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Compatibility of amsacrine (Amsidyl®) concentrate for infusion with polypropylene syringes

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Amsacrine, an acridine derivative with antitumour activity, is supplied in Germany as Amsidyl® concentrate for infusion. The drug is only minimally soluble in water and is dissolved in anhydrous *N,N*-dimethylacetamide (DMA) which is known to interact with plastic materials. Consequently the manufacturer of Amsidyl® recommends the use of glass syringes for the initial steps in the preparation of Amsidyl® infusion solutions. However, handling of cytotoxic agents with glass syringes has definite disadvantages. The purpose of our study was to evaluate the compatibility of Amsidyl® infusion concentrate with rubber-free, plastic syringes. The study was conducted with the DMA-vehicle for amsacrine solution instead of Amsidyl® concentrate for infusion, and B | Braun Injekt syringes (manufacturer: B. Braun Melsungen, Germany, barrel: polypropylene, plunger: polyethylene). The methods, employed in our study were high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring (SIM). One extraction product was detected. This product was identified as oleic acid amide, a lubricant contained in the barrel material of the syringes. Approximately 50 µg oleic acid amide were measured after storage of DMA in 2 ml B | Braun Injekt syringes for 24 h at 37 °C. Under conditions comparable to the preparation of amsacrine infusion solutions, in cytotoxic reconstitution services, with shorter contact time and lower temperatures, oleic acid amide leaching was not quantifiable. There is no evidence of toxic properties of oleic acid amide in the current literature. Our conclusion is that transfer of the Amsidyl® concentrate for infusion to the diluent, with B | Braun Injekt syringes, can be recommended as safe for all concerned.

1. Introduction

Amsacrine exhibits significant antitumour activity in xenograft animal tumour models. With its three coplanar rings, amsacrine is known to intercalate between DNA base pairs, resulting in the inhibition of DNA synthesis. In clinical practice, the drug is used primarily in chemotherapy for acute non-lymphocytic leukemia, e.g. 120 mg/m^2/d for 3 consecutive days [1]. The drug is usually administered as an intravenous infusion over a period of 1 h or more.

Amsacrine (4′-(9-acridinylamino)methansulphon-m-anisidide, AMSA, m-AMSA), is a synthetic derivative of the acridine dye class. The lipophilic molecule is poorly soluble in water (0.3 mg/ml) and ethanol (2.3 mg/ml). Enhanced solubility of amsacrine is obtained with *N,N*-dimethylacetamide (DMA) (100 mg/ml). Mixing of amsacrine-DMA-solution with aqueous solutions, as is necessary for infusion solutions, results in immediate precipitation. Amsacrine forms a water soluble salt with lactic acid. However gelatinous material forms and precipitates in aqueous AMSA-lactate solutions within a few hours. The phenomenon can be prevented by the presence of DMA. In consequence, amsacrine is supplied in a formulation consisting of two sterile liquids that are aseptically mixed before use, an ampoule containing 1.7 ml of 50 mg/ml amsacrine in anhydrous DMA (85 mg/vial) and a 20 ml amber vial with 13.5 ml of 0.0353 mol/l (S)-lactic acid diluent.

This formulation is marketed in Germany by the Gödecke company and is named Amsidyl® concentrate for infusion and diluent solution. Adding 1.5 ml of the orange drug solution to the lactic acid diluent, results in a solution containing 5 mg/ml of amsacrine in 10% v/v DMA. The lactate salt of amsacrine is formed in situ. The combined solution is reported to be stable for at least 48 h at room temperature under ambient light [1, 2]. Stability is enhanced by an acidic pH value (2.5–3.5), which is provided by surplus lactic acid diluent [3, 4]. Reconstituted solutions may be further diluted with 5% dextrose solution and were reported to be stable for 48 h [1, 5] or at least 72 h [6]. In addition, an admixture of amsacrine in 5% dextrose, containing 1 mEq sodium bicarbonate, was reported to be stable for at least 96 h at room temperature [2]. The hydrochloride salt of amsacrine is poorly soluble in water. Consequently amsacrine solutions are incompatible with 0.9% sodium chloride injection and other chloride-containing solutions [7].

