Cancer Chemother Pharmacol (2006) 57: 125–128
DOI 10.1007/s00280-005-0022-7

SHORT COMMUNICATION

Seppo W. Langer · Annemette V. Thougaard
Maxwell Sehested · Peter Buhl Jensen

Treatment of anthracycline extravasation in mice with dexrazoxane with or without DMSO and hydrocortisone

Received: 16 January 2005 / Accepted: 18 March 2005 / Published online: 5 July 2005

Abstract Dexrazoxane has been reported to be protective against anthracycline induced subcutaneous ulceration in mice. It is currently under clinical investigation as an acute antidote in accidental anthracycline extravasation, for which indication topical dimethylsulfoxide (DMSO) and intralesional hydrocortisone are used empirically. We studied the effect in 72 mice of monotherapy with and combined therapy of intraperitoneal dexrazoxane, topical DMSO, and intralesional hydrocortisone as acute antidotes against ulceration after subcutaneous daunorubicin. Dexrazoxane completely prevented wounds from occurring, while neither DMSO nor hydrocortisone had any preventive effect. The addition of topical DMSO actually reduced the efficacy of dexrazoxane. In conclusion, the present study does not support the concomitant use of topical DMSO + systemic dexrazoxane or intralesional hydrocortisone + systemic dexrazoxane. Monotherapy with systemic dexrazoxane seems preferable and is highly efficacious in preventing ulceration.

Keywords Extravasation · Anthracycline · Dexrazoxane · DMSO · Hydrocortisone

Introduction

Dexrazoxane is a topoisomerase II catalytic inhibitor and an iron chelating agent that has been reported to be useful for cardioprotection against anthracycline induced cardiomyopathy. We have demonstrated that dexrazoxane is highly efficacious in protecting against anthracycline induced subcutaneous necrosis in mice [1, 2]. The efficacy of dexrazoxane in ameliorating the devastating effects of accidental extravasation of anthracycline containing chemotherapy in humans has been suggested in published case stories [3–6]. Moreover, the efficacy and toxicity of dexrazoxane for this indication is currently assessed in an international phase II multicenter study [7]. In animal studies, dimethylsulfoxide (DMSO) and hydrocortisone showed inconsistent effect against anthracycline induced extravasation injuries. Despite the lack of definitive clinical evidence of effect, topical DMSO and intra- or perilesional infiltration with hydrocortisone are used in many institutions to treat accidental extravasation of anthracyclines [8].

The aim of this study was to compare the efficacy of topical DMSO, intralesional hydrocortisone and systemic dexrazoxane as monotherapy as well as in combination against anthracycline induced ulceration in mice.

Material and methods

Drugs were obtained commercially. Dexrazoxane hydrochloride (Zinecard, SP Pharmaceuticals, Albuquerque, NM, USA); Daunorubicin hydrochloride (Cerubidin, Aventis Pharma A/S, Hoersholm, Denmark); Hydrocortisone (SoluCortef, Pfizer ApS, Ballerup, Denmark, Dimethylsulfoxid (Merck KGaA, Darmstadt, Germany); Fentanyl-fluanisone (Hypnorm, Janssen-Cilag, Birkeroed, Denmark); Midazolam (Dormicum, Roche, Hvidovre, Denmark).

Individually marked female B6D2F1 (M & B, Ry, Denmark) weighing 19–21 g were caged in groups of nine in a temperature and light controlled environment with ad libitum access to food and water. The pre-experimental acclimatization period was 1–2 weeks.

S. W. Langer (✉) · P. B. Jensen
Laboratory of Experimental Medical Oncology, The Finsen Center 5073, Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark
E-mail: swlanger@dadlnet.dk

M. Sehested · P. B. Jensen
Department of Pathology, The Diagnostic Center 5431, Rigshospitalet, Copenhagen, Denmark

A. V. Thougaard · M. Sehested
Topotarget A/S, Symbion Science Park, Fruebjergvej 3, 2100, Copenhagen, Denmark

Animal handling and daily observation was carried out according to animal welfare guidelines [9]. A standard solution containing one part fentanyl-fluanisone, one part midazolam, and two parts isotonic saline was used for anaesthesia in an intraperitoneal (IP) dose of 0.1 ml/10 g. The caudal back of the mice were shaved with an electrical hair clipper. Based on previous experiments [1, 2], daunorubicin 3 mg/kg was injected SC with a 50 µl fixed-volume Hamilton syringe (Bonaduz AG, Bonaduz, Switzerland) and a 27 G × 3/4“ needle one centimetre cranially from the root of the tail after grabbing the dorsal skin.

