Cancer Chemother Pharmacol (2012) 69:573–576
DOI 10.1007/s00280-011-1794-6

SHORT COMMUNICATION

Treatment of experimental extravasation of amrubicin, liposomal doxorubicin, and mitoxantrone with dexrazoxane

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Received: 27 October 2011 / Accepted: 25 November 2011 / Published online: 6 December 2011

Abstract
Purpose Dexrazoxane is an established treatment option in extravasation of the classic anthracyclines such as doxorubicin, epirubicin, and daunorubicin. However, it is not known whether the protection against the devastating tissue injuries extends into extravasation with new types of anthracyclines, the anthracenediones, or the liposomal pegylated anthracycline formulations. We therefore tested the antidotal efficacy of dexrazoxane against extravasation of amrubicin, mitoxantrone, and liposomal pegylated doxorubicin in mice.
Methods A total of 80 female B6D2F1 mice were tested in an established mouse extravasation model. The mice had experimental extravasations of amrubicin, mitoxtanrone, and Caelyx and were immediately hereafter treated with systemic dexrazoxane or saline.
Results and conclusion Systemic treatment with dexrazoxane resulted in significant protection against extravasation injuries from all three drugs. Moreover, the vesicant potential of the three test drugs was weaker than seen in previous experiments with the classic anthracyclines.

Keywords Extravasation · Dexrazoxane · Amrubicin · Liposomal pegylated doxorubicin · Mitoxantrone

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Introduction

The unintentional instillation into the surrounding tissues of anthracycline-based chemotherapy, that is, anthracycline extravasation (EV), is a feared complication of cancer therapy. Previously, we have demonstrated that systemic treatment with dexrazoxane offers significant protection against the tissue damage after EV of doxorubicin, epirubicin, daunorubicin, and idarubicin in mice [1–4]. These observations have successfully been translated into clinical practice [5]; dexrazoxane for extravasation is now the recommended treatment of accidental EV of anthracyclines [6, 7].

The continuous process of developing new anti-cancer agents has resulted in the introduction of new synthetic anthracyclines, new formulations of existing compounds, as well as chemically related compounds. Amrubicin is a synthetic anthracycline compound with limited cardiac toxicity compared with the classical anthracyclines. It is currently under investigation in many clinical cancer trials. Doxorubicin in the pegylated liposomal formulation has a lower rate of cardiac toxicity, and extravasation injuries tend to be less vesicant. Mitoxantrone is an anthracenedione compound with both anticancer effect and therapeutic indications in multiple sclerosis. Like the anthracyclines, mitoxantrone is classified as a DNA topoisomerase II poison.

Both mitoxantrone, pegylated liposomal doxorubicin, and amrubicin are vesicant drugs, that is, they have the potential to induce severe tissue damage after

extravasation. However, the vesicant potential is probably weaker compared with the classic anthracyclines. Only very few case reports exist on extravasation of these threes drugs [8, 9]. The antidotal effect of dexrazoxane in EV of these three compounds has not previously been tested. Accordingly, this study aimed at testing whether dexrazoxane protects against experimentally induced EV in an established mouse model.

Materials and methods

Mouse model

All mouse experiments were performed in accordance with national and EU legislation, and a permit to perform the specific type of experiments was obtained from the Experimental Animal Inspectorate, Danish Ministry of Justice. Female B6D2F1 mice (Taconic, Ry, Denmark) weighing 19–21 g were kept in type III cages (9 mice per cage) with wood chip bedding and nesting material (Brogaarden, Gentofte, DK) in a 12-hour light/12-hour dark cycle. The mice had free access to feed (Altromin 1234, Brogaarden, Gentofte, DK) and tap water, and were allowed to acclimatize for at least 5 days before entering the experiment.

Compounds

Pegylated liposomal doxorubicin hydrochloride (Caelyx®, SP Europe, Brussels, Belgium), mitoxantrone (Mitoxantron "Meda", Allerød, Denmark), amrubicin (Sumitomo Pharmaceuticals Co Ltd, Japan) were dissolved in sterile isotonic saline at the appropriate concentrations. Dexrazoxane (Savene, Topotarget A/S, Copenhagen, Denmark) was dissolved in sterile isotonic saline.