Glass syringes are recommended for the transfer of amsacrine concentrate to the lactic acid diluent [8], because of the interaction of concentrated DMA with plastic materials. DMA may extract UV-absorbing compounds from plastics and rubber used in plastic syringes [9], and DMA containing solutions may leach the plasticizer di(2-ethylhexyl)phthalate (DEHP) from polyvinylchloride (PVC) plastic infusion infusion devices [10]. These authors did not investigate the compatibility of DMA with polypropylene syringes, and data provided from Gödecke [11] are ambiguous. The manufacturer's compatibility studies with anh. DMA in plastic syringes, using a GC assay, revealed the extraction of a fatty acid amide after 10 min contact time. Type, amount, and toxicity of the fatty acid amide were not determined.

In preparing a ready-to-administer cytotoxic drug solution, glass syringes have serious disadvantages. For preparation and administration of hazardous drug solutions, syringes and i.v. sets with Luer-lock type fittings are generally recommended [12], because they are less prone to accidental separation than are friction fittings. However at the tip of a glass syringe the needle is held only by friction. This is a special problem in handling the amsacrine concentrate, because of the difficulty of inserting the needle into the hard rubber stopper of the diluent vial. Moreover, there is an increased risk of contamination for personnel handling the cytotoxic drug with a glass syringe.

In view of the lack of detailed information on the compatibility of Amsidyl® concentrate with plastic syringes, and in view of the safety problems in handling the concentrate with glass syringes, we saw the need for specific experimental studies. The purpose of this study was the detection and identification of potential extraction products involved in the transfer of amsacrine concentrate to the diluent vial, using 2 ml plastic syringes. For the tests we chose a rubber-free, plastic syringe (B | Braun Injekt syringes), which is widely used in European hospitals. The tests were performed using HPLC and GC-MS operated in selective ion mode (GC-MS-SIM).

2. Investigations and results

Storage of concentrated DMA in the B | Braun Injekt syringes over different periods (15 min to 43 h), and at different temperatures (ambient, 37 °C), resulted in no visible changes of the contents or syringes themselves. The physical performance of the Injekt syringes was not affected.
Looking for UV-absorbing compounds potentially extracted from the plastic material by the incubated DMA, PDA chromatogramms were performed over a wavelength range of 200 to 600 nm. None of the analysed DMA samples showed any evidence of an UV-absorbing extraction product. The assay was not suitable to investigate the leaching of oleic acid amide. Even in reference samples containing 1 mg/ml, oleic acid amide could not be detected.

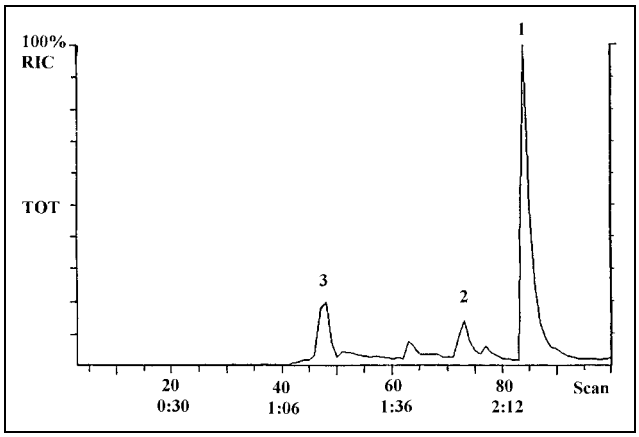

Fig. 1: Total ion chromatogramm of 1 mg/ml oleic acid amide (injection volume 1 µl). ① oleic acid amide, ②, ③ impurities

