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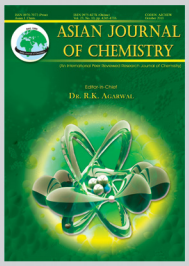

Stability of New Anticancer Agents in Intravenous Solutions

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The purpose of this study is to determine the stability of daunorubicin derivative containing in the amidine group a piperidine (DD-1), morpholine (DD-2), pyrrolidine (DD-3) or hexahydroazepine (DD-4) moiety in intravenous solutions. For DD-2, the influence of its concentration and of the light-protective effect of packaging on the stability of that compound was also investigated. The influence of intravenous solutions and conditions of storage on the stability of new derivatives of daunorubicin was studied by HPLC method. After storage at room temperature DD-3 was the most stable (in 84.6 % of the intravenous solutions) whereas the DD-2 was stable only in two of the solutions over a study period of 2 h (5 % glucose and 0.9 % NaCl). When stored at 2-6 ºC all derivatives of daunorubicine in the intravenous solutions were more stable. The unfreezing of the intravenous solutions to room temperature caused additional degradation. The concentration of DD-2 did not have any significant influence on the rate and kinetic mechanism of degradation. Exposure to light also did not have any significant effect on the degradation of DD-2 when it was stored in polypropylene syringes.

Key Words: Daunorubicin derivative, Stability, HPLC.

INTRODUCTION

Daunorubicin is an important member of the group of antracycline antibiotics and is used in treatment of solid tumors and hematologic malignancies. It produces a dose-dependent cardiotoxicity, which results in severe cardiomyopathy and limits its clinical usefulness[1].

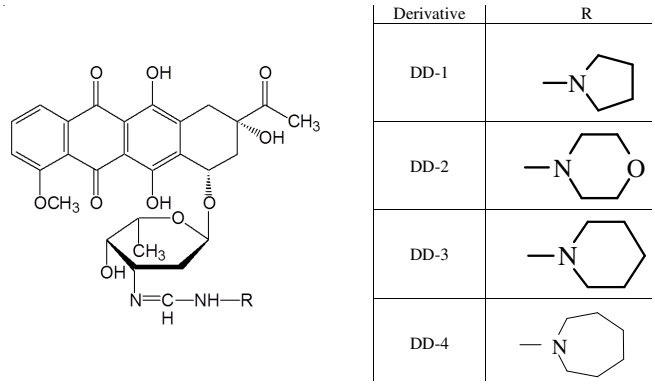
Chemical modification of a daunorubicin molecule can be one of the ways to reduce its cardiotoxicity and to overcome the resistance of cancer cells to the drug. Among the new deri-vatives of daunorubicine, compounds containing a formamidine group ($N=CH-NR^1R^2$) with a heterocyclic ring instead of an $-NH_2$ group in the daunosamine moiety exhibited better biological properties, lower toxicity, particularly cardiotoxicity and higher antiproliferative activity[2-4], in comparison to daunorubicin.

Because a new derivatives, similarly to daunorubicin, may only be administered intravenously, it is important to evaluate the influence of pH, ionic strength and buffers on their stability in aqueous solutions. It has been found that daunorubicin is degraded in aqueous solutions. Its degradation has been studied at 50 ºC and pH 0-14. The influence of buffers, ionic strength and temperature on the degradation of daunorubicin has also been described[5]. The influence of H^+ and OH^- as well as ionic strength on the stability of derivatives containing in the amidine group a morpholine (DD-2), pyrrolidine (DD-3) and hexahydroazepine (DD-4) moiety has been investigated[6-9]. It was proved that DD-2 was the least stable in solutions therefore its stability in solid state was investigated[10].

The stability of daunorubicin in 5 % dextrose injection, 0.9 % sodium chloride injection, lactated Ringer's solution and normosol-R pH 7.4 has been studied[11]. The influence of packaging (plastic syringes, minibags, PVC infusion bags)[12,13] and the compatibility of cytarabine, etoposide epirubicin, doxorubicin and pirarubicin with daunorubicin have also been established[14].