Based on previous experiments [1, 2], dexrazoxane was administered IP in a volume of 0.2 ml in a dose of 62.5 mg/kg immediately after SC injection of daunorubicin and after 3 h and 6 h. Hydrocortisone 50 mg/ml (based on [10]) was injected intralesionally (IL) immediately after daunorubicin in 0.05 ml. DMSO 99% was applied topically with a cotton swap and allowed to air dry, 3 drops 3 times a day for 4 days. First dose was applied immediately after the SC daunorubicin. Saline served as control treatment; i.e. IP 0.2 ml, IL 0.05 ml, and topically 3 drops 3 times a day for 4 days. There were eight treatment groups ($n = 72$) as shown in Table 1.

Injection sites were observed daily. A wound was defined as loss of hair and dermal disruption of at least 2 mm^2 . Healing was defined as complete regrowth of hair in a wound area. The observation period ended, when all lesions had healed.

Data handling and statistical methods

Sizes of lesions were measured every 2 days with a ruler. The wound areas were calculated from the product of the two longest perpendicular diameters. For each mouse, the wound area in square millimeter was plotted against time in days. Similarly, the mean area of wounds in each study group was calculated and plotted against time, and the area under the ulceration curve (AUC) was calculated. The mean AUCs were compared using the Mann-Whitney's nonparametric test.

Results

Results are presented numerically in Table 2, and a graphic overview of wound areas is displayed in Fig. 1.

No mice developed wounds after subcutaneous daunorubicin when treated with dexrazoxane IP as monotherapy given three times within the first 6 h after daunorubicin. In contrast, 9/9 (100%) of the mice in the control group (saline IP) developed ulcers with a mean AUC of 630 $mm^2 \times$ days and a mean duration of 21.2 days.

The addition of topical DMSO to the systemic dexrazoxane treatment resulted in the occurrence of wounds in six of nine (66%) of the mice compared to none for dexrazoxane alone. However, the wounds where small with a mean AUC of 55 $mm^2 \times$ days and of short duration, i.e., mean 4.7 days. When intralesional hydrocortisone was added to systemic dexrazoxane, one wound of very short duration (2 days) appeared.

There were no statistically significant differences in the number of wounds, sizes of wounds, and duration of wounds between treatment with topical DMSO and the control groups, i.e topical and IP saline ($P = 0.55$ and $P = 0.55$, respectively for the mean AUC)

There were no differences between the wound frequency, sizes and duration after IL treatment with hydrocortisone and the corresponding control groups, i.e. IL and IP saline ($P = 0.06$ and $P = 0.73$, respectively for the mean AUC).

Discussion

The study confirms the efficacy of dexrazoxane in ameliorating the devastating effects of anthracycline extravasation in the subcutaneous (subpannicular) mouse model as also shown in [1, 2].

Although inflammation is minimal in the histological evaluation of acute and late doxorubicin induced lesions, hydrocortisone has been tested extensively as an adjuvant drug; however, a beneficial effect has hitherto not been documented. Thus, in a rat study,

Table 1 Overview of treatment groups

Vesicant	Adjuvant treatment	No. of mice
Daunorubicin 3 mg/kg, 0.05 ml SC on day 1 at $t = 0$	Dexrazoxane 62.5 mg/kg, 0.2 ml IP on day 1 at $t = 0$, $t = 3$ h, and $t = 6$ h	9
	Hydrocortisone 50 mg/ml, 0.05 ml IL on day 1 at $t = 0$	9
	DMSO topically 99%, 3 drops 3 times a day on day 1, 2, 3, and 4	9
	Saline 0.2 ml IP on day 1 at $t = 0$	9
	Saline 0.05 ml IL on day 1 at $t = 0$	9
	Saline topically 3 drops 3 times a day on day 1, 2, 3, and 4	9
	Dexrazoxane 62.5 mg/kg, 0.2 ml IP on day 1 at $t = 0$, $t = 3$ h, and $t = 6$ h *combined with* hydrocortisone 50 mg/ml, 0.05 ml IL on day 1 at $t = 0$	9
	Dexrazoxane 62.5 mg/kg, 0.2 ml IP on day 1 at $t = 0$, $t = 3$ h, and $t = 6$ h *combined with* DMSO topically 99%, 3 drops 3 times a day on day 1, 2, 3, and 4	9