Treatments

Before subcutaneous anthracycline/anthracenedione injections, the mice were anaesthetized with fentanyl 0.01 mg/kg/fluanison 0.37 mg/kg (Janssen/Cilag A/S, Birkerød, Danmark)/midazolam 0.19 mg/kg (Roche, Basel, Switzerland) injected intraperitoneally (i.p.). The coat of the caudal back of the mice was shaved with an electric hair clipper (Oster® Golden A5, clipper blade no. 15, Oster Professional Products, McMinnville, TN, USA). Subcutaneous (SC) anthracycline/anthracenedione injections were given in the same standardized site in the midline of the dorsal skin 1 cm above the tail base using a tuberculin syringe.

Drug doses were chosen based on results from pilot experiments aiming at inducing ulcers in at least two-thirds of the mice. The resulting relative SC doses of the three drugs mirrored the differences in potency and hence the doses in daily clinical practice. Amrubicin was injected SC in doses of 7 mg/kg with IP saline (control, $n = 12$), and 7 mg/kg with IP dexrazoxane ($n = 12$), as well as 10 mg/kg SC with IP saline (control, $n = 12$) and 10 mg/kg with IP dexrazoxane ($n = 12$). Caelyx (pegylated liposomal doxorubicin) was injected SC in a dose of 4 mg/kg with IP saline (control, $n = 7$) and 4 mg/kg with IP dexrazoxane ($n = 7$). Mitoxantrone was injected in a dose of 2.5 mg/kg with IP saline (control, $n = 15$) and 2.5 mg/kg with IP dexrazoxane ($n = 12$).

Within 5 min after the subcutaneous injection, the groups of mice were injected with isotonic saline (controls) or 62.5 mg/kg dexrazoxane IP in a volume of 0.1 mL/10 g body weight, followed by two further injections given with 3-h intervals. Two diameters of the developing skin wound were measured daily to calculate the area of the wound in square mm. The area under the curve (AUC) of the skin wound area versus days graph from each individual mouse was calculated to express the wound severity.

Statistical analysis

Statistical analysis and the graphical presentations were performed using the software GraphPad Prism® v. 4.0 (GraphPad Software, San Diego, CA, USA). The effect of dexrazoxane on the sizes and durations of induced skin

Table 1 Results of the experiments

Drug	*N*	Frequency of wounds		AUC (SD)	
Amrubicin 7/10	24	15/24	$P < 0.0001$	52.0 (77.4)	$P = 0.0026$
Amrubicin 7/10 + dexrazoxane	23	1/23		0.43 (2.0)	
Mitoxantrone 2.5	15	15/15	N/A	1,170 (220.6)	$P < 0.0001$
Mitoxantrone 2.5 + dexrazoxane	14	14/14		605.4 (219.2)	
Caelyx 4	7	5/7	$P = 0.021$	279.8 (353.0)	N/A
Caelyx 4 + dexrazoxane	7	0/7		0	

Amrubicin data are compiled from two experiments, see "Results" section. Subcutaneous doses of the vesicants are shown in mg/kg. Dexrazoxane was administered 62.5 mg/kg intraperitoneally × 3

AUC mean wound area over time (square mm × days), *SD* standard deviation

wounds was evaluated using a student's *t* test. Frequencies of wounds were compared using Fischer's exact test.

Results

Amrubicin 7 and 10 mg/kg produced ulcers in 6/12 and 9/12 mice, respectively. With IP dexrazoxane treatment, the fractions were 0/12 and 1/11 (one mouse died under general anesthesia), respectively. The corresponding mean AUCs were 37.5 and 66.5, respectively without antidote and 0 and 0.9, respectively with dexrazoxane. The reductions in frequency of wounds and in the AUC conferred by dexrazoxane treatment were statistically significant. The compiled data are shown in Table 1 and Fig. 1.

Data from treatment of extravasation of mitoxantrone and Caelyx are shown in detail in Table 1: dexrazoxane did not reduce the frequency of wounds induced by mitoxantrone extravasation; however, the reduction in AUC was significant, as was the reductions in frequency as well as AUC in Caelyx-extravasation.

Discussion

Acute treatment with dexrazoxane against anthracycline EV is already implemented into daily clinical practice guidelines. However, neither experimental studies nor clinical studies have hitherto been carried out to investigate whether dexrazoxane reduces the damaging effects after EV of amrubicin, mitoxantrone, and pegylated liposomal doxorubicin.