The purpose of this study is to determine the stability of daunorubicin derivatives containing in the amidine group a piperidine (DD-1), morpholine (DD-2), pyrrolidine (DD-3) or hexahydroazepine (DD-4) moiety using stability-indicating HPLC procedures[15] in intravenous solutions. Regarding the most unstable derivative, [(N-morpholine) methylene]daunorubicin (DD-2) the influence of concentration, light-protective effect of infusion packaging on its kinetic degradation

was investigated. The chemical structures of the daunorubicin derivatives (DD-1 to DD-4) are given below :



Chemical structure of derivatives of daunorubicin

EXPERIMENTAL

The daunorubicine derivatives DD-1 to DD-4 were synthesized in the Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, Warsaw, Poland[16]. They were reddish powders, freely soluble in water and common intravenous solutions. Sodium laurisulfate (A.C. reagent, Sigma-Aldrich Logistic GmbH) and other chemicals (Merck, KGaA) were of analytical or HPLC grade. The solutions for injections were sterile. During preparation and kinetic studies all solutions of DD-1 to DD-4 were protected from light.

The stability of DD-1 to DD-4 was assessed visually by appearance (clarity and colour changes). A studied derivative was considered stable if its concentration was between 90 and 110 % of the labeled concentrations.

Changes in the concentrations of derivatives of DD-1 to DD-4 were measured with a validated isocratic reversed-phase HPLC method at 22 ºC. A LiChrospher RP 18 e column (125 mm × 4 mm, particle size 5 µm, Merck, Germany) was used as the stationary phase. The mobile phase consisting of acetonitrile, methanol, solution containing 2.88 gL^{-1} sodium laurisulfate, 1.6 mL L^{-1} 85 % H_3PO_4 (9:1:10 v/v/v), at a flow rate of 1.5 mL min^{-1}, was used. Detection wavelength was 254 nm[15].

Assay procedure: During the stability study polyethylene minibags were filled with a solution containing 5 mg of a studied derivative diluted with 5 mL to a nominal concentration of 1 mg mL^{-1} at the site of the experiment of sterile water, sodium chloride (0.9 %), glucose (5, 10, 20 %), Ringer's solution, Ringer's lactate solution, mixture of sodium chloride (0.9 %) and glucose (5 %) /1:1; 2:1/, pedriatic solution, multielectrolytic solution, Jonosteril®Bacis solution and 20 % mannitol. Solutions for the study were stored at room temperature (25 ± 2 ºC, protected from light) for 2, 6 and 24 h, refrigerated (5 ± 3 ºC, protected from light) for 6 h and 24 h or frozen (-12 ± 3 ºC, 60 ± 5 % relative humidity, protected from light) for 30 days and then unfreezed at room temperature.

The influence of DD-2 concentration (0.2-1.5 mg mL^{-1}) in a 0.9 % solution of sodium chloride on stability was studied. The light-protective effect of packinging (polyethylene minibags and polypropylene syringes) on DD-2 stability in solution of 0.9 % sodium chloride, sterile water and 5 % glucose was also studied.

At specified time intervals, samples of solutions of the studied substances were taken and to each sample 1 mL of the internal standard was added. In order to verify whether the values of observed rate constant - k_{obs} determined during degradation of DD-2 were statistically significant the parallelism test was used.

RESULTS AND DISCUSSION

The HPLC method with UV detection used in this study had previously been found suitable for the determination of derivatives DD-1 to DD-4 during stability studies[15]. Although this method had already been validated, its selectivity during our stability studies in intravenous solutions was confirmed when the influence of concentration and light on the degradation of DD-2 was investigated. The method proved selective for all derivatives in the presence of their degradation products and the internal standard (Fig. 1).

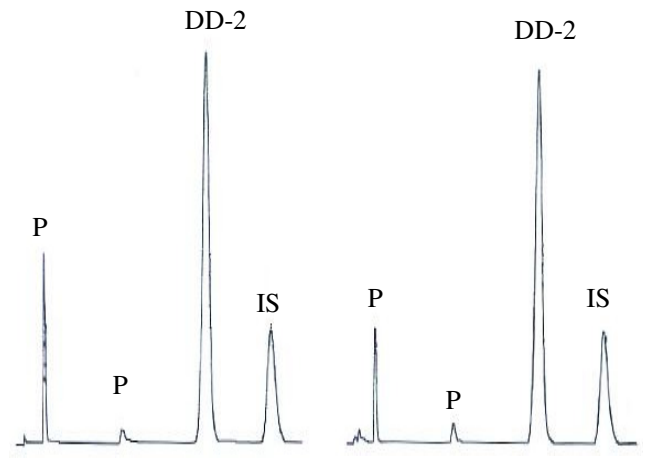


Fig. 1. Chromatograms for: DD-2 in sterile water (0.5 mg mL^{-1}) (a) and (b) in 5 % glucose (0.5 mg mL^{-1}) notprotected from light (b).; P-degradation products, DD-2 [(N-Morpholine)methylene]daunorubicin hydrochloride, IS- internal standard.