Fig. 1 shows the GC-MS total ion chromatogramm of the reference compound oleic acid amide. Oleic acid amide was eluted at a retention time (r_t) of 2:18 min. In the reference solution, two additional products were detected (r_t = 1:19 min, r_t = 1:59 min), most probably ordinary impurities like saturated fatty acid compounds. The detection limit of oleic acid amide in the GC-MS assay was found to be 0.3 mg/ml and no extraction products were detected in the samples.
In order to determine the presumed lower concentrations of oleic acid amide, the sensitivity of the assay was increased by selected ion monitoring (SIM) technique. We chose the ion fragment 59 for analysis (Fig. 2). The retention time of oleic acid amide was 2:22 min. The limit of quantification was 0.025 µg per injection (= 25 µg/ml). When analysing the incubated samples of DMA by means of the SIM assay, oleic acid amide could be identified in each sample. A semi-quantitative analysis of our chromatographic data showed a duration of contact, and temperature dependent extraction of oleic acid amide from the plastic syringes (Fig. 3). The amount of leached oleic acid amide was in the range of the detection limit (short contact time, ambient temperature), or the quantification limit of the assay. Only in samples stored for 24 h at 37 °C, the amount of extracted oleic acid amide was quantifiable. The peak area corresponded to the area after injection of 0.025 µg of oleic acid amide. Consequently, the amount of leached oleic acid amide from 2 ml B | Braun Injekt syringes filled with DMA can be calculated as approximately 50 µg. Storage at ambient temperature resulted in considerably less oleic acid amide extracted.

3. Discussion

Leaching and absorption have to be considered in the clinical use of lipophilic, poorly water soluble drugs like amsacrine and its drug formulation. The problem of the leaching of plasticizers, monomers, or other compounds (e.g. initiators, catalysts) from plastic infusion devices may be worsened by organic co-solvents. Ready-to-administer amsacrine infusion solutions contain less than 1% v/v DMA. Negligible amounts of DEHP were found to be leached from i.v. sets and PVC bags into 0.9% sodium chloride and 5 or 10% DMA solutions [10]. However, contact of the undiluted amsacrine solution with plastic items, including filters and syringes is still to be avoided because of the concentrated DMA content [2]. Interactions

