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Toxicological Aspects of a Novel 9-Aminoanthracycline, SM-5887

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The toxicological characteristics of SM-5887 were evaluated in mice after a bolus intravenous injection, and compared with those of adriamycin (ADR). The acute toxic signs observed after SM-5887 administration were body weight decrease, ataxia, hair loss, and myelosuppression. They were qualitatively comparable to those induced by ADR. The 50% lethal dose values determined by 14-day observation after drug administration were in the range of 32 to 50 mg/kg for SM-5887 and 16 to more than 20 mg/kg for ADR in four strains of mice. The maximum tolerated doses (MTD) were estimated to be 25 mg/kg for SM-5887 and 12.5 mg/kg for ADR (no death or body weight loss of more than 3 g occurred). When 14-day survivors were further observed until 90 days after drug administration, ADR frequently and dose-independently showed delayed-type lethal toxicity at doses of more than 10 mg/kg, whereas SM-5887 did not. The myelosuppression of SM-5887 was more severe even at a half of the MTD than that of ADR at the MTD, but its recovery was more rapid than that after ADR. In addition, when the drugs were injected into the subplantar region of mouse hind paws, ADR induced a severe inflammatory reaction, whereas SM-5887 yielded only a slight one. The data suggest that toxic effects of SM-5887 are more reversible and more controllable than those of ADR.

Key words: Anthracycline — SM-5887 — Toxicity

Adriamycin (ADR) is one of the most widely used anticancer agents, but its clinical usefulness is restricted by various side-effects; myelosuppression, mucositis, alopecia, gastrointestinal toxicity, local tissue toxicity, and cardiotoxicity. In the last decade, therefore, a number of anthracycline analogs have been synthesized or isolated, but none better than ADR in terms of both antitumor activities and toxicities has yet been found.

SM-5887 is a novel, totally synthetic 9-aminoanthracycline derivative.[1] It has excellent antitumor activities in murine experimental tumor systems and human tumor-nude mouse systems.[2] In addition, it showed much less cardiotoxicity than ADR in a rabbit chronic experimental model.[3]

The present study was carried out to evaluate the toxicological aspects of SM-5887 other than cardiotoxicity by administering a single bolus intravenous (iv) injection to mice. The toxic effects proved to be more reversible and more controllable than those of ADR.

MATERIALS AND METHODS

Chemicals SM-5887 was prepared by Sumitomo Pharmaceuticals Co., Ltd. (Osaka). ADR was purchased from Kyowa Hakko Co., Ltd. (Tokyo). SM-5887 was dissolved in citrate-phosphate buffer containing 5% lactose (pH 3.8), and ADR was dissolved in 0.85% NaCl solution. These drugs were administered iv to mice at 0.1 ml/10 g of body weight.

Animals ICR/Jcl mice were purchased from Shizuoka Experimental Animal Corp. (Hamamatsu). BALB/c×DBA/2 F1 (hereafter called CDF1), C57BL/6×DBA/2 F1 (hereafter called BDF1), and BALB/c mice were obtained from Charles River Japan, Inc. (Kanagawa).

Toxicological study SM-5887 and ADR were injected iv into 4 strains of mice (female BALB/c, male CDF1, male BDF1, and male ICR/Jcl). The doses were 20 to 50 mg/kg for SM-5887 and 8 to 20 mg/kg for ADR. Each treatment group was composed of 6 mice. The lethality within 90 days after drug administration was observed. The mice were weighed every 2 days for 14 days and once a week from 15 to 90 days, and toxic signs were observed for 14 days.

Measurement of total number of femoral bone marrow cells As an index of marrow toxicity, the total number of femoral bone marrow cells was measured after an administration of SM-5887 or ADR. The drugs were given iv to groups of male BALB/c mice, and the animals were sacrificed at definite intervals. The femurs were removed rapidly, and any adherent tissues were wiped away. Bone marrow cells were flushed out with 2.5 ml of 0.85% NaCl solution by injection into one end of the bone. The bone marrow cell suspension was diluted 100-fold with ISOTON II, and the cell number of this suspension was counted with a Coulter counter. The relative number of bone marrow cells of the treated groups (composed of 3 mice) with respect to that of the control groups (composed of 5 mice) was calculated.

