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Modification of the physicochemical and pharmacological properties of anticancer platinum compounds by commercial 5-fluorouracil formulations: a comparative study using cisplatin and carboplatin*

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Summary. The influence of a commercial formulation of 5-fluorouracil (5-FU) on the stability and pharmacological properties of two platinum derivatives, cisplatin and carboplatin, was studied to determine whether the drugs could be mixed in containers or intravenous lines. When cisplatin was incubated in a French commercial formulation of 5-FU (Fluoro-uracile, Roche, France), high-performance liquid chromatographic (HPLC) studies demonstrated a rapid disappearance of the parent platinum compound, the extent of the degradation being 75% after 3.5 h. These studies also revealed that the degradation was not caused by a reaction between 5-FU and cisplatin but rather resulted from an interaction between cisplatin and trometamol, the excipient used in the French 5-FU formulation to buffer the solution at pH 8.2. The sole presence of trometamol in a cisplatin solution for 24 h at 30° C resulted in the complete inhibition of both the ability of cisplatin to bind in vitro to human serum albumin and the antitumor activity of the cytostatic agent against P388 leukemia in mice (T/C% = 88% for cisplatin + trometamol vs >333% for cisplatin). When cisplatin was incubated at the same pH in trometamol-free sodium hydroxide solutions (the excipient used in 5-FU formulations in several countries, including the United States and the United Kingdom), the parent compound was transformed into reactive species that were toxic to mice (T/C% = 40% in P388 leukemia). The degradation determined for a carboplatin-trometamol admixture using HPLC was similar to that found for cisplatin but occurred at a slower rate (0 after 3.5 h incubation and 55% after 24 h). The antitumor activity of carboplatin in P388-bearing mice was not significantly altered by a 24-h period of preincubation in the presence of trometamol (T/C% = 209% vs 241% for treatment with carboplatin in the absence of trometamol). As in the case of cisplatin, incubation of carboplatin for 24 h in a sodium hydroxide solution resulted in a toxic effect (T/C%=64%). Our results thus demonstrate the incompatibility of both cisplatin and carboplatin with commercial formulations of 5-FU.

Introduction

The promising results obtained in a variety of malignancies using combinations of cisplatin and 5-fluorouracil (5-FU) have led to clinical trials designed to optimize the administration of these drugs [1, 8, 9]. With this in mind, continuous infusions and the use of portable syringe pumps are often proposed. The ease of long-term administration would be greatly improved by either mixing both of the drugs in the same syringe or at least injecting them through a single catheter. Such methods raise questions concerning the compatibility of the two drugs in the same solution over relatively long periods. Lokich et al. [10] recently reported that the admixture of cisplatin with 5-FU at pH 8.7 resulted in the loss of >80% of the former after 24 h incubation, whereas there was no significant loss of 5-FU. These results were subsequently confirmed by Stewart and Fleming [4, 18], who concluded that cisplatin and 5-FU should not be mixed in the same container for continuous infusion. These previous studies established the absence of a direct interaction between 5-FU and cisplatin in the same solution and indicated that the alkaline pH of the 5-FU solution could be responsible for the degradation of cisplatin. However, no information was available concerning the pharmacological properties of the products generated by mixing the two drugs before their administration.

Carboplatin is also used in combination with 5-FU for the treatment of several malignancies [2, 5, 14]. It would therefore be of interest to determine whether carboplatin would also interact with a 5-FU solution and to compare the stability of cisplatin and carboplatin under the same conditions. The present study was thus initiated to evaluate alterations in the chemical and biological properties of

