The Effect of Tetrandrine and Extracts of Centella asiatica on Acute Radiation Dermatitis in Rats


Department of Radiation Oncology, Pathology, and Medical Research, Mackay Memorial Hospital, 10449 Taiwan and Department of Martial Art, Chinese Culture University, 11114 Taiwan, Republic of China.

Received October 19, 1998; accepted April 12, 1999

Radiation injury to the skin is one of the major limiting factors in radiotherapy. We designed this study using Sprague-Dawley rats to evaluate the reduction in skin injury achieved using natural products from plant extracts as protection. The acute skin reaction in tetrandrine- and Madecassol-treated animals appeared earlier, but was significantly less severe, than in the control group. The peak skin reactions in the tetrandrine group were less serious than those of the control group at three different radiation doses. At a high dose irradiation, the healing effect of tetrandrine is better than Madecassol and vaseline. The histologic findings indicate that tetrandrine and Madecassol are able to reduce acute radiation reactions by their anti-inflammatory activity.

Key words acute radiation dermatitis; tetrandrine; Centella asiatica

Radiation injury to the skin is one of the most common adverse effects of radiation therapy. The acute skin reaction, involving swelling, desquamation and ulceration, usually causes significant pain, thereby becoming a limiting factor for radiation therapy. Topical application of steroidal or non-steroidal anti-inflammatory agents is the most common treatment for radiation injury of the skin, yet the results are unsatisfactory and local toxicity has been noted. Several experimental and clinical studies have suggested that extracts from plants can reduce radiation injury of the skin. Tetrandrine, a bisbenzylisoquinoline alkaloid isolated from the dried root of Stephania tetrandra S. Moore, possesses a remarkable pharmacological profile. It has been shown to inhibit proliferation and induce apoptosis in human leukemic U937 cells, protect normal human mononuclear cells against ionizing irradiation and inhibit production of inflammatory mediators, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α and nitric oxide, by monocytes/macrophages and lymphocytes. The antitumor potential of tetrandrine has been further confirmed by inducing apoptosis in human malignant glioma U138MG cells and producing a radiosensitizing effect on both leukemic and glioma cells (data not shown).

Extracts of Centella asiatica (Linn.) exhibit various pharmacological effects including A) inhibition of gastric ulceration, B) improving capillary permeability in patients with venous hypertension, C) stimulating collagen synthesis in fibroblast cultures, D) reducing the healing time after repeated surgical excision in rats, E) a therapeutic effect in hepatic fibrosis, and F) preventing hypertrrophy of burn and postoperative scars. Centella extracts have been marketed for many years under various trade names, including Madecassol. Because it exhibits both antitumor activity and a protective effect against radiation injury, tetrandrine may be an effective agent for use with radiation therapy. Therefore, we designed this study to assess whether or not tetrandrine can reduce acute radiation reactions in rat skin. In a pilot study, we found that Madecassol reduced the healing time after radiation-induced skin injury and, therefore, we also included it in the present study.

MATERIALS AND METHODS

Rats and Anesthesia Adult male Sprague-Dawley (S-D) rats, purchased from the animal center of the National Science Council of Taiwan, weighing 350—400 g at the time of irradiation, were used in these experiments. Each rat was caged alone and allowed chow and water ad libitum. They were anesthetized with pentobarbital (Nembutal) 50 mg/kg i.p. before irradiation. The skin over the gluteal area was shaved completely and radiation fields were outlined with a marking pen just prior to irradiation. There were three groups of 5 animals each, one group to be treated with tetrandrine, one with Madecassol, and the third with vaseline as a control. The gross skin reactions were evaluated in all rats and only 3 rats in each group underwent histological examination.

Drugs and Treatment Tetrandrine (C$_{32}$H$_{47}$O$_{8}$N$_{3}$) was purchased from Aldrich Co. (Milwaukee, WI, U.S.A.). The powder was dissolved in vaseline at 60°C and then cooled to room temperature to obtain a gel containing 0.1% tetrandrine.