All infusion solutions of derivatives DD-1 to DD-4 were clear and orange-red but after being dissolved in the pediatric and multielecrolytic solutions precipitation was observed. After unfreezing the solutions were also clear and orange-red. The highest pH change relative to the initial pH was 0.03. In this study solutions of derivatives DD-1 to DD-4 were defined as stable when the substrate loss was not greater than 10 % relative to the initial value.

Stability of derivatives DD-1 to DD-4 were studied in polyethylene minibags containing intravenous solutions. The DD-3 was the most stable, in 66 % of the solutions (sterile water, 0.9 % sodium chloride, 5 % glucose, Lactated Ringer's solution, 5 % glucose: 0.9 % sodium chloride /1:1/, jonosteril®Bacis solution) whereas the DD-2 was stable only in 10 % glucose during 2 h (Table-1). The stability of all the 4-derivatives of daunorubicin intravenous solutions stored in 5 ºC was satisfactory (Table-2). The unfreezing process after 30 days at -30 ºC storage of intravenous solutions of daunorubicine derivatives decreased their stability significantly (Table-3).

TABLE-1
STABILITY OF DAUNORUBICINE DERIVATIVES STORED AT ROOM TEMPERATURE IN POLYETHYLENE MINIBAGS, PROTECTED FROM LIGHT

Intravenous solution	DD-1	DD-2	DD-3	DD-4
Sterile water				
2 h	100	75.4	98.7	88.0
4 h	100	60.3	98.6	84.0
6 h	100	44.0	89.4	83.3
24 h	100	5.7	86.9	76.7
0.9 % Sodium chloride				
2 h	99.2	88.9	98.4	78.2
4 h	97.8	75.3	98.4	78.1
6 h	97.5	66.0	98.1	77.0
24 h	84.1	20.2	90.5	76.1
5 % Glucose				
2 h	98.1	85.2	100	91.2
4 h	98.0	64.9	100	90.8
6 h	98.3	50.0	100	90.2
24 h	93.6	12.1	100	84.4
10 % Glucose				
2 h	87.4	91.1	91.13	93.8
4 h	82.2	86.9	86.9	91.8
6 h	80.8	74.4	84.1	91.5
24 h	68.3	24.2	80.5	84.0
Ringer's solution				
2 h	87.2	77.7	90.3	97.1
4 h	84.6	64.2	88.0	95.6
6 h	84.3	53.3	87.9	91.6
24 h	84.0	10.19	86.04	89.6
Lactated Ringer's solution				
2 h	100	44.6	100	85.4
4 h	100	24.4	100	84.4
6 h	100	9.84	100	84.8
24 h	100	0	100	72.9
5 % Glucose:0.9 % sodium chloride /1:1/				
2 h	75.4	77.2	95.6	98.3
4 h	74.9	69.9	92.0	98.0
6 h	74.5	54.8	90.6	96.5
24 h	71.0	12.6	90.5	92.6
Jonosteril®Bacis solution				
2 h	90.2	72.5	95.2	90.7
4 h	89.9	38.8	94.0	88.8
6 h	88.5	34.5	93.1	85.5
24 h	87.6	12.8	92.7	73.8
20 % Mannitol				
2 h	81.7	69.1	88.5	81.8
4 h	73.0	50.9	86.6	78.6
6 h	72.4	42.4	82.0	77.6
24 h	70.3	1.6	80.1	65.2

Results obtained by calculating the average value from three determinations (SD < 3 %).

The degradation of DD-2 was a pseudo first order reaction described by the following equation:

$$\ln (P_{DD\text{-}2}/P_{IS}) = \ln (P_{DD\text{-}2}/P_{IS})_0 - k_{obs}t$$

where, $(P_{DD\text{-}2}/P_{IS})$ and $(P_{DD\text{-}2}/P_{IS})_0$ are the ratio of the areas of the peaks of DD-2 to those of the internal standard, at time t = 0 and t, respectively. The study of the effect of concentration of DD-2 (0.2-1.5 mol mL^{-1}) and packing on the stability of DD-2 demonstrated that those factors had no influence on the kinetic mechanism of its degradation. The differences between the observed rate constants compared by using the parallelism test were statistically significant, yet of the same magnitude (Table-4).