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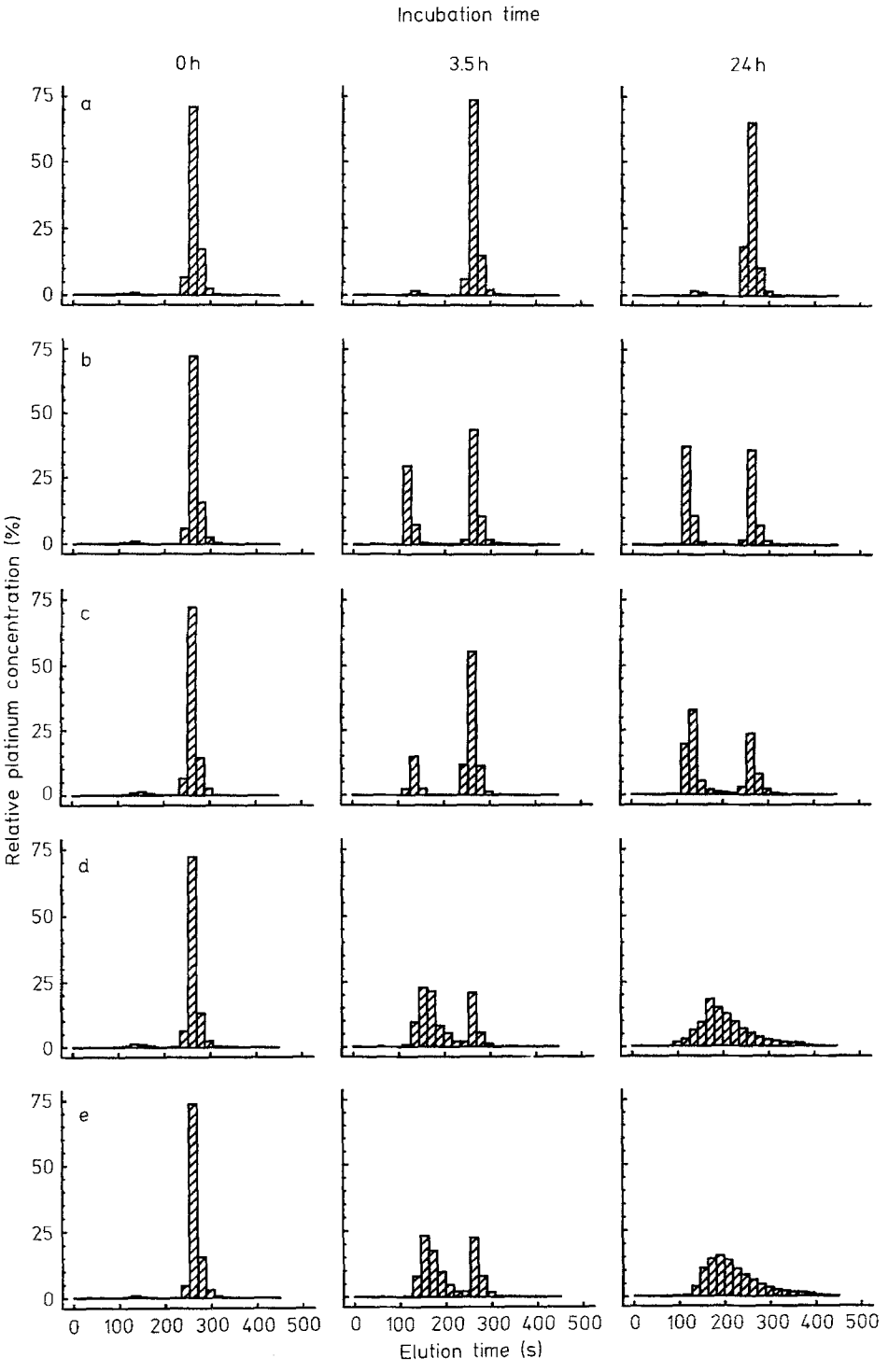

Fig. 1 a–e. HPLC study of the stability of cisplatin in various media (see Table 1 for the composition of admixtures). After different periods of preincubation at 30° C (**a** CP1, **b** CP2, **c** CP3, **d** CP4, **e** CP5), column effluents were collected in fractions and platinum was measured off-line by FAAS

cisplatin and carboplatin during incubation with commercial 5-FU. The changes in solutions containing the excipients of a commercial formulation of 5-FU were also studied to determine the mechanism of degradation. High-performance liquid chromatographic (HPLC) monitoring was used to measure the evolution of the degradation process with time, and the different cisplatin admixtures were tested for both their in vitro protein-binding capacity and their antitumor efficacy in P388 lymphocytic leukemia in mice.