IP Intraperitoneal; *IL* Intralesional; *SC* Subcutaneous; *t* time

Table 2 Results of the wound assessment in 72 mice after 0.05 ml daunorubicin 3 mg/kg SC and different adjuvant treatments. Doses and schedules are explained under material and methods and further elucidated in this table

Adjuvant treatment	Frequency[a] of wounds (%)	Mean AUC[b] (±SEM)	Duration[c] (±SEM)
Dexrazoxane IP	0/9 (0)	0	0
Hydrocortisone IL	9/9 (100)	630 (±144)	21.2 (±2.8)
DMSO topically	9/9 (100)	557 (±102)	20.8 (±1.7)
Saline IP	8/9 (89)	739 (±171)	21.8 (±3.0)
Saline IL	9/9 (100)	961 (±121)	24.9 (±1.9)
Saline topically	8/9 (89)	745 (±149)	23.4 (±3.2)
Dexrazoxane IP + hydrocortisone IL	1/9 (11)	23 (±23)	2.0 (±2.0)
Dexrazoxane IP + DMSO topically	6/9 (67)	55 (±19)	4.7 (±1.7)

IL intralesional; *IP* intraperitoneal
[a]Number of mice with wounds/treated mice
[b]Area under the curve of mean wound area in mm^2 over time in days ± standard error of the mean
[c]Mean duration of wounds in days ± standard error of the mean

multiple local injections of hydrocortisone actually increased wound sizes after intradermal doxorubicin, but a single intralesional injection of hydrocortisone was reported to result in minor reduction of ulcer sizes [11]. A single intralesional injection of hydrocortisone increased wound lesions after doxorubicin in mice, whilst an indication of protection was seen at the lowest doses used, i.e. 2.5 mg of hydrocortisone, and topical hydrocortisone had no effect [10]. Hydrocortisone did not ameliorate lesions caused by intradermal doxorubicin in rats and mice [12–14] and also proved to be useless in daunorubicin lesions in mice [15]. In accordance with this, the present study could not demonstrate any beneficial effect of a single dose of intralesional hydrocortisone. The risk of infection in the extravasation area is high, which may be related to the absence of white blood cells and poor wound healing, as well as to necrotic tissue serving as a medium for bacteria. Thus, the concept of intralesional treatment of anthracycline extravasation injuries may be questioned.

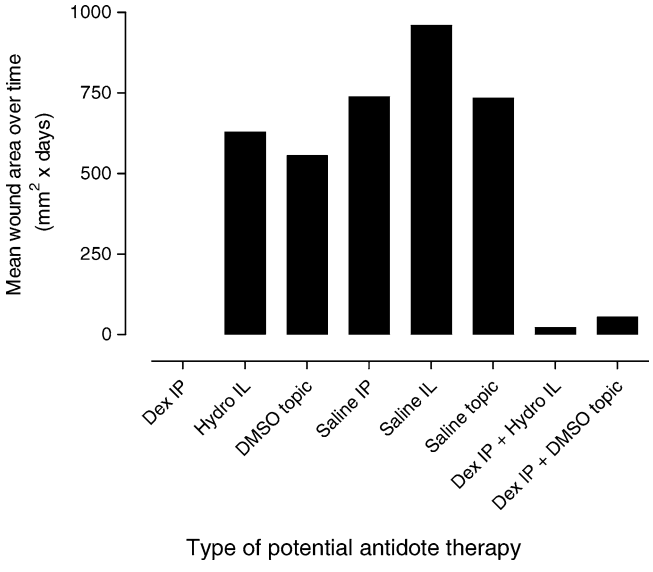

Fig. 1 Bar plot of the mean AUC of wound area over time after subcutaneous daunorubicin and antidote treatment. The treatments are detailed in Table 1. *Dex* Dexrazoxane; *Hydro* Hydrocortisone; *IP* Intraperitoneal; *IL* Intralesional; *Topic* Topical

Animal studies of the efficacy of DMSO have been somewhat contradictory. Thus, complete failure of topical, intralesional, and systemic DMSO in protecting against lesions induced by intradermal doxorubicin in mice was reported by Dorr et al. [16]. The wound diameter, but not the frequency of wounds, resulting from doxorubicin induced lesions was slightly reduced in rats [17], and a statistically non significant protective role for intralesional DMSO against doxorubicin induced ulceration in pigs and rats was suggested in [18]. In the present study, topical DMSO did not protect against the effects of SC daunorubcin. Furthermore, concomitant topical DMSO actually decreased the efficacy of systemic dexrazoxane, and this result could actually have clinical implications. The present animal study questions the use of DMSO, however this does not translate directly into drop DMSO from clinical use [8].

