Safety of Systemic Acridine Orange Administration with Low-dose Radiation in Mice

Takuya Maruo[1]

Tetsushi Tezuka[2]

Haruyuki Kondo[1]

Shiori Nakamura[1]

Kensuke Orito[3]

Takuo Shida[1,3]

Tomohiro Nakayama[4]

1) Veterinary Teaching Hospital, Azabu University 1-17-71, Fuchinobe, Chuo, Sagamihara, Kanagawa 229-8501, Japan
2) Takashimadaira Tezuka Animal Hospital, 1-35-11, Takashimadaira, Itabashi, Tokyo 175-0082, Japan
3) Veterinary Medicine, Azabu University 1-17-71, Fuchinobe, Chuo, Sagamihara, Kanagawa 229-8501, Japan
4) Laboratory of Veterinary Radiology, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, 1866, Kameino, Fujisawa, Kanagawa 252-0813, Japan

Corresponding author: Tomohiro NAKAYAMA
College of Bioresource Science, Nihon University, 1866, Kameino, Fujisawa, Kanagawa 252-0813, Japan
Tel: 0466-84-3422
Fax: 0466-84-3422
E-mail: nakayama.tomohiro80@nihon-u.ac.jp

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ABSTRACT

Acridine orange (AO) is a known radiation sensitizer, and concomitant local administration of AO and irradiation (5 Gy) after cytoreductive surgery results in good local control of musculoskeletal sarcomas in humans. Local administration of AO solution into the surgical field can act directly on residual tumor cells, and it is thought that systemic administration of AO may affect tumor cells invading neighboring tissues. However, the toxicity of AO and concomitant X-ray irradiation is not well described. The purpose of the present study was to evaluate the safety of intravenous (IV) administration of AO (1 mg/kg) and simultaneous radiotherapy in mice.

To this end, mice were randomly assigned to the following 3 groups (n = 6 in each group): radiotherapy alone (RT alone), IV AO alone (AO alone), and AO and radiotherapy (AO-RT). AO (1 mg/kg) was administered to the AO alone group and the AO-RT group. The mice in the RT alone and AO-RT groups were irradiated with 5 Gy 15 min after injection. The mice were euthanized 72 h after the experiment, and histopathological examinations of the duodenum,

jejunum, and ileum were performed.

Reductions in crypt proliferative zone and cell division were observed most frequently in the AO alone group, followed by the RT alone and AO-RT groups. Increased apoptotic bodies in crypts and villous atrophy were seen most frequently in the RT alone group, followed by the AO alone and AO-RT groups. Similar results were observed in the jejunum and ileum. However, no significant differences were detected except for reduction of the proliferative zone in the duodenal crypt between the AO-RT and AO alone groups.

Histopathological findings of the AO-RT group were not significantly different from those of the RT alone and AO alone groups. Concomitant administration of IV AO (1 mg/kg) and X-ray irradiation appears to be safe for mice.

INTRODUCTION

Acridine orange (AO) was first extracted from coal tar as a weak basic dye over 100 years ago.[1] Several acridine compounds such as acridine, proflavine, quinacrine, and AO exert photodynamic DNA strand-breaking activity in yeast.[2] Photosensitizing proflavine and AO have a photobactericidal effect in several pathogenic organisms,[3] and AO photodynamic therapy is effective against mouse osteosarcoma cells in vitro.[4]

Almost all solid tumors produce energy via glycolysis and lactic acid fermentation, which perpetuates an acidic environment.[5-7] In a model of osteosarcoma, AO selectively accumulates in tumor tissue due to reversed pH gradients.[1] AO has the ability to rapidly and specifically accumulate in malignant tumors.[8]

AO combined with low-dose X-ray irradiation of 1–5 Gy has a strong cytocidal effect on cultured mouse osteosarcoma cells (radiodynamic therapy with AO, AO-RDT).9 Kusuzaki et al. (2005) employed local administration of AO solution after intralesional or partially marginal tumor excision, and AO photodynamic and radiodynamic therapy resulted in good local control of musculoskeletal sarcomas in humans.[10] Locally administered AO solution into the surgical field can act directly on residual surface tumor cells, but there is insufficient penetration due to low permeability. Thus, it was hypothesized that systemic administration of AO may be useful for preventing tumor cell invasion into neighboring tissues. However, the toxicity of concomitant AO and X-ray irradiation is not sufficiently known.