In this study, we demonstrate that all three compounds have a weaker ulcerogenic potency in an established mouse model than the classic anthracyclines, that is, doxorubicin and daunorubicin. The mean AUC (expressing the sizes of wounds over time) is generally smaller than in earlier experiments with classic anthracyclines in equitoxic doses using the same mouse model. The AUCs of amrubicin 7–10 mg/kg, mitoxantrone 2.5 mg/kg, and Caelyx 4 mg/kg were 52, 1,170, and 280 ($mm^2 \times$ days), respectively, compared with 800–1,200 and 400–500 ($mm^2 \times$ days) for daunorubicin 2–3 mg/kg and doxorubicin 2–3 mg/kg, respectively, in earlier studies [1–4]. Amrubicin seems to be less prone to produce ulcers in the experimental model, which is indicated by the smallest AUC despite highest drug concentrations. However, it was possible to produce wounds with relatively high consistency in the control groups. Dexrazoxane effectively reduced sizes and duration as well as the frequency of wounds induced by amrubicin.

In mitoxantrone EV, however, ulceration could not be prevented from occurring by dexrazoxane. The duration of mitoxantrone-induced wounds was considerably longer (Fig. 1) than those induced by the other drugs, and also longer than those induced by the classic anthracyclines in previously published experiments. However, the sizes and durations were significantly reduced by the antidotal treatment with dexrazoxane.

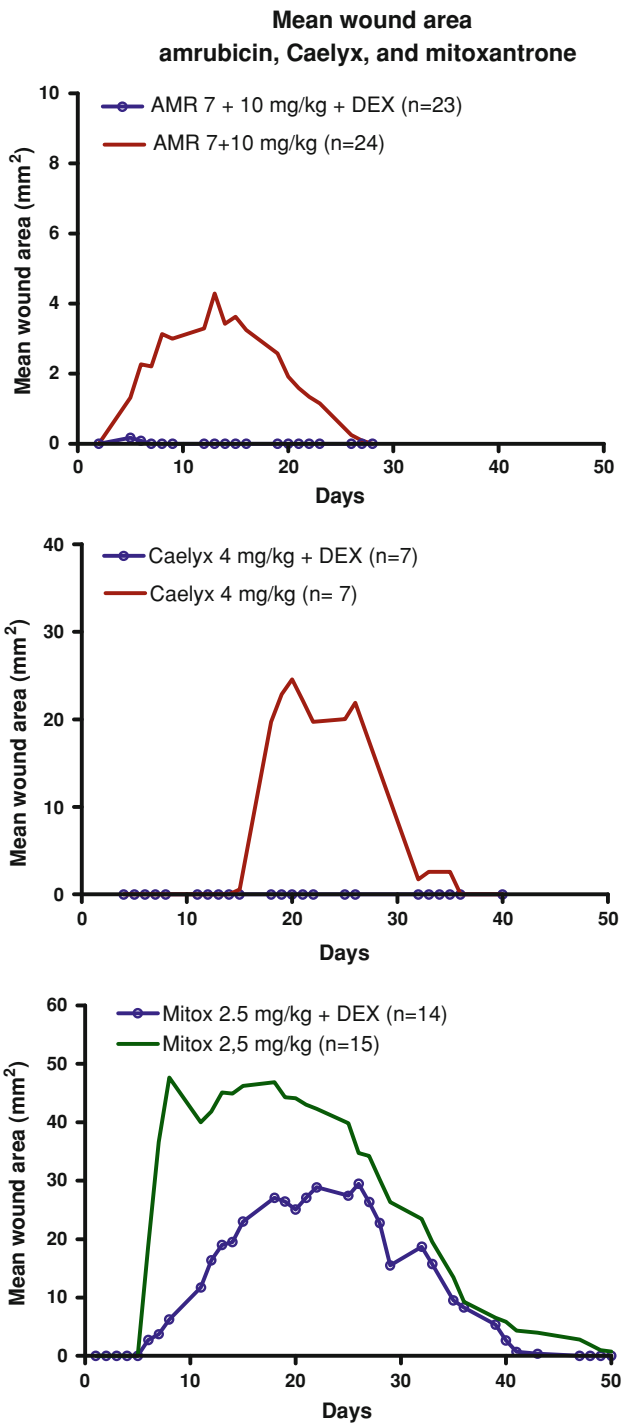

Fig. 1 Mean wound area over time for the three treatment groups. *AMR* amrubicin, *DEX* dexrazoxane, *Mitox* mitoxantrone

Caelyx-induced EV wounds were smaller and occurred later (Fig. 1) than doxorubicin-induced wounds in historical experiments. No wounds occurred in the dexazoxane-treated mice.

Conclusion

Dexrazoxane offers significant protection against tissue destruction induced by amrubicin, mitoxantrone, and liposomal pegylated doxorubicin in an established mouse model for extravasation. The experimental vesicant properties are weaker for these compounds than for the classic anthracyclines.

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