Local tissue toxicity Local tissue toxicity was evaluated by the method previously reported.[4] SM-5887 was dissolved in distilled water or 5% mannitol, and ADR was dissolved in 0.85% NaCl solution. Male CDF1 mice were

injected subcutaneously (sc) with 10 μl of drug solution in the plantar region of the left hind paw using a microsyringe, and with 10 μl of vehicle into the right hind paw. Inflammation was determined by measuring the thickness of the paw with a Mitsutoyo dial caliper. The degree of inflammation was expressed as the difference between the thickness of the drug-injected paw and the thickness of the paw injected with solvent.

RESULTS

Acute toxicity SM-5887 and ADR were given to four mouse strains by single iv injection, and the lethality and toxicological signs were observed for 14 days. SM-5887 caused dose-dependent body weight loss, ataxia and hair loss qualitatively comparable to those caused by ADR. As shown in Fig. 1, the 50% lethal dose (LD_{50}) values of

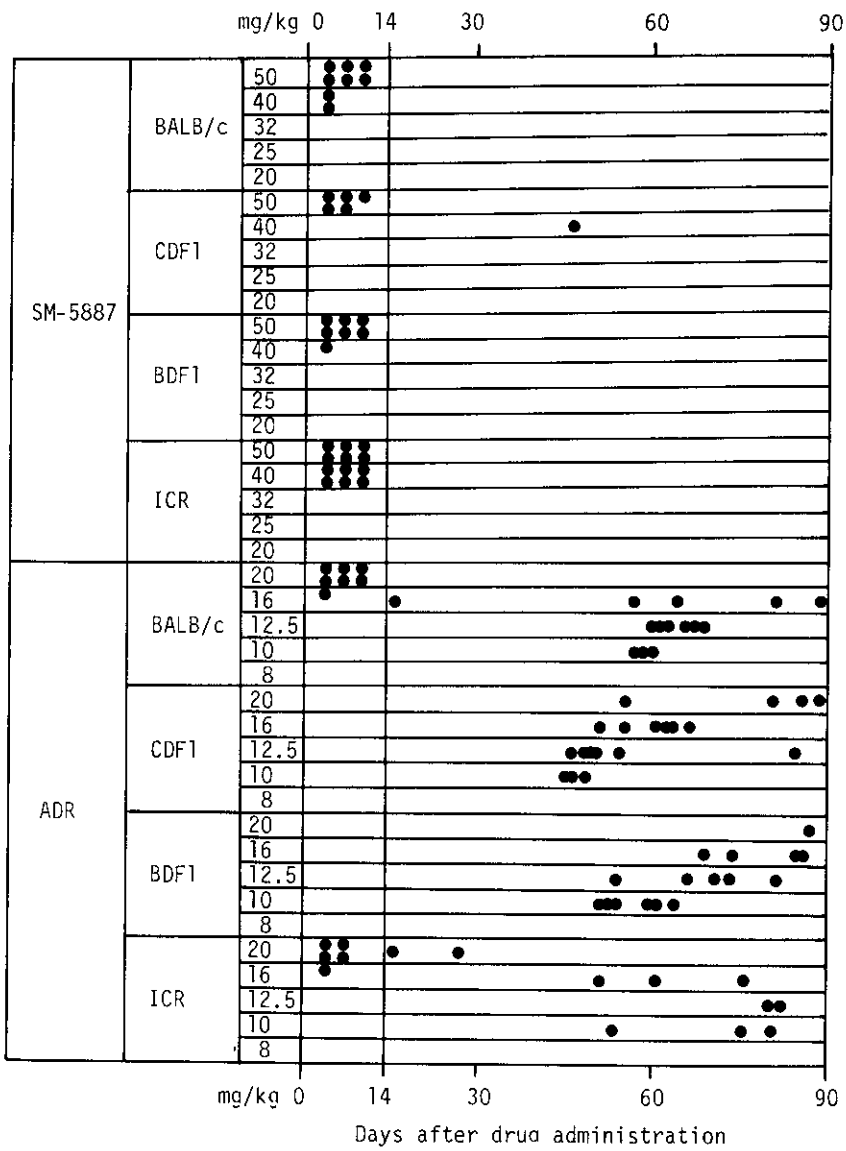


Fig. 1. Toxic deaths of mice treated with SM-5887 and ADR for 90 days after single iv injection. Closed circles (●) indicate dead mice. Each group consisted of 6 mice.