In conclusion, systemic treatment with dexrazoxane prevented all wounds from occurring after SC daunorubicin in this mouse model. Addition of topical DMSO resulted in decreased efficacy of the dexrazoxane treatment. Monotherapy with topical DMSO and monotherapy with intralesional hydrocortisone had no effect against wounds induced by the SC anthracycline. This experimental study establishes dexrazoxane as an effective treatment for anthracycline extravasation and demonstrates that DMSO and cortisone may be detrimental when used with dexrazoxane. Clinical studies are needed to firmly establish optimal dosing and frequency of administration of dexrazoxane in various conditions and amounts of extravasation.

Acknowledgements We are indebted to Annette Nielsen for her expert technical support.

References

1. Langer SW, Sehested M, Jensen PB (2000) Treatment of anthracycline extravasation with dexrazoxane. Clin Cancer Res 6:3680–3686

2. Langer SW, Sehested M, Jensen PB (2001) Dexrazoxane is a potent and specific inhibitor of anthracycline induced subcutaneous lesions in mice. Ann Oncol 12:405–410
3. Langer SW, Sehested M, Jensen PB, Buter J, Giaccone G (2000) Dexrazoxane in anthracycline extravasation. J Clin Oncol 18:3064
4. Jensen JN, Lock-Andersen J, Langer SW, Mejer J (2003) Dexrazoxane—a promising antidote in the treatment of accidental extravasation of anthracyclines. Scand J Plast Reconstr Surg Hand Surg 37:174–175
5. Bos AM, van der Graaf WT, Willemse PH (2001) A new conservative approach to extravasation of anthracyclines with dimethylsulfoxide and dexrazoxane. Acta Oncol 40:541–542
6. El Saghir N, Otrock Z, Mufarrij A, Abou-Mourad Y, Salem Z, Shamseddine A, Abbas J (2004) Dexrazoxane for anthracycline extravasation and GM-CSF for skin ulceration and wound healing. Lancet Oncol 5:320–321
7. Topotarget A/S, Copenhagen Denmark (2005) TT02—a clinical trial on Topotect® (dexrazoxane) in the treatment of accidental extravasation of anthracycline anti-cancer agents
8. Bertelli G, Gozza A, Forno GB, Vidili MG, Silvestro S, Venturini M, Del Mastro L, Garrone O, Rosso R, Dini D (1995) Topical dimethylsulfoxide for the prevention of soft tissue injury after extravasation of vesicant cytotoxic drugs: a prospective clinical study. J Clin Oncol 13:2851–2855
9. The Council of Europe Convention, Strasbourg (1986) The Council of Europe Convention for the protection of vertebrate animals used for experimental and other scientific purposes, 18 March 1986
10. Dorr RT, Alberts DS, Chen HS (1980) The limited role of corticosteroids in ameliorating experimental doxorubicin skin toxicity in the mouse. Cancer Chemother Pharmacol 5:17
11. Upton J, Mulliken JB, Murray JE (1979) Major intravenous extravasation injuries. Am J Surg 137:497–506
12. Loth TS, Eversmann WW Jr (1986) Treatment methods for extravasations of chemotherapeutic agents: a comparative study. J Hand Surg Am 11:388–396
13. Coleman JJ III, Walker AP, Didolkar MS (1983) Treatment of adriamycin-induced skin ulcers: a prospective controlled study. J Surg Oncol 22:129–135
14. Cohen MH (1979) Amelioration of adriamycin skin necrosis: an experimental study. Cancer Treat Rep 63:1003–1004
15. Soble MJ, Dorr RT, Plezia P, Breckenridge S (1987) Dose-dependent skin ulcers in mice treated with DNA binding antitumor antibiotics. Cancer Chemother Pharmacol 20:33–36
16. Dorr RT, Alberts DS (1983) Failure of DMSO and vitamin E to prevent doxorubicin skin ulceration in the mouse. Cancer Treat Rep 67:499–501
17. Svingen BA, Powis G, Appel PL, Scott M (1981) Protection by alpha-tocopherol and dimethylsulfoxide (DMSO) against adriamycin induced skin ulcers in the rat. Res Commun Chem Pathol Pharmacol 32:189–192
18. Desai MH, Teres D (1982) Prevention of doxorubicin-induced skin ulcers in the rat and pig with dimethyl sulfoxide (DMSO). Cancer Treat Rep 66:1371–1374