Materials and methods

Drug admixtures. Admixtures of cisplatin (Cisplatyl, Roger Bellon), carboplatin (Paraplatine, Bristol), 5-FU (Fluoro-uracile, Roche), sodium chloride, trometamol (TRIS buffer), and sodium hydroxide were prepared as indicated in Table 1. The resulting concentrations were 0.8 g/l for cisplatin and carboplatin (2.7 and 2.2 mM, respectively), 20 g/l for 5-FU (0.15 M), 7.2 g/l for NaCl in CPl and CP3, 13.2 g/l for NaCl in CP4 and CP5, 100 g/l for trometamol, and 0.136 g/l for NaOH in CP3 and CB4. In the presence of trometamol or NaOH, the pH of the solutions was 8.2, whereas it was 6.4 in the other cases. To test drug interactions,

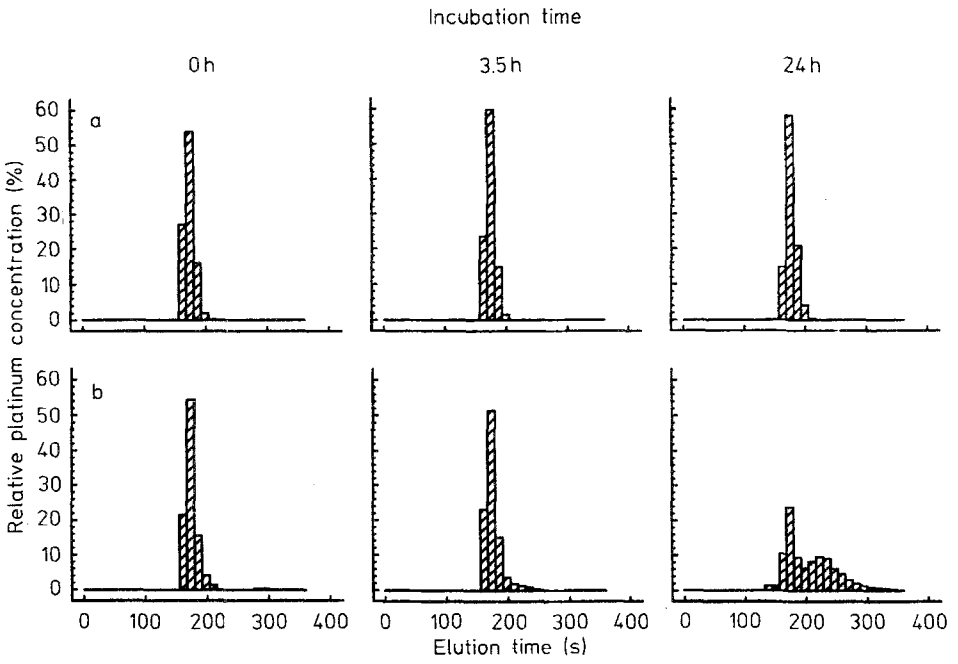

Fig. 2 a, b. HPLC study of the stability of carboplatin in **a** CB1 and **b** CB2 (see Table 1 for the composition of the admixtures)

all admixtures were incubated at 30° C in amber-colored flasks for 1 min and for 0.5, 3.5, 24, and 96 h.

HPLC assay. Cisplatin, carboplatin, and 5-FU were analyzed using an HPLC system consisting of a constant-flow-rate pump (model 6000A, Waters), an anion-exchange column (8P SAX 10 µm, Waters) in a radial compression module (Z-module, Waters), an ultraviolet (UV) detector (LKB), and a fraction collector (LKB). The mobile phase consisted of 0.2 M aceto-acetic buffer (pH 3.8)/methanol (20:80, v/v) run at a flow rate of 2.0 ml/min [11]. 5-FU was monitored at 254 nm. Column effluents were collected in fractions every 6 s, and the platinum content of each fraction was quantitated using flameless atomic absorption spectrophotometry (FAAS) according to a previously published protocol [7].

In vitro binding of cisplatin to HSA. Drug binding was studied by mixing cisplatin solutions (previously incubated for 24 h at 30° C) with human serum albumin (HSA, Sigma) in 0.9% sodium chloride. The final concentrations were 6.5 mg/l (22 µM) for platinum and 50 g/l (0.8 mM) for HSA. Admixtures of cisplatin and HSA were incubated at 37° C and ultrafiltered (Centrifree, Amicon; molecular-weight cutoff, 30,000 Da) after 0.25, 0.75, 1.5, 3, and 5 h incubation. Ultrafiltration (1000 g) was performed at 4° C for 25 min. The platinum content in ultrafiltrates was determined using FAAS, and the results were analyzed according to first-order kinetics of binding.