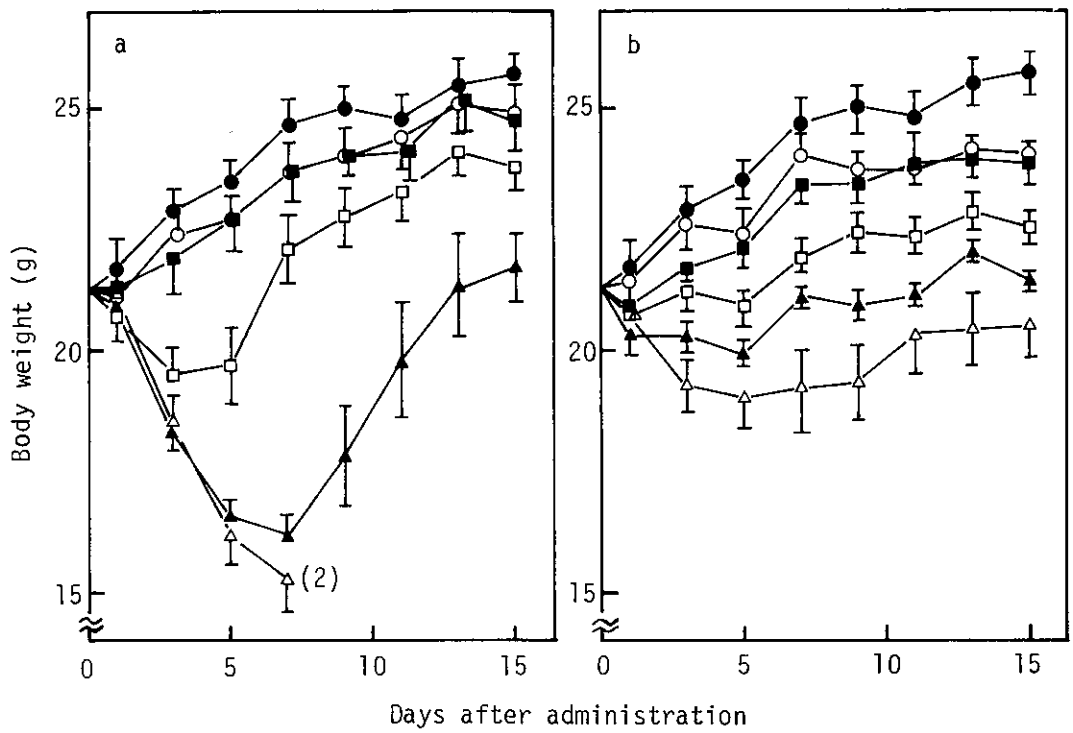


Fig. 2. Time-course of body weight for 15 days after single iv injection of SM-5887 (a) and ADR (b) in CDF1 mice. (a) Groups of 6 mice were untreated (●) or given SM-5887 at doses of 20 mg/kg (○), 25 mg/kg (■), 32 mg/kg (□), 40 mg/kg (▲), or 50 mg/kg (△). (b) Groups of 6 mice were untreated (●) or given ADR at doses of 8 mg/kg (○), 10 mg/kg (■), 12.5 mg/kg (□), 16 mg/kg (▲), or 20 mg/kg (△). Each point represents the mean±SE of 4 to 6 mice. Numbers in parentheses indicate the number of dead mice.

SM-5887 were in the range of 32 to 50 mg/kg for the four strains of mice, although they could not be definitely determined because of the steep dose-response curves. The LD_{50} values of ADR were between 16 and 20 mg/kg for BALB/c and ICR mice, and more than 20 mg/kg for CDF1 and BDF1 mice. Although the lethal toxicities of these drugs thus varied according to the strains of mice, SM-5887 and ADR induced similar body weight changes in all four strains; the case of CDF1 mice is illustrated in Fig. 2. SM-5887 and ADR caused severe body weight loss of more than 3 g compared with the control group at doses of more than 32 mg/kg and 16 mg/kg, respectively. In terms of lethality and body weight loss of more than 3 g, therefore, the maximum tolerated doses (MTD) by a single iv injection were estimated to be 25 mg/kg for SM-5887 and 12.5 mg/kg for ADR in the four mouse strains tested.