Madecassol ointment was a gift of the manufacturer (Laboratories Roche Nicholas S.A., Gaillard, France). It contains three constituents of Centella extracts: madecassic acid, asiatic acid and asiaticoside. The ointment base was vaseline and the total concentration of these constituents altogether was 1%.

Tetrandrine gel, Madecassol ointment and vaseline were applied topically to the irradiated area every day after radiation. The mean dosages per day in the respective groups were 1.6 mg tetrandrine per cm$^2$ skin, 16.0 mg Madecassol constituents per cm$^2$ skin, and an equivalent amount of vaseline for the control group.

Electron Beam Irradiation The gluteal area prepared for radiation was divided into three sections, each receiving a different radiation dose (20, 40 and 80 Gy). An electron beam with 6 MeV energy produced by a linear accelerator (Clinac® 1800, Varian Associates, Inc., CA, U.S.A.) was
used. The dose was delivered on day 0 at 4 Gy/min to the prepared area after the rats were anesthetized.

**Gross Evaluation of Skin Reactions** Acute skin reactions were evaluated and scored every other day until the 30th day after irradiation using the modified skin score system proposed by Abe et al., as follows: 0=normal, 0.5=slight epilation, 1.0=epilation in about 50% of the radiated area, 1.5=epilation in greater than 50% of the area, 2.0=complete epilation, 2.5=complete epilation with definite edema or dry desquamation in more than 50% of the area, 3.0=moist desquamation in a small area, 3.5=moist desquamation in most of the area.

The skin reaction was scored three times a week by a radiation oncologist and a pathologist, respectively.

**Histologic Evaluation of Skin Reactions** Skin samples were taken after sacrificing the rats on day 30. Each specimen was embedded in a paraffin block and thin sections were prepared, stained by the hematoxylin–eosin method and examined by a pathologist under a light microscope (400×).

The microscopic findings involving the skin reactions were categorized into 3 grades as follows: mild, moderate and severe. The mild reaction was characterized by minimal dermal fibrosis, swelling of the endothelium and mild atrophy of the epidermis. Moderate dermal fibrosis with radiation atypia was classified as a moderate reaction. A severe reaction included the following findings: advanced dermal fibrosis with radiation atypia, advanced atrophy of skin appendages, ulceration and necrosis with bullae formation of the epidermis near the ulcer.

**Statistical Analysis** The means of each group were analyzed by a two-way analysis of variance (ANOVA) with repeated measurement. We performed our statistical analysis using a SAS/STAT program (SAS Institute Inc., NC, U.S.A.). A p<0.05 was considered as being significant.

**RESULTS**

The drugs were well tolerated without any toxic effects. All the rats gained weight during the period of observation and the average weights in the control, tetranehrine and Madecassol groups did not differ significantly at the end of the study (Table 1).

The average skin reaction scores in each group are shown in Figs. 1—3. The skin reactions in the tetranehrine and Madecassol groups tended to be less marked than those in the control group at all three irradiation dosages.

As illustrated in the Figs. 1—3, the tetranehrine group exhibited greater skin reaction than the controls early on (day 11 at 20 Gy, day 11 at 40 and 80 Gy). However, within 10 days, the scores in the control group were higher. The scores in the Madecassol group exhibited a similar, though less pronounced, early increase compared with controls. By day 21, the dermatitis in the control group treated with 20 Gy had progressed to complete epilation whereas, in the tetranehrine and Madecassol groups, healing had begun (Fig. 1).

Toward the latter part of the study there were significant differences between the treated and control groups (day 19 at 40 Gy in the tetranehrine group, day 23 at 40 Gy in the Madecassol group, day 21 at 80 Gy in the tetranehrine group and day 25 at 80 Gy in the Madecassol group). Thereafter, the skin reactions in the control group progressed to moist desquamation in most areas, but in both treated groups it continually improved (Figs. 2, 3).

Madecassol seemed to reduce early skin reactions (day 13—15) better than tetranehrine at all 3 radiation dosages. However, this difference became insignificant by day 15 at 20 and 40 Gy (Figs. 1, 2). At 80 Gy, the therapeutic effect of tetranehrine was significantly better than Madecassol by day 25 after irradiation (Fig. 3).