Antitumor effect in mice. Evaluation of the antitumor activity of the different admixtures was performed using the murine P388 lymphocytic leukemia model. Mice (DBA/2, Charles River France) were inoculated i.p. with 0.2 ml diluted ascitic fluid (10^6 cells) obtained from rodents in which the tumor had been propagated. The date of tumor implantation was designated as day 0. Admixtures were preincubated at 30° C for 24 h prior to their i.p. administration on days 1–6. The daily dose was 2 mg/kg for cisplatin and 40 mg/kg for carboplatin. Experiments were evaluated on day 30 unless there were survivors, in which case the evaluation was done on day 45. Results were reported as the median survival of test animals relative to untreated controls ($\times 100$ = T/C%).

Results

HPLC analysis of cisplatin admixtures

The stability of cisplatin in different admixtures was initially studied by ion-exchange HPLC following preincubation for various periods (0, 0.5, 3.5, 24, and 96 h). The results are summarized in Fig. 1. For the sake of clarity, data corresponding to incubation intervals of 0.5 and 96 h were not represented, since they were very similar to those obtained after incubation periods of 0 and 24 h, respec-

Table 1. Composition of the different drug admixtures prepared to test the degradation of cisplatin or carboplatin in the presence of a commercial form of 5-FU containing trometamol

Admixtures	Cisplatin	carboplatin	5-fluorouracil	TRIS buffer	NaCl	NaOH
CP1	+	–	–	–	+	–
CP2	+	–	–	–	–	–
CP3	+	–	–	–	+	+
CP4	+	–	+	+	+	–
CP5	+	–	–	+	+	–
CB1	–	+	–	–	–	–
CB2	–	+	+	+	–	–
CB3	–	+	–	+	–	–
CB4	–	+	–	–	–	+

In the presence of trometamol (TRIS buffer) or sodium hydroxide, the pH of the solutions was 8.2; in the other cases it was 6.4

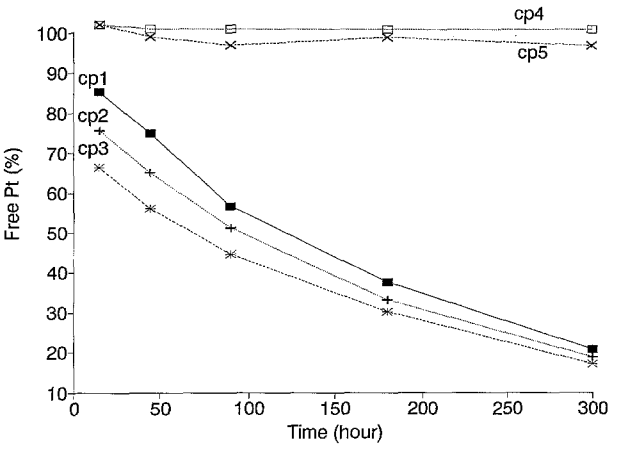

Fig. 3. Inhibitory effects of trometamol-containing solutions (CP4 and CP5) on the in vitro binding of cisplatin to HSA. All admixtures (CP1–CP5) were preincubated at 30° C for 24 h prior to binding experiments. Free platinum species were evaluated by ultrafiltration with Centrifree micropartition units (molecular-weight cutoff, 30,000 Da)

Table 2. Effect of treatment with different platinum admixtures on P388-bearing mice

Treatment[a]	Animals (*n*)	Median survival (days)	T/C%	LTS (number of mice)
Cisplatin admixtures:				
None	10	13.5	–	0/10
CP1	6	>45.0	>333	6/6
CP3	6	5.5	40	0/6
CP5	6	12.0	88	0/6
Carboplatin admixtures:				
None	10	11.0	–	0/10
CB1	6	26.5	241	2/6
CB3	6	23.0	209	1/6
CB4	5	7.0	64	0/5

Admixtures were preincubated for 24 h at 30° C and were given i. p. to mice from day 1 to day 6. The daily dose was 2 mg/kg for cisplatin and 40 mg/kg for carboplatin

[a] See Table 1 for explanations on groups

LTS, Long-term survival

tively. When cisplatin was incubated in saline solution (CP1), the retention time (RT) of platinum species (260 s) remained unchanged for at least 96 h (Fig. 1 a). In contrast, chromatograms of cisplatin incubated in distilled water (CP2, Fig. 1 b) revealed the appearance of a new peak (RT = 110 s) corresponding to the well-known aquation of the parent compound. After 96 h incubation, this new peak represented 53% of the total amount of platinum. In alkaline saline solution (pH 8.2; CP3, Fig. 1 c), a similar profile of hydrolysis was observed, and the corresponding peak represented 63% of the total platinum content after 96 h incubation.