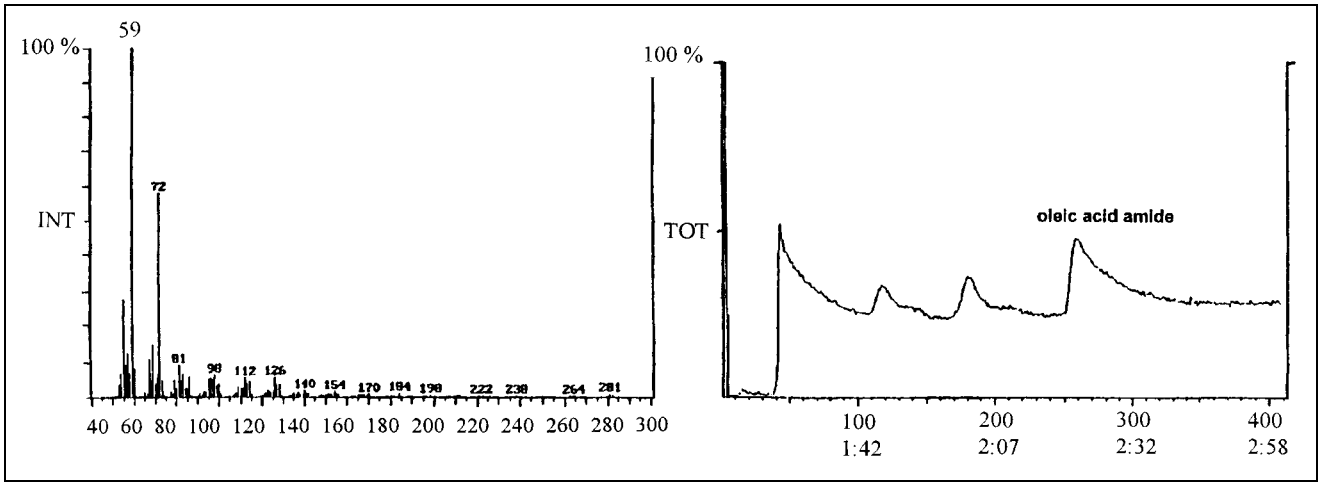

Fig. 2: Mass spectrum of oleic acid amide and mass fragmentogramm of m/z 59

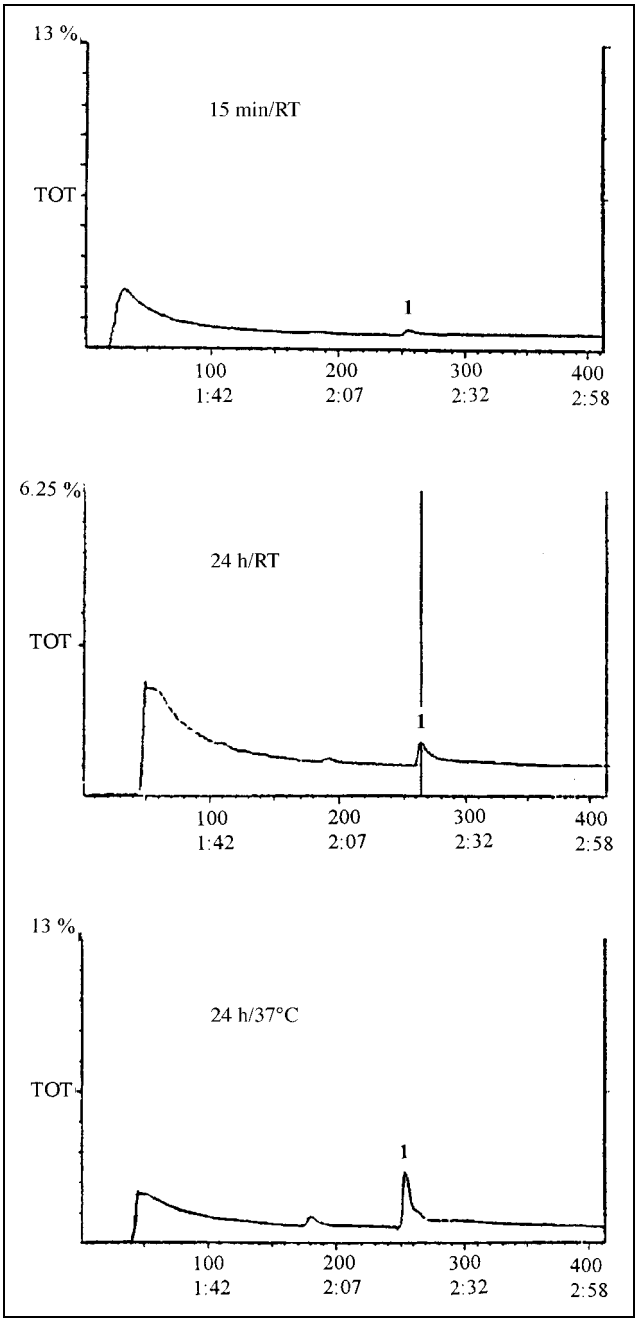

Fig. 3: Mass fragmentogramms (m/z 59) of incubated DMA samples (injection volume 1 µl) showing time and temperature dependent leaching of oleic acid amide. ① oleic acid amide

with plastic syringes are occasioned by DMA and not by the presence of amsacrine in the solution [9].

Therefore we conducted our study, investigating the compatibility of amsacrine concentrate with plastic syringes, using the anh. DMA alone instead of the amsacrine concentrate. We selected B | Braun Injekt syringes for our leaching tests. Although these syringes lack a Luer-lock fitting, they have various advantages over glass syringes, like the leak proof and self-arresting plunger, and disposibility. The syringes are rubber-free and this is associated with a lower risk of extraction by DMA. Rubber-free, plastic syringes with Luer-lock fitting are unfortunately not available.