It was thought that the effect of AO-RDT on the skin was minimal because the irradiation dose was only 5 Gy. Therefore, we elected to examine the small intestine due to its high radiosensitivity and rapid cell division. The purpose of this study was to evaluate the safety of acute intravenous (IV) AO administration (1 mg/kg) and concomitant radiotherapy (AO-RT) in mice.

MATERIALS AND METHODS

Healthy Slc:ICR mice (4-week-old males) were obtained from Japan SLC, Inc. (Shizuoka, Japan). All procedures were performed in compliance with the guidelines of the Animal Research Committee of Azabu University (No. 101119-2). Two weeks later, the 6-week-old mice weighed 22–27 g and were randomly assigned to one of 3 groups (RT alone, IV AO alone, and AO-RT), and each group included 6 mice.

AO solution (0.1 mg/ml) was made by mixing AO (Merck Ltd., Japan, Tokyo) with saline, and the resulting solution was sterilized by microfiltration using a membrane filter (25AS020AS, Advantex MFS, Inc., Dublin, CA, USA). AO (1 mg/kg) was administered into the caudal vein in both the AO alone and AO-RT groups. Saline (10 ml/kg) was administered into the caudal vein in the RT alone group, and RT was performed 15 minutes after injection.

Each mouse was placed in a plastic container (15 cm × 15 cm), and 6-MV linear accelerator (Primus: Toshiba Medical Systems, Tokyo, Japan) was used. Boluses (15 cm × 15 cm × 1 cm, homogenous tissue-equivalent gel with a density of 1.03 g/cc, CIVCO Medical Solutions, Kalona, IA, USA) were

Table 1: *Histopathological findings of the duodenum, jejunum, and ileum*

			RT					AO						AO-RT		
Duodenum	0	1	2	3	4		0	1	2	3	4		0	1	2	3	4
Villous atrophy	2	1	0	2	1		2	1	3	0	0		4	0	1	1	0
Reduction of goblet cells	0	0	2	4	0		0	1	0	5	0		0	0	5	1	0
Reduction of crypt proliferative zones	2	1	0	0	3		0	0	1	3	2*		4	0	2	0	0*
Reduction of cell division in crypts	2	0	3	1	0		1	0	0	5	0		5	1	0	0	0
Increased apoptotic bodies in crypts	3	0	2	0	1		3	0	3	0	0		4	0	1	1	0
Jejunum																	
Villous atrophy	1	1	2	0	2		1	1	0	1	3		2	0	0	4	0
Reduction of goblet cells	0	0	4	2	0		0	1	3	2	0		0	1	5	0	0
Reduction of crypt proliferative zones	1	2	0	2	1		0	0	0	3	3		6	0	0	0	0
Reduction of cell division in crypts	1	0	2	0	3		0	0	0	5	1		6	0	0	0	0
Increased apoptotic bodies in crypts	1	0	5	0	0		4	0	2	0	0		5	0	1	0	0
Ileum																	
Villous atrophy	0	2	1	3	0		0	1	0	3	2		0	0	2	4	0
Reduction of goblet cells	1	0	4	1	0		1	0	5	0	0		1	2	3	0	0
Reduction of crypt proliferative zones	2	0	1	3	1		1	0	0	1	4		4	0	2	0	0
Reduction of cell division in crypts	2	0	0	3	1		1	0	0	2	3		6	0	0	0	0
Increased apoptotic bodies in crypts	3	0	2	1	0		6	0	0	0	0		4	0	2	0	0

Lesions were classified into 5 grades: normal (0), extremely mild (1), mild (2), moderate (3), and severe (4)
RT, radiotherapy alone; AO, acridine orange alone; AO-RT, concomitant acridine orange and radiotherapy
**: In the duodenum, reduction of the proliferative zone in crypts was significantly different (p= 0.049) only between the AO-RT and AO groups.*

set at the upper and lower sides of the plastic container, and the mice were irradiated in a 5-Gy opposing portal field.