Cisplatin was then incubated with the French commercial formulation of 5-FU (Fluoro-uracile, Roche; CP4). The resulting pH was 8.2 due to the presence of trometamol (TRIS buffer) in the solution. The chromatogram obtained after a 0.5-h period of incubation (data not shown) was identical to that obtained for cisplatin in saline solution at pH 6.4 (CP1). However, the chromatograms obtained after longer incubation periods showed a broad distribution of platinum species over the elution times (Fig. 1 d). The peak corresponding to intact cisplatin represented only 25% of the total amount after 3.5 h incubation and was no longer apparent after 24 h. For the detection of possible molecular interactions between cisplatin and 5-FU, concurrent UV monitoring of the eluent was performed. No modification of the 5-FU peak could be observed and no new peak appeared. Moreover, no significant amount of platinum could be detected in column eluents corresponding to the retention time of 5-FU.

When cisplatin was incubated with trometamol in the absence of 5-FU (CP5, pH 8.2), the chromatogram (Fig. 1 e) demonstrated a platinum-distribution pattern that was similar to that obtained in the presence of 5-FU plus trometamol (CP4) but quite different from that obtained at pH 8.2 in NaOH (CP3).

HPLC analysis of carboplatin admixtures

Carboplatin was stable in distilled water for at least 96 h (CB1, Fig. 2 a). In admixtures with commercial 5-FU (CB2, pH 8.2), degradation products appeared as in the case of cisplatin. However, the rate of loss of the parent compound was significantly lower for carboplatin than for cisplatin. After 3.5 h incubation, at least 50% of the cisplatin had degraded, whereas the carboplatin remained unchanged. After 24 h incubation, the peak corresponding to carboplatin represented 45% of the total platinum content. The retention time of 5-FU showed no change over 96 h, and no platinum could be detected in the column eluent corresponding to 5-FU, as previously observed with cisplatin.

Studies of protein binding in cisplatin admixtures

Cisplatin admixtures (Table 1) were preincubated for 24 h at 30° C, after which the ability of platinum compounds to bind to proteins was tested using HSA. After preincubation with either distilled water, NaCl, or NaOH, platinum species reacted with HSA according to first-order kinetics (Fig. 3). The reaction rate constants were 0.32, 0.31, and 0.32 h^{-1}, respectively. In contrast, complete inhibition of binding occurred following the preincubation of cisplatin with either commercial 5-FU (5-FU plus trometamol) or trometamol alone.

Antitumor activity of admixtures in P388-bearing mice

Treatment of leukemic mice with cisplatin that had been preincubated at 30° C for 24 h in sodium chloride solution (CP1) resulted in 100% survival at 45 days (Table 2). Under the same conditions, treatment with the NaOH admixture (CP3) was toxic and the mice died before day 6 (T/C% = 40%). After treatment with cisplatin that had been preincubated in trometamol (CP5), the median survival (12 days) was not significantly different from that of un-

treated mice. Conversely, treatment with carboplatin that had been preincubated for 24 h in trometamol at 30° C (CB3) resulted in improved survival as compared with untreated mice (T/C% = 209%). This effect did not differ much from that obtained using carboplatin that had been preincubated in water (CB1; T/C% = 241%). In contrast, treatment with the NaOH admixture (CB4) was toxic (T/C% = 64%).

Discussion

Interaction of cisplatin with commercial 5-FU

Two types of excipients are currently used in injectable 5-FU solutions to solubilize and stabilize the drug at an alkaline pH: trometamol (TRIS buffer) in France and Japan, and sodium hydroxide in other countries, including the United States and the United Kingdom.

The addition of cisplatin to a trometamol-containing 5-FU solution at pH 8.2 resulted in a rapid decrease in native cisplatin as demonstrated by HPLC analysis (Fig. 1 d). This decrease began soon after the preparation of the admixture, and almost all of the cisplatin disappeared after 24 h incubation. Identical chromatograms were obtained for a trometamol solution at the same pH in the absence of 5-FU (Fig. 1 e), which suggests that this degradation was actually attributable to an interaction between trometamol and cisplatin. This interaction can be readily explained by the chemical structure of the trometamol molecule, which contains amine groups that are known to react with cisplatin [13, 15]. Moreover, the absence of an interaction between cisplatin and 5-FU was confirmed by HPLC studies: first, 5-FU elutes in a single sharp peak that does not decrease with time during incubation with cisplatin; and second, the platinum content in column effluents corresponding to the 5-FU peak remained negligible until at least 96 h incubation.