TABLE-2
STABILITY OF DAUNORUBICINE DERIVATIVES STORED AT 5ºC IN POLYETHYLENE MINIBAGS, PROTECTED FROM LIGHT

Intravenous solution	DD-1	DD-2	DD-3	DD-4
Sterile water				
6 h	100	84.1	100	100
24 h	100	62.6	100	98.8
0.9 % Sodium chloride				
6 h	100	81.98	99.3	99.3
24 h	100	66.23	96.1	96.1
5% Glucose				
6 h	100	92.9	100	100
24 h	100	73.6	100	100
10 % Glucose				
6 h	100	95.1	100	94.7
24 h	100	80.1	100	92.8
Ringer's solution				
6 h	91.1	79.9	100	98.4
24 h	89.1	64.1	100	97.6
5 % Glucose:0.9 % sodium chloride /1:1/				
6 h	100	89.2	98.5	100
24 h	96.5	72.8	97.2	95.8
Jonosteril®Bacis solution				
6 h	78.4	38.7	98.9	94.3
24 h	74.5	31.2	95.8	90.7

Results obtained by calculating the average value from three determinations (SD < 3 %)

TABLE-3
STABILITY OF DAUNORUBICINE DERIVATIVES STORED FOR 30 DAYS AT –30 ºC IN POLYETHYLENE MINIBAGS

Intravenous solution	DD-1	DD-2	DD-3	DD-4
Sterile water	69.5	75.1	81.2	93.9
0.9% Sodium chloride	86.4	75.3	82.0	96.0
5% Glucose	100	75.9	98.47	87.4
10% Glucose	99.5	100	89.5	95.2
Ringer's solution	95.6	82.1	97.0	100
5% Glucose: 0.9 % sodium chloride /1:1/	81.9	85.0	92.0	96.3
Jonosteril®Bacis solution	98.2	81.5	92.2	85.8

Results obtained by calculating the average value from three determinations (SD < 3 %)

Although the derivatives of daunorubicin show better biological properties, lower toxicity, particularly cardiotoxicity and higher antiproliferative activity than daunorubicin[3-5], their stability in intravenous solutions is lower than that of daunorubicin. For example, the stability of daunorubicin solutions (0.9 % NaCl) stored in polyvinyl chloride minibags at ambient temperature for 43 days was found to be significantly higher than the stability of solutions of daunorubicin derivatives[12]. As demonstrated in this study, when intravenous solutions of daunorubicin derivatives are unfreezed after being stored for 30 days at -30 ºC, their stability is significantly decreased. By contrast, solutions of daunorubicin under the same conditions are stable. Wood *et al.*[12] suggested that the loss daunorubicin in PVC minibags was an effect of a combination of sorption and degradation. A similar process occurred during the storage of daunorubicin derivativers in polyethylene minibags.

Based on the present results, it was demonstrated that the new derivatives of daunorubicin are more susceptible to degradation in intravenous solutions and during freezing and unfreezing polypropylene syringes and polyethylene minibags

TABLE-4
OBSERVED RATE CONSTANTS AND COEFFICIENTS α FOR DEGRADATION OF DD-2 IN SOLUTIONS STORED IN POLYPROPYLENE SYRINGES AND POLYETHYLENE MINIBAGS, NOT PROTECTED FROM LIGHT

Intravenous solution	Polypropylene syringes		Polyethylene minibags		t_0^*
	a [min] × 10^{3-}	k_{obs} [s^{-1}] × 10^{-5}	a [min] × 10^{-3}	k_{obs} $s^{-1}10^{3-}$	
Sterile water	- 1.64 ± 0.29	2.73 ± 0.49	- 2.08 ± 0.14	3.47 ± 0.24	1.2384
0.9 % Sodium chloride	- 1.75 ± 0.20	2.91 ± 0.33	- 2.27 ± 0.25	3.78 ± 0.42	0.5870
5 % Glucose	- 1.57 ± 0.01	2.62 ± 0.20	- 1.64 ± 0.02	2.74 ± 0.34	0.4224

t_0^* calculated with parallelism test

were found to ensure acceptable stability. [(N-morpholine) methylene]daunorubicin (DD-2) a derivative known as the most active biologically, was the least stable.

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