No extraction products were detected in any sample by means of HPLC or GC-MS. Only when the sensitivity of the GC-MS assay was enhanced by SIM, oleic acid amide, an internal lubricant in the B | Braun Injekt syringes, was identified. Even with our most sensitive method no further extraction products were detected. Our detection of oleic acid amide specifies the results from the Gödecke company, which reported the extraction of an unspecified fatty acid amide after 10 min contact of anh. DMA with plastic syringes, e.g. Injekt syringes. Time and concentration dependency of extraction was already shown for DEHP-leaching from PVC materials [14, 15]. In the present study, the extent of oleic acid amide leaching was directly dependent on the solution contact time with the barrel surface, and storage temperature. The amount of oleic acid amide leached under the conditions of ordinary pharmacy practice (contact time 2–5 min, ambient temperature) is far less than 50 µg and such negligible amounts were not quantifiable, even using the GC-MS-SIM assay.

Established limits for human exposure to oleic acid amide do not exist. Oleic acid amide is seldom used in the production of medical plastics and little is known about the toxicology of this substance. As reported by the manufacturer [16], the B | Braun Injekt syringes passed the tests according to the USP monograph ⟨88⟩ [17], which are designed to determine the biological response of animals to medical-grade polymeric materials. The different tests (systemic injection test, intracutaneous test, implantation test, safety test) are directly related to the intended use of the plastic articles. The different extract media listed in the USP monograph (sodium chloride injection, 1 in 20 solution of alcohol in sodium chloride injection, polyethylene glycol 400, vegetable oil) were used in the tests. The Braun company also performed cell-culture tests in order to detect cytotoxicity, and animal tests in guinea pigs to define the allergenic potential of the syringe materials. Extraction of the syringes was performed with dimethylsulfoxide (DMSO), cell culture medium for the *in vitro* tests or cotton seet oil for the animal studies. The extracts failed to show any measurable cytotoxic or allergenic effects [16]. The assumption is that the extraction qualities of DMA are similar to those of lipophilic extract media used in the tests. Most probably, the extracts in the tests performed by the company, contained leached oleic acid amide, and this suggests that DMA extracts are harmless, too. A review of the literature suggests that oleic acid amide does not possess any toxic properties.

Our study was restricted to B | Braun Injekt syringes. The type and amount of the extraction products may be vary with syringes from other manufacturers using other grades of oleic acide amide or even other lubricants.

Transfer of the Amsidyl® concentrate for infusion to the diluent, with B | Braun Injekt syringes, can be recommended. Even with the method with the highest sensitivity only one extraction product, namely oleic acid amide, was detected. No risk for patient safety is to be expected, since toxic effects of oleic acid amide have not been reported. Moreover the use of plastic syringes is more convenient, and they are associated with low contamination risks for the personnel handling the cytotoxic drug.

4. Experimental

4.1. Reagents and chemicals

N,N-Dimethylacetamide (DMA) was purchased from Fluka Chemika, Buchs, Switzerland. Water, acetonitrile and methanol used were of HPLC-grade (Mallinckrodt Baker, Deventer, Holland). The phosphate buffer was prepared of KH_2PO_4 (Merck, Darmstadt, Germany) and pH adjusted by addition of 36% hydrochloride acid (DAB 10). Oleic acid amide was obtained from B. Braun, Melsungen, Germany (Crodamide® OR) as a grant.

All tests were performed with 2 ml B | Braun Injekt syringes (B. Braun, Melsungen, Germany, lot 96I0201 and 96J1401A). The barrels of the syringes consist of polypropylene (Novolen 3245PC) mixed with 0.1% oleic acid amide as lubricant. The plungers of the syringes are composed of polyethylene (Hostalen GA7260). Plungers of the syringes with the lot-number 96JI401A are green coloured (contained dye: Masterbatch). Since January 1997 only B | Braun Injekt syringes with green plungers have been available on the market.