As demonstrated in Fig. 4, the peak skin scores of rats

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**Table 1. Weight of S-D Rats at Day 0 and Day 30 after Irradiation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at day 0 (g)</th>
<th>Weight at day 30 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>382.6±6.0</td>
<td>443.0±6.8</td>
</tr>
<tr>
<td>Tetranehrine group</td>
<td>388.2±5.2</td>
<td>453.4±11.1</td>
</tr>
<tr>
<td>Madecassol group</td>
<td>384.0±5.3</td>
<td>448.0±6.0</td>
</tr>
<tr>
<td>p value</td>
<td>0.59</td>
<td>0.44</td>
</tr>
</tbody>
</table>

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**Fig. 1. Time-Course of the Average Skin Score after 20 Gy Irradiation**

Error bars indicate ±S.E. * p<0.05, ** p<0.01 in comparison with tetranehrine and control groups. * p<0.05, ** p<0.01 in comparison with Madecassol and control groups. * p<0.05, ** p<0.01 in comparison with tetranehrine and Madecassol groups.

**Fig. 2. Time-Course of the Average Skin Score after 40 Gy Irradiation**

Error bars indicate ±S.E. * p<0.05, ** p<0.01 in comparison with tetranehrine and control groups. * p<0.05, ** p<0.01 in comparison with Madecassol and control groups. * p<0.05, ** p<0.01 in comparison with tetranehrine and Madecassol groups.
treated with tetrandrine were significantly lower than the control group at all radiation doses. The peak skin scores in the Madecassol group tended to be lower than control group, but the difference did not reach statistical significance. There was no difference in peak skin scores between the 2 drug groups.

There are apparent changes in the histologic findings between the control and drug-treated groups. In the control group, 20 and 40 Gy radiation results in mainly moderate and severe skin reactions, respectively. Moreover, all the rats whose skin received 80 Gy radiation exhibited severe reactions in the same group. In contrast, 20 Gy radiation only produced mild changes in both tetrandrine- and Madecassol-treated rats. Skin reactions after 40 Gy radiation were mainly mild and only one rat exhibited a moderate reaction. Although 80 Gy radiation resulted in severe reactions in the drug-treated groups, but the reactions were still less prominent than those in the control group receiving the same dose of radiation (Table 2).

**DISCUSSION**

Radiation injury to the skin is mainly caused by cell damage secondary to the production of free radicals, release of inflammatory mediators/cytokines and the subsequent inflammatory reaction. The mechanism of radiation dermatitis is likely to be a multi-step process involving unspecified cellular and humoral events.\(^{15,16}\)

Eicosanoid synthesis is one part of the inflammatory process and glucocorticoids can interfere with this pathway by inhibiting phospholipase A\(_2\).\(^{17,18}\) However, the effect of steroids on radiation dermatitis remains controversial as shown by previous clinical trials. Gloces et al. suggested that neither hydrocortisone nor clobetasone creams should be used as a treatment of choice to control radiation dermatitis,\(^{9}\) and Potera et al. found that topical cortisone cream had no prophylactic effects to radiation dermatitis.\(^{20}\)

Vitamin C, as an anti-oxidant, also has significant ability to prevent radiation dermatitis.\(^{21}\) Rather than focusing on agents acting only on a few steps in the inflammatory reaction, attempts to find effective anti-inflammatory agents to treat radiation dermatitis should focus on drugs with a broad anti-inflammatory profile.

![Fig. 3. Time-Course of the Average Skin Score after 80 Gy Irradiation](image)

Error bars indicate S.E. \(p<0.05\), \(p<0.001\) in comparison with tetrandrine and control groups. \(p<0.05\), \(p<0.001\) in comparison with Madecassol and control groups.

![Fig. 4. Dose-Dependent Curves for Peak Skin Reaction](image)

Error bars indicate S.E. \(p<0.05\) in comparison with tetrandrine and control groups.

