Highly Sensitive Differential Pulse Amperometric Detection of Second Generation Anti-Tumor Platinum Compounds in HPLC Effluents

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Key Words

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Summary

A highly sensitive on-line amperometric detection of platinum compounds in HPLC effluents was possible with the use of a polarographic detector. For TNO-1 (=cis-1,1-di(aminomethyl)-cyclohexane Pt(II)chloride) a linear dynamic range of at least three decades could be obtained (0.034-108 µg TNO-1/ml) with a detection limit of three times the noise signal at 0.7 ng TNO-1 (20 ng Pt/ml). The sensitivity was 0.1 nA/ng TNO-1 (0.2 nA/ng Pt). The within-day precision was 1.1% at a concentration of 10.8 µg TNO-1/ml (n = 10).

With this system the compatibility was measured of TNO-6 with infusion fluids. In 0.15 M NaCl, TNO-6 (=cis-1,1-di(aminomethyl)-cyclohexane Pt(II)sulphate) was converted into TNO-1 within 40 minutes. Molecular changes of TNO-6 were also observed in a 5% aqueous glucose solution.

Introduction

Cisplatin is well recognized as a potent anti-tumor agent for several solid tumors [1, 2, 3]. Its side effects and especially its nephrotoxicity, brought on a search for second generation platinum compounds with less nephrotoxicity and greater anti-tumor activity [4]. Two potential compounds are TNO-1 (=cis-1,1-di(aminomethyl)cyclohexane Pt(II)chloride) and TNO-6 (=cis-1,1-di(aminomethyl)-cyclohexane Pt(II)sulphate) of which the structural formulas are shown in Fig. 1. Because of its higher aqueous solubility and favourable characteristics [5] TNO-6 was selected for a phase I study in the Netherlands [6]. It is administered as an i.v. infusion in a 5% glucose solution at a concentration of 120 µg/ml. TNO-1 is considered as one of the main metabolites.

To study the stability of TNO-6 in 5% glucose and its conversion to TNO-1 in the presence of chloride a selective analysis was developed for both compounds.

Up to the present, many pharmaceutical and pharmacological studies on platinum compounds were performed with the use of non-selective platinum analysis by atomic absorption spectrometry [7, 8]. Recently, HPLC procedures were developed for cisplatin using either an off-line AAS detection [9, 10] or an on-line UV-detection with loss of sensitivity [9, 10] or loss of specificity by precolum derivatisation [11, 12]. Therefore, the purpose of our study was, the development of a selective HPLC procedure for TNO-1 and TNO-6 with on-line polarographic detection having the features of being sensitive, selective and applicable to other platinum compounds as well [13, 14, 15].

Experimental

Materials

The chemicals used were of analytical grade. TNO-1 and TNO-6 were pure for at least 99.6% as measured by elementary analysis and were kindly supplied by Dr. E. Bulten (Institute of Applied Chemistry TNO, Utrecht). TNO-1 solutions were made in 0.15 M NaCl and TNO-6 solutions in bidistilled water unless indicated otherwise. The TNO-6 solutions in glucose were constituted with glucose (Ph. Eur. quality, Brocacef, Amsterdam) currently used in the hospital for the preparation of infusion fluids. The mobile phase, pump head and polarographic detector were purged with nitrogen, purified on the spot by subsequent purging through water, alkaline pyrogallol and mobile phase. The alkaline pyrogallol solution consisted of 105 gr potassium hydroxide and 15 gr pyrogallol solubilized in 105 gr water.

Fig. 1
Structural formulas of TNO-1(I) and TNO-6(II).

Instrumentation

The chromatographic system consisted of a Model 6000A pumping system (Waters, Etten-Leur), a Valco Universal inlet (Chrompack, Middelburg) provided with a 20 μ l sample loop and a stainless-steel KNAUER column of 40mm $\times$ 4.6mm i.d. (Salm & Kipp, Breukelen) filled with Nucleosil 10C18 (Machery Nagel, Hicol, Rotterdam) by means of a KNAUER column packing assembly (Salm & Kipp, Breukelen). The mobile phase consisted of 10% methanol in an aqueous solution of 0.1M NaNO₃. The flow rate was 1 ml/min. The detector was a model 310 polarographic detector controlled by a model 174A Polarographic Analyzer (EG & G Instruments (PARC), Nieuwegein). The electrochemical detector was operated in the differential pulse mode using a 100mV pulse, medium drop size, a pulse interval of 0.5 sec and the low pass filter off. The electrode potential was held constant at predetermined values vs. Ag/AgCl. Chromatographic recording was made on a Model PM8251 strip chart recorder (Philips, Eindhoven) with a chart speed of 10mm/min. The E-i-t curve was made on a BD 30 X-Y-t recorder (Kipp, Delft). A diagram of the whole system is given in Fig. 2.