4.2. Sample preparation for the HPLC assay

Ten B | Braun Injekt syringes of the volume 2 ml (lot 96I0201) were filled with DMA. Five syringes were stored at ambient temperature, and 5 syringes were stored at 37 °C. After a storage time of 43 h the contents of the 5 syringes stored at the same temperature were mixed. A sample of each mixture was taken and diluted with water to a DMA concentration of 30 μg/ml. Samples of oleic acid amide 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml in methanol were analysed without further dilution.

4.3. Sample preparation for the GC-MS assay

Fifteen B | Braun Injekt syringes with a volume of 2 ml (lot 96I0201), and 15 B | Braun Injekt syringes (lot 96J1401A) with green plungers were filled with DMA. Five syringes of each lot were stored 15 min at ambient temperature, 5 for 24 h at ambient temperature, and 5 for 24 h at 37 °C. Prior to analysis the contents of the 5 syringes from th same lot stored under the same conditions, were mixed. Samples of 1 μl were taken from each mixture, and GC-MS analysis was performed without further dilution. As a negative control DMA not stored in plastic syringes was analysed. Oleic acid amide was diluted with methanol to concentrations of 1 mg/ml, 0.3 mg/ml, 0.1 mg/ml and 0.01 mg/ml, and GC-MS analysis was performed with 1 μl samples.

4.4. HPLC-assay

DMA was identified using an HPLC-assay described by Snorek et al. [13], slightly modified to achieve acceptable chromatography in our laboratory. The assay was established on a Waters/Millipore system (Waters: HPLC-pump 510; photodiode array detector 996; 717 plus-autosampler; software: Waters Millenium 2010 version 2.0). The system was equipped with a Waters Novapak C18, 60 Å, 4 mm, 3.9 × 150 mm column. The mobile phase consisted of 5% acetonitrile and 95% 0.1 M phosphate buffer pH 2.5. The flow rate was 1.0 ml/min. Under these conditions DMA eluted at approximately 2.5 min with an absorption maximum of 210 nm. PDA chromatogramms (wavelength 200–600 nm) were performed in order to identify extraction products. The injection volume was 10 μl.

4.5. GC-MS-assay

GC-MS analysis was performed on a Varian 3700 gas chromatograph interfaced with a Finnigan MAT 44 S quadrupole mass spectrometer. GC separation was performed on a Durabond DB-1 column (J & W Scientific), 30 m × 0.32 mm internal diameter and 0.25 mm film thickness. The injector temperature was 300 °C. The column temperature was programmed to rise from an initial temperature of 250 °C to a final temperature of 300 °C (gradient 20 °C/min). Helium was used as carrier gas. The interface temperature was 200 °C. Electron impact was used as ionization method (ionization energy 70 eV). Oleic acid amide was identified by a combination of the full scan spectrum (40–300 atomic mass units) and the retention time. Mass spectral fragmentation of the peak with the retention time 2:18 min and a sample of oleic acid amide (50 μg) analysed by direct probe MS was identical: m/z (%): molecular peak (M^+) 281 (1), 154 (1), 140 (1.5), 126 (6.5), 112 (6.5), 98 (7), 81 (10), 72 (59), 68 (16), 59 (100), 55 (29).
A scan of oleic acid amide was performed on the selected-ion fragment 59, which was abstracted from the full scan spectrum. For quantification the peak area method was used.

Acknowledgements: We are grateful to the Department of Pharmaceutical Biology at the Institute of Pharmacy, Johannes Gutenberg-University Mainz for providing the GC-MS equipment and especially acknowledge the support of Dr. H. Falkenhagen during the analysis. We thank the B. Braun Melsungen company for the useful exchange of data and ideas.

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Received November 11, 1998
Accepted December 28, 1998

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