**Table 2. Histologic Findings in Irradiated Rat Skin**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Control 20 Gy</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Control 40 Gy</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Control 80 Gy</td>
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</tr>
<tr>
<td>Madecassol 20 Gy</td>
<td>++</td>
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<tr>
<td>Madecassol 40 Gy</td>
<td>++</td>
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<td>Madecassol 80 Gy</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tetrandrine 20 Gy</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Tetrandrine 40 Gy</td>
<td>++</td>
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</tr>
<tr>
<td>Tetrandrine 80 Gy</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The histologic findings on skin specimens from 3 irradiated rats in each group are represented as follows: A, minimal dermal fibrosis; B, swelling of endothelium; C, mild atrophy of epidermis; D, dermal fibrosis with radiation atypia, moderate; E, dermal fibrosis with radiation atypia, advanced; F, advanced atrophy of skin appendages; G, ulceration; H, necrosis and bullae formation of the epidermis near the ulcer. The "++, +++" means specimens from one, two or three rats showed the reaction as indicated above.
Tetrandrine possesses a remarkable anti-inflammatory profile. It has been shown to suppress phosphoinositide turnover in human mononuclear cells, inhibit production of IL-1, TNF-α by monocytes/macrophages, inhibit both oxygen consumption and superoxide release by activated alveolar macrophages, protect against radiation-induced mononuclear cell injury accompanied with suppression of superoxide production and inhibit nitric oxide production in activated mice macrophages. The therapeutic effect of tetrandrine that we found in radiation dermatitis may be due to this range of anti-inflammatory activities.

The potential effect of Madecassol on radiation dermatitis may be mediated by gradually converting scars from the inflammatory phase to the maturation phase as reported in the literature.

The histologic findings demonstrated that tetrandrine and Madecassol can reduce inflammation in irradiated rat skin. In general, the severe reactions in the control group occurred at a lower dose and were more prominent than in the tetrandrine and Madecassol groups. For example, necrosis and bullae formation of the epidermis near the ulcer was observed in the control group irradiated with 40 and 80 Gy whereas the tetrandrine and Madecassol groups exhibited no such effects. A radiation dose-dependent effect was obvious in all 3 groups. These histologic findings in the tetrandrine- and Madecassol-treated rats implies that their effects in reducing acute radiation dermatitis may be via anti-inflammatory processes. Considering these histologic findings and previous pharmacological reports on tetrandrine and Madecassol, it appears that the anti-inflammatory actions of these compounds may be produced by inhibiting the production of radiation-induced free radicals and the release of inflammatory mediators from mononuclear cells. However, the mechanism involving the earlier damage-promoting effects of these compounds is unknown. It may due to some type of chemical interaction between these compounds, radiation and radiation-induced free radicals which occur somewhat earlier in skin injury.

Effective drugs for radiation dermatitis described in previous reports have no antitumor activity at all. Tetrandrine can induce apoptosis and inhibit clonogenicity in leukemic U937 cells, and 
Centella extractscan inhibit the proliferation of transformed cell lines and retard the development of solid and ascites tumors. These 2 agents could be of benefit in clinical oncology.

To treat clinical radiation dermatitis, topical drugs should be applied cautiously to avoid establishing a "build-up" region by scattering rays, an effect which may increase the skin dose.

The doses we used (1.6 mg/cm²/d tetrandrine, 16.0 mg/cm²/d 
Centella extractswere effective in treating radiation dermatitis, but the optimal dose remains to be determined in further experiments. We are currently conducting other studies with tetrandrine focusing on its radioprotecting effect, reversal of drug resistance and prevention of radiation pneumonitis. In conclusion, acute radiation dermatitis developed earlier following both tetrandrine and Madecassol treatment, but it was less severe and repair began earlier and was more marked than in the control group. At high dose irradiation, the healing effect of tetrandrine is better than Madecassol and baseline.

Acknowledgments This study was supported by grant MMH-8710 from the Mackay Memorial Hospital.

REFERENCES