Delayed-type lethal toxicity Delayed lethality (15–90 days after a single iv administration) was observed in four strains of mice (Fig. 1). ADR caused frequent and dose-independent delayed death at doses of more than 10 mg/kg, which is less than the MTD of ADR. SM-5887, however, hardly ever caused delayed death. Figure 3 shows the body weight change of CDF1 mice treated with SM-5887 and ADR. The ADR-treated mice exhibited a remarkable loss of body weight followed by delayed death. Intestinal discoloration was often observed in ADR-treated mice surviving till day 90.

Myelosuppression Effects of SM-5887 and ADR on the bone marrow were investigated by administering a single iv injection to mice as shown in Fig. 4. SM-5887 caused more potent myelosuppression even at half the MTD (12.5 mg/kg) than ADR at the MTD (12.5 mg/kg). The time needed for recovery from myelosuppression caused by SM-5887 to the level of the untreated mice was 6 days at 1/2MTD (12.5 mg/kg) and 8 days at the MTD (25 mg/kg). In the case of ADR, on the other hand, the recovery from myelosuppression took 10 days at 1/2 MTD (6.25 mg/kg) and more than 11 days at the MTD (12.5 mg/kg). The data show that there was faster

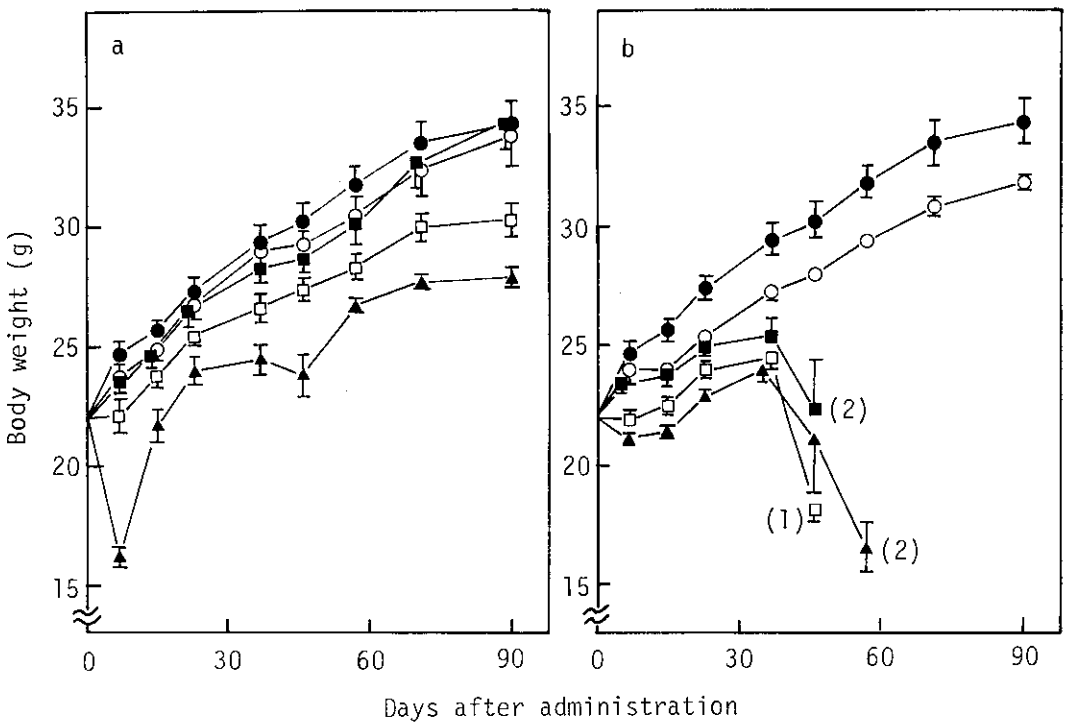
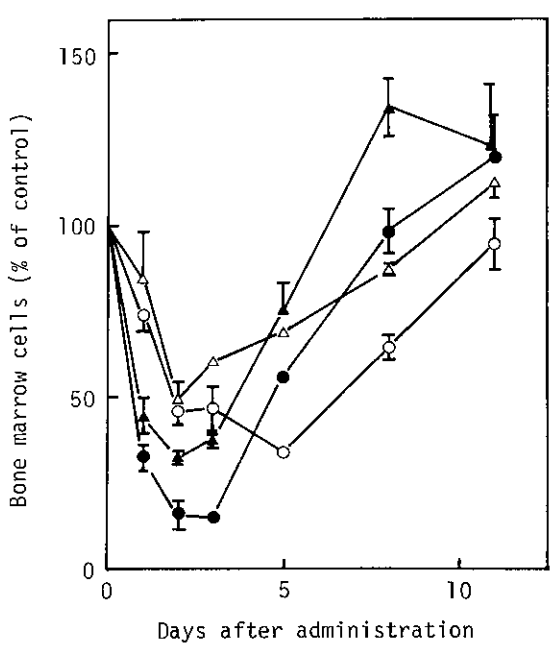


