed against saline for 24 h. The saline was stirred four times during this period. After dialysis, an aliquot (10 µl) of the [^{3}H]-penbutolol solution was added to each sample and penbutolol binding was measured following the above-mentioned procedure.

Statistical analysis. All data are presented as mean values ± SEM. Student's *t*-test for paired data was used to compare means. The criterion for significance was $P < 0.05$.

Results

In the presence of BCNU, the free fraction of penbutolol increased significantly (5.27%±0.47% vs 1.98%±0.18% in samples without BCNU; $P < 0.001$). In the presence of mechlorethamine, the free percentage of penbutolol increased (5.23%±0.17% vs 1.98%±0.18% in samples without the drug; $P < 0.001$). In addition, after incubation with BCNU (2 h), the free fraction of penbutolol continued increasing (10.96%±0.70% vs 5.27%±0.47% at time 0; $P < 0.001$), whereas it remained unchanged after incubation with mechlorethamine (5.08%±0.28% vs 5.23%±0.18% before incubation; Fig. 1).

It is noteworthy that the binding of penbutolol was not modified after the dialysis of serum samples against saline for 24 h (free fraction, 9.05%±1.24%, vs 11.04%±1.55% non-dialyzed), which suggests that the binding capacity of AAG was irreversibly affected by BCNU.

Discussion

Cancer is a pathological condition that allows increased levels of AAG, and this increase is closely related to augmented binding of basic drugs [1]. It has been observed that the binding capacity of AAG decreases in this situation, although the mechanism of this phenomenon was not studied [3]; however, a structural alteration of AAG has been observed in cancer, consisting of many abnormalities in the carbohydrate chains that form the molecule [11], and these changes may be related to the binding alterations mentioned.

Moreover, experimental studies in rabbits have demonstrated that repeated doses of carmustine modify the binding and kinetics of sulfisoxazole, an acidic drug that shows specific binding to albumin [5]. However, it has thus far not been determined whether treatment with carmustine modifies the binding of basic drugs, which bind to AAG. Our results show that BCNU binds to AAG and modifies its capacity to bind to penbutolol, and this effect is increased by incubation. In contrast, the effect of mechlorethamine on binding does not increase after such incubation. Mechlorethamine is an anticancer agent that acts by alkylating guanine residues into DNA chains, which leads to an error in the codification process [9]. Carmustine possesses this mechanism of action and also releases isocyanate by spontaneous degradation that carbamylates lysine residues. This explains the increase in its effect on penbutolol binding observed after incubation.

These results should be taken into account in the treatment of cancer patients with mechlorethamine or carmustine because these agents could affect the binding of other basic drugs like penbutolol, e.g., methadone or mianeserin, used simultaneously in these patients. The clinical relevance of these findings in humans should be investigated.

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