The ultimate demonstration of an interaction between cisplatin and trometamol would involve chemical analysis of the resulting compounds. This analysis was not performed, since the major goal of the study was to evaluate the pharmacological consequences of mixing the commercial drugs. To this end, we first evaluated alterations in the reactivity of cisplatin by studying its in vitro protein binding. Figure 3 shows the well-known binding kinetics of intact cisplatin to HSA. In this figure it can also be seen that the binding was completely inhibited following the preincubation of cisplatin for 24 h in the trometamol-containing buffer in either the presence or the absence of 5-FU. It can thus be concluded that the interaction of trometamol with cisplatin results in the inhibition of all or part of cisplatin's chemical reactivity. The mechanism of inhibition probably involves the formation of stable complexes between cisplatin and trometamol as previously shown.

Another potential consequence of cisplatin degradation in the presence of trometamol could be a loss of antitumor activity or the occurrence of toxic side effects, which have been reported for hydrolysis products of cisplatin [3]. A 24-h preincubation period in trometamol leads to the complete loss of cisplatin's antitumor effect on P388 lymphocytic leukemia in mice (T/C% = 88; Table 2) as compared with the efficacy found for cisplatin that had been preincubated in physiologic saline solution for the same time (T/C% = >333%).

The results obtained after the incubation of cisplatin in sodium hydroxide solutions at pH 8.2 were quite different from those found following its preincubation with trometamol at the same pH. First, HPLC analysis revealed a degradation process similar to that obtained in distilled water (Fig. 1 b, c), which corresponded to the well-known hydrolysis of cisplatin [17]. The higher content of hydrolyzed species in sodium hydroxide solution (63% vs 55% after 24 h) was attributable to the pH-dependent reaction of aquation. The second difference with respect to trometamol involved the reactivity of cisplatin after incubation in sodium hydroxide. In this case, the binding of cisplatin to HSA was not inhibited and was comparable with that found for cisplatin in physiologic saline solution or in distilled water at neutral pH (Fig. 3). The main difference concerned the experiments on P388 leukemia in mice, since lethal toxicity was observed following the preincubation of cisplatin for 24 h in sodium hydroxide at pH 8.2 (T/C% = 40%; Table 2).

In summary, the main findings were that both trometamol and sodium hydroxide degrade cisplatin, the former yielding nonreactive products that have lost the antitumor activity of the parent drug and the latter producing reactive species that exhibit high and acute toxicity.

Interaction of carboplatin with commercial 5-FU

The chromatographic platinum profiles shown by carboplatin following its incubation in a trometamol-containing 5-FU solution suggest a mechanism of degradation similar to that found for cisplatin, which also involved a direct role for trometamol (Fig. 2). However, the rate of degradation of carboplatin was slower, with the intact drug remaining stable for at least 3.5 h. Carboplatin is less reactive than cisplatin as shown by its very slow protein binding rate [6, 12]. Thus, the slow degradation of carboplatin could be consistent with a low reaction rate with trometamol to produce complexes. The major consequence of the higher stability of carboplatin could be demonstrated by studying the antitumor effect of the admixtures. The results obtained using the P388 murine leukemia model in mice indicated that the presence of trometamol only slightly decreased the antitumor activity of carboplatin (Table 2), at least for a 24-h preincubation period and at the dose used. Under these conditions, the T/C% values obtained were 241% for carboplatin alone and 209% for the carboplatin-trometamol admixture, which correspond to the data in the literature [16]. This result is surprising, since HPLC experiments showed that only half of the initial carboplatin remained intact after 24 h incubation (Fig. 2). One possible explanation might be that the amount of intact drug remaining was sufficient to produce a cure in our experimental model. In contrast, the incubation of carboplatin in a sodium hydroxide solution for 24 h resulted in a toxic effect similar to that observed for cisplatin.

Conclusion

Cisplatin and carboplatin should not be mixed with commercial solutions of 5-FU that contain sodium hydroxide (e.g., American and British commercial formulations), since the admixture induces the degradation of the parent drugs and the subsequent formation of reactive and toxic species. In 5-FU solutions containing trometamol rather than sodium hydroxide (French and Japanese commercial formulations), another type of degradation occurs, whereby cisplatin forms products that are nontoxic but have lost their antitumor activity and carboplatin is degraded in a similar manner, albeit only after 3–4 h. However, even in the latter case, it seems advisable to avoid any admixture with 5-FU.

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