Fig. 2

Diagram of the chromatography system: gas lines (---), liquid lines (—), signal lines (-.-.-), V = needle valves, W = wash bottles (1. water, 2. alkaline pyrogallol, 3. mobile phase), R = reservoir with mobile phase, p = HPLC pump, I = injector, C = column, D = detector, A = analyser, R = recorder.

Results and Discussion

Chromatographic Conditions

The hydrophobic moieties of the platinum compounds were prone to reversed phase chromatography. Several bonded phase materials were tried out (Nucleosil 10NH₂, 10C8 and 10C18). The best results were obtained with Nucleosil 10C18 yielding well shaped peaks for the platinum compounds preceded by a sharp oxygen peak from which they were well separated. Even with a column length of only 4cm good separations were obtained between the platinum compounds and oxygen (capacity factors k' were 1.2, 6.0 and 8.0 for oxygen, TNO-1 and TNO-6 respectively).

The mobile phase was degassed by sonification and extensively purged by purified nitrogen. After this preparation the mobile phase and polarographic cell were continuously

kept under a gentle stream of purified nitrogen. In this way the background current never exceeded 5nA, which allowed a stable baseline and a low detection limit. Samples were not deoxygenated, while the window applied in the differential pulse mode only allowed a small oxygen signal to get through, which was still sufficiently separated from the platinum peaks in the most sensitive full scale current range.

When operated with the Liquid Chromatography Analysis Cell, a flow rate of 1ml/min and 20 μ l injections of both platinum solutions, the HMDE appeared about 4% more sensitive than the SMDE in both sampled direct current polarography (CDCP) and differential pulse polarography (DPP). The HMDE was chosen for its higher sensitivity, a lower noise level and reduced mercury consumption compared to the SMDE.

TNO-1

Optimum conditions for TNO-1 were established for the HMDE in the DPP mode and operating as HPLC detector by means of the LC adapter assembly. Further fixed parameters were a modulation pulse of 100mV and a medium drop size. The modulation pulse of 100mV caused a higher sensitivity (lowest signal to noise level) than the smaller modulation pulses. Loss of selectivity with the high modulation pulse was no problem because of the uncomplicated chromatographic task. The medium drop size instead of the large drop size was chosen for its higher stability in the effluent, although some sensitivity was lost. The optimal potential was established by the measurement of chromatograms at potentials in the range of 0 to -800mV vs E_{Ag/AgCl} with intervals of 100mV (Fig. 3) and more detailed from -400mV to -600mV in steps of 20mV. A potential of -540mV vs Ag/AgCl was chosen because at this potential the highest sensitivity was obtained for TNO-1 with minimal sensitivity for the oxygen peak. Under these circumstances a linear dynamic range was obtained from 0.034 - 108 μ g TNO-1/ml as shown in Fig. 4. The upper limit was determined by the solubility of TNO-1. An indication for the repeatability of the peak-height at the measured concentrations is given in Table I. A more precise within-day precision was determined by a ten times injection of 20 μ l containing 10.8 μ g TNO-1/ml 0.15M NaCl(5.2 μ g Pt/ml). The coefficient of variation was 1.1%. The sensitivity was 0.1 nA/ng TNO-1(=0.2 nA/ng Pt) and the experimental detection limit 0.7 ng TNO-1(=20 ng Pt/ml). Under comparable circumstances the theoretical detection limit with the HMDE in the SDPC mode was 6.2ng TNO-1(=190ng Pt/ml). It is evident that the analysis with the HMDE in the DPP mode has to be preferred by its high sensitivity and its potential selectivity. Both features may be important when the analysis will be applied for the analysis of TNO-1 present in low concentrations in complicated mixtures like body fluids. Because the electrode reaction concerns the reduction of platinum(II) to metallic platinum it may be expected that other anti-tumor platinum(II) compounds can be analyzed in the same way with a comparable sensitivity. This means that the differential pulse amperometric detection technique will be generally applicable for on-line mobile phase analysis of platinum

Fig. 3

E-I-t chromatograms of TNO-1. Column: 40mm X 4.6mm i.d. packed with Nucleosil 10C18. Mobile phase: 10% methanol in an aqueous solution of 0.1 M NaNO₃.