The mice were euthanized with intraperitoneal pentobarbital sodium injections (50 mg/kg, Somnopentyl, Kyoritsu Seiyaku Co. Ltd., Tokyo, Japan) 72 hours after the experiment. The duodenum, jejunum, and ileum were collected and analyzed.

Histopathological analysis of the duodenum, jejunum, and ileum revealed the following: villous atrophy; reduction of goblet cells, crypt proliferative zones, and crypt cell division; and increased apoptotic bodies in the crypts. The lesions were classified into 5 grades: normal (0), extremely mild (1), mild (2), moderate (3), and severe (4).

Mann–Whitney U-tests were used to compare findings in the duodenum, jejunum, and ileum. Bonferroni correction for multiple comparisons was also performed. The level of significance was set at $p < 0.05$.

RESULTS

None of the mice showed any signs of

clinical illness during the experiment. The histopathological findings are summarized in Table 1. In the crypts, reductions in the proliferative zone and cell division were seen most frequently in the AO alone group, followed by RT alone and AO-RT. Increased apoptotic bodies in the crypts and villous atrophy were most common in the RT alone group, followed by AO alone and AO-RT. Similar results were observed in the jejunum and ileum. However, villous atrophy was similar in the jejunum and ileum among all 3 groups. Moreover, reductions of the proliferative zone and cell division and increased apoptotic bodies were reduced in the jejunum and ileum of the AO-RT group compared to the AO only and RT only groups. However, the only statistically significant difference ($p = 0.049$) was observed for duodenal crypt proliferative zone loss, which was lower in the AO-RT animals compared to the AO group.

DISCUSSION

Here, we demonstrate that acute IV AO (1 mg/kg) and concomitant radiotherapy (5 Gy) is safe. None of the animals showed evidence of adverse effects over the 3 days after treatment. The histopathological findings in the AO-RT group were not greater than those in the RT alone and AO alone groups.

Although AO was originally considered to have a radiation sensitizer effect, but the concomitant AO-RT group did not show any significant deterioration compared to the other 2 groups. In fact, it is possible that AO has a radioprotective effect. Maenhaut-Michel (1975) reported that proflavine, an acridine derivative, was radioprotective against the indirect effects of γ-irradiated bacteriophage λ.[11] The conclusion was that these effects were due to the extensive scavenging of radio-induced water radicals within the medium.[11] This may explain why the AO-RT group did not develop more severe lesions than the other 2 groups.

The toxicity of AO appears to be mild. The International Agency for Research on Cancer (IARC) of the World Health Organization reported that it could not be classified as carcinogenic (class 3).[12] Some authors reported that AO solution has been applied to surgical sites in humans without any associated toxicity.[10, 13] Oral (PO) AO was safely administered at 500 mg PO in humans, and the only side effects were mild gastrointestinal symptoms (nausea in 3 cases and vomiting in 1 case out of 35 patients total).14 It was possible to administer 15 mg PO daily for 4 days in tumor-bearing mice.15 Furthermore, the median lethal dose of IV AO was reported to be 27.3 mg/kg in mice.[8] Previous studies have employed a 1 mg/kg dose.[16, 17] In the present study, 1 mg/kg AO was given IV without any adverse events.

Hashiguchi et al. (2002) suggested that AO might be excited by X-rays and kill osteosarcoma cells by releasing cytotoxic singlet oxygen.[9] However, the histopathological findings in the AO–RT group were not significantly different than those of the RT alone and AO alone groups. The results do not support the hypothesis that AO has a radiation sensitizer effect, but they do suggest that acute AO–RT is safe in mice.

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