Fig. 3. Time-course of body weight for 90 days after single iv injection of SM-5887 (a) and ADR (b) in CDF1 mice. (a) Groups of 6 mice were untreated (●) or given SM-5887 at doses of 20 mg/kg (○), 25 mg/kg (■), 32 mg/kg (□), or 40 mg/kg (▲). (b) Groups of 6 mice were untreated (●) or given ADR at doses of 8 mg/kg (○), 10 mg/kg (■), 12.5 mg/kg (□), or 16 mg/kg (▲). Each point represents the mean±SE of 4 to 6 mice. Numbers in parentheses indicate the number of dead mice.



recovery from myelosuppression induced by SM-5887 than by ADR, although SM-5887 caused more severe myelosuppression than ADR.

Local tissue toxicity When the drugs were injected into mouse tail vein, local tissue injury was observed in the case of ADR but not with SM-5887. The local tissue toxicity of SM-5887 and ADR, therefore, was quantitatively evaluated by determining the degree of inflammation in mouse hind paw injected with drug solution. Figure 5 shows the time-course of the increase in paw thickness after drug injection of 100 μg/paw of SM-5887 and ADR. The initial inflammatory responses were seen 2 h after drug injection, but they decreased within 24 h. After that, the paw thickness increased again and reached the peak level 2 to 4 days later. The second peak was severe and more long-lasting than the initial peak.

Fig. 4. Time-course of the number of bone marrow cells after single iv injection of SM-5887 and ADR in BALB/c mice. Mice were treated with SM-5887 (▲, 12.5 mg/kg; ●, 25 mg/kg), or ADR (△, 6.25 mg/kg; ○, 12.5 mg/kg). Each point represents the mean±SE of 3 mice.

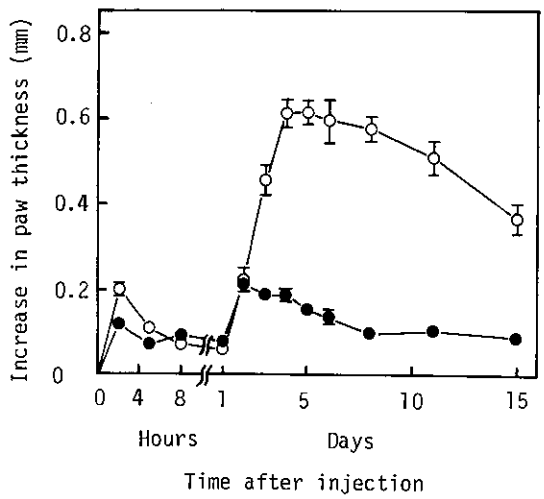


Fig. 5. Time-course of hind paw inflammation induced by SM-5887 and ADR. CDF1 mice were injected sc with 100 μg of SM-5887 (●) or ADR (○) in the plantar region of the hind paw. Each point represents the mean±SE of 9 or 10 mice.

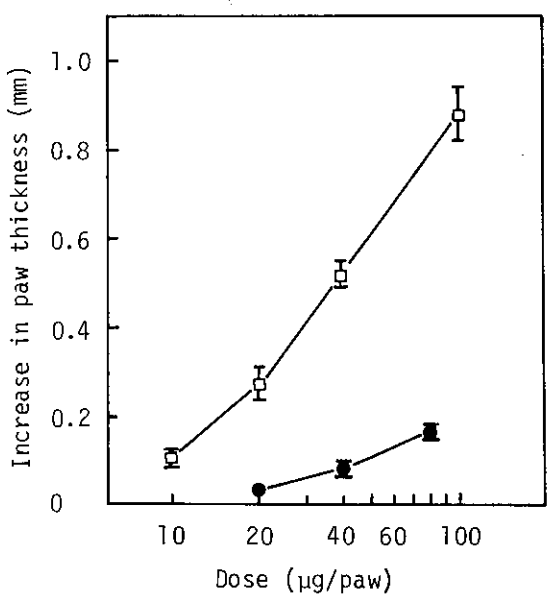


Fig. 6. Dose-response relationship for CDF1 mouse hind paw inflammation induced by sc injection of SM-5887 and ADR. CDF1 mice were injected sc with the indicated doses of SM-5887 (●) or ADR (□). Each point represents the mean±SE of 9 or 10 mice.