Flow rate: 1ml/min. Polarographic detector: PAR 310 in DPP mode, 100mV pulse, medium drop size, 0.5sec pulse interval. Samples: 20μl of 108μg TNO-1/ml 0.15M NaCl. Peaks: A = oxygen, B = TNO-1.

Fig. 4

Linear dynamic range of TNO-1. The interval indicates ± 1 S.D.

Table I. Repeatability of the peakheight for TNO-1 at different concentrations

Concentration (μg Pt/ml)	Number of injections	C.V. (%)
51.8	4	2.7
17.3	5	3.9
5.2	3	1.2
1.7	5	2.5
0.52	5	4.1
0.17	2	6.2
0.052	2	20.3
0.017	3	9.0

compounds present in small amounts after HPLC fractionation of body fluids like plasma ultrafiltrate. A feature that has not been realized by other detection techniques up to now. The technique will be further evaluated by our group for other platinum compounds and will be applied for the analysis of platinum species in body fluids.

TNO-6

The procedure developed for TNO-1 was also applied for TNO-6 (Fig. 5). Under the same conditions a sensitivity

of 6pA/ng TNO-6(=0.013nA/ng Pt) and a detection limit of 0.4μg Pt/ml was obtained. The apparent lower sensitivity for TNO-6 could be explained by the low recovery of TNO-6 from the HPLC system as measured by means of atomic absorption spectrometry. Correction for the recovery yielded the same high sensitivity as for TNO-1. Studies for a better column system are ongoing.

Fig. 5

HPLC chromatograms of TNO-6. HPLC conditions as in Fig. 3. Peaks: A = oxygen, C = TNO-6.

Fig. 6

Chromatograms at 6(I), 13(II), 21(III) and 28(IV) min after mixing of equal volumes of TNO-6(105.5μg/ml water) and 0.30M NaCl. Peaks: A = oxygen, B = TNO-1, C = TNO-6.

Fig. 7

Conversion of TNO-6(x) into TNO-1(.) as measured under the conditions of Fig. 6 Encircled values represent a second independent measurement.

Fig. 8a

Chromatograms of (I) a 5% glucose solution and (II) an aqueous TNO-6 solution (50 µg/ml).

Fig. 8b

Chromatograms at various times after mixing of equal volumes of aqueous solutions of TNO-6 (98.8 µg/ml) and glucose (10% w/v):

III. 10 min, IV. 2 hr 20 min, V. 23 hr. Chromatographic conditions as in Fig. 3. Peaks: C = TNO-6, the other peaks have not been identified yet.

Compatibility of TNO-6 with Infusion Fluids

The conversion of TNO-6 to TNO-1 was measured under the described conditions after mixing equal volumes of an aqueous TNO-6 solution (105.5 µg TNO-6/ml) with 0.30 M NaCl. Fig. 6 shows the chromatograms obtained at 6, 13 and 21 min after mixing. The TNO-6 peak decreased rapidly, while the TNO-1 peak increased at the same time. The experiment was repeated and peakheights of TNO-1 and TNO-6 of both experiments were plotted against time (Fig. 7). It is clear that TNO-6 is completely converted to TNO-1 within 40 minutes. The results mean that TNO-6 is incompatible with chloride containing infusion fluids. Therefore, TNO-6 is administered to patients as an i.v. infusion of drug solubilized in a 5% glucose solution. For that reason the chromatograms of TNO-6 in 5% glucose were also studied. To that purpose aqueous solutions of TNO-6 (98.8 µg/ml) and 10%(w/v)glucose were mixed in equal volumes followed by HPLC chromatography at various time after constitution. The chromatograms show a slow changing picture with time (Fig. 8). Equilibrium is reached after about 4 hours with a picture equal to that at 23 hours. Perhaps, these pictures have to be interpreted as possible shifts in aquated and hydroxylated products of TNO-6 as they are also known for cisplatin [16, 17]. Further experiments for the elucidation of the character of the peaks will be done. The present results clearly indicate that changes in the character of the drug may occur after fresh preparation of the infusion fluids.

Conclusion

Differential pulse amperometry is a highly sensitive technique for the on-line detection of TNO-1 and TNO-6 in HPLC effluents with the expectancy to be applicable to other anti-tumor platinum compounds as well. Preliminary results indicate that TNO-6 is a reactive compound in various common infusion fluids.

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