SM-5887 showed a much smaller secondary inflammatory response than ADR.

As shown in Fig. 6, second inflammatory responses were dose-dependently induced by SM-5887 and ADR. The degree of inflammation was lower even at 80 μg/paw of SM-5887 than at 20 μg/paw of ADR.

DISCUSSION

ADR is one of the most effective antitumor agents, but it has been found to have various side effects: myelosuppression, mucositis, alopecia, gastrointestinal toxicity, local tissue toxicity, and cardiotoxicity, for example. Cardiotoxicity at high cumulative doses is the most serious complication. SM-5887 has much less cardiotoxicity than ADR, at least in a rabbit chronic experimental model.[3] The data, therefore, suggest that the toxicological properties of SM-5887 are considerably different from those of ADR, despite the similarity in chemical structure. This suggestion was also supported by the present study, because these two drugs were remarkably different in terms of delayed-type lethal toxicity and local tissue toxicity.

ADR frequently caused deaths 15 to 90 days after a single iv administration at the dose of more than 10 mg/kg, which is less than the MTD of ADR (12.5 mg/kg), while SM-5887 almost never did. Similar delayed toxicity of ADR has also been reported by Corbett *et al.*,[5] although the frequency in the case of iv administration was much less than in our experiment. Probably, this is due to the fact that the delayed-type toxicity is caused more frequently by a single massive administration of ADR rather than by intermittent injections of divided doses as carried out by Corbett *et al.* The delayed-type lethal toxicity, therefore, is considered to be an intrinsic effect of ADR in mice. SM-5887 did not cause this type of toxicity even in mice given more than the MTD, and it has also been shown that SM-5887 was able to be repeatedly injected without cumulative toxic effects at the MTD at intervals of 10 days.[6] These data suggest that SM-5887 is a compound without substantial delayed-type lethal toxicity.

It has been reported that ADR induces severe local tissue necrosis in the area of injection upon accidental extravasation.[7-9] This is one of its most serious complications. In the present study, therefore, the local tissue toxicities of SM-5887 and ADR were compared quantitatively by determining the degree of inflammation in the mouse hind paw injected with drug solution. ADR induced a dose-dependent and long-lasting severe inflammatory reaction, whereas the inflammation caused by SM-5887 was milder even at a four-fold greater dosage than by ADR. Daunorubicin caused the same degree of inflammatory reaction as ADR, and aclarubicin at a two-fold dosage was almost equal to ADR in terms of toxicity, altough the data are not shown. The low local tissue toxicity of SM-5887, therefore, is a

unique and favorable feature different from other anthracyclines.

As described above, SM-5887 was quite different from ADR with regard to delayed-type toxicity and local tissue toxicity as well as cardiotoxicity in a rabbit chronic experimental model, as reported by Suzuki *et al.*[3] Nevertheless, the acute toxicities of SM-5887 and ADR, such as body weight decrease, ataxia, hair loss, and myelosuppression, were qualitatively very similar, and the myelotoxicity induced by SM-5887 was more severe than that by ADR, because SM-5887 caused more potent myelosuppression even at half the MTD than ADR at the MTD. The number of bone marrow cells, however, recovered more rapidly to the normal level in mice treated with SM-5887 than with ADR, when compared at the MTD. The data show that myelotoxicity of SM-5887 is more severe yet more reversible than that of ADR.

In conclusion, the acute toxicities of SM-5887 and ADR were qualitatively similar, but the toxic effects of SM-5887 were much less than those of ADR in terms of delayed-type toxicity and local tissue toxicity, as also reported for cardiotoxicity.[3] The data suggest that the toxic effects of SM-5887 are more reversible and more controllable than those of ADR.

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