

Clinical study of recombinant adenovirus-p53 (Adp53) combined with hyperthermia in advanced cancer(a report of 15 cases)

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Abstract The study was to evaluate the efficacy of the Adp53 combined with hyperthermia on advanced cancer. Fifteen patients with advanced cancer were enrolled in this clinical trial. Thirteen patients with recurrent tumours failed in conventional treatments and the two other patients with primary tumour received no treatment before they were enrolled. Recombinant adenovirus-p53 (Adp53) is a E1 substituted replication-incompetent recombinant adenovirus encoding the human wild-type p53 (wtp53) gene. The 15 patients were intra-tumourally injected with Adp53, 1×10^{12} vp (virus particle) once a week, with a total of 4~8 times was given. The temperature being set hyperthermia every week 3 days after the injection of Adp53 at 43~44°C using 915MHz microwave machine for superficial tumour for 1 h or at 42~43°C using 41MHz radiofrequency machine for deep-seated tumour for 1 h. Among the 15 patients, five concurrently were added with radiotherapy and three were added with cisplatin-based chemotherapy. The treatment achieved CR in two cases, PR in four cases, SD in eight cases and PD in one case and, after the treatment, tumours of two cases disappeared and seven of the other 13 cases (54%) had low-density area (LDA) of more than 50% on CT images in tumours. In the 15 patients, no dose-limiting toxicity and adverse events were noted, except transient fever after Adp53 administration. In conclusion, Adp53 combined with hyperthermia was safe and effective in patients with advanced cancer and p53 gene therapy was potential to thermosensitize in advanced cancer.

Keywords: Advanced cancer, hyperthermia, recombinant adenovirus, p53

Introduction

p53 gene is a well understood structure and the strongest functional tumour suppresser gene. Wild-type p53 (wtp53) gene plays a key role on cell cycle arrest and apoptosis, especially for cells stressed by irradiation, DNA damage agents or hyperthermia (HT) and inhibits proliferation of tumour cells. Hyperthermia suppresses the proliferation of tumour cells via a way of inducing cell cycle arrest and apoptosis. Wtp53 promotes heat-inducing cell cycle arrest and apoptosis of tumour cells; therefore, it enhances intrinsic thermosensitivity of tumour cells. While p53

mutation abrogates these responses and increases resistance to heat stress [1–3]. A previous study proved that wtp53 mediated by adenovirus transfers into the two human gastric carcinoma cell lines with different p53 status, could express p53 protein in cell's nuclear, increase heat-inducing G2/M arrest and apoptosis and increase thermosensitivity in vitro [4]. Another study proved that recombinant adenovirus-p53 (Adp53) promotes heat-inducing apoptosis in a nasopharyngeal carcinoma cell line [5]. p53 gene mutation occurring in more than 50% of all human tumours induces refractory to conventional treatment including hyperthermia. Thus, Adp53 transfection restoring wtp53 gene into tumour cell becomes a new strategy in cancer gene therapy combining with radiotherapy (RT) or chemotherapy [6, 7]. The present study is a clinical study on Adp53 combined with hyperthermia conducted at the Beijing Cancer Hospital.

Patients and method

The study was a non-randomized and non-controlled clinical trial of Adp53 combined with hyperthermia. The protocol was approved by the institutional review board (IRB) of SFDA China (SFDA China project #2000-54). The study was conducted according to the Declaration of Helsinki (amended version, Hong Kong, 1989). Patients all signed the informed consent.

Patients enrolled in this clinical trial were histologically diagnosed as having advanced cancer and measurable disease and had no distant metastasis. Age ranged from 18–80 years. Pregnant or nursing women, patients with uncontrolled serious infections or with serious heart and lung failure were excluded. The presence of p53 mutation in the tumour was not a requirement for study entry. Patients must have a projected life expectancy of at least 3 months and a Karnofsky performance score of at least 60. Patients were required to have adequate bone marrow function (WBC count $\geq 3.0 \times 10^9 L^{-1}$, haemoglobin $\geq 7g L^{-1}$, platelet count $\geq 70 \times 10^9 L^{-1}$) and adequate liver and renal function (AST, ALT, BUN and Cr < 1.5 times the upper limit of normal). An interval of at least 4 weeks after the previous chemotherapy, radiotherapy or major surgery was mandatory.

Between October 2001–June 2004, 15 patients (11 males, four females) with advanced cancer confirmed by patho-histological examination were enrolled in this clinical trial. The mean age was 58 years (range 36–73). Among the 15 patients, three had liposarcoma, one had malignant neurinoma, one thyroid adenocarcinoma, one ovarian adenocarcinoma, one cheek mucous adenocarcinoma, one lung squamous cell carcinoma, one skin squamous cell carcinoma, three nasopharyngeal squamous cell carcinoma, one hypopharyngeal squamous cell carcinoma and two laryngeal squamous cell carcinoma.

Adp53 is a E1 substituted replication-incompetent recombinant adenovirus encoding wtp53 gene. Adp53 was supplied by ShenZhen Sibiono Genetech Co. Ltd,

Beijing, PR China and stored at -20°C at concentrations of 1×10^{12} vp ml⁻¹ (vp: Virus particle). Adp53 solution was thawed and diluted moderately in normal-saline according to tumour size.

All patients were intra-tumourally injected with Adp53 1×10^{12} vp once a week, with a total of 4~8 times. Adp53 was injected weekly 3 days before hyperthermia at $43 \sim 44^{\circ}\text{C}$ using a 915MHz microwave machine for superficial tumour for 1 h or at $42 \sim 43^{\circ}\text{C}$ using a 41MHz radiofrequency machine for deep-seated tumour for 1 h. Body surface temperature was maintained at $43 \sim 44^{\circ}\text{C}$ which was confirmed by a thermo-sensor during hyperthermia for superficial tumours. And esophagus or rectum temperature was maintained at $42 \sim 43^{\circ}\text{C}$, which was confirmed by a thermo-sensor during hyperthermia for deep-seated tumours. The oesophagus temperature represented that of the thorax tumour and the rectum temperature represented that of the abdominal tumour.

Five patients concurrently were added with radiotherapy using the 6 or 8 MV linear accelerator X-ray with the conventional fractionation 2 Gy once a day from Monday–Friday to a total dose 50–70 Gy/25–35 f/5–8 w. Another three patients were added with cisplatin-based chemotherapy treated with intravenous administration of cisplatin 50mg/5-fluorouracil 1.25 g once a week for 3~8 times (Table I).

Two orthogonal diameters A and B of each tumour were measured according to CT scan of tumour. The tumour size was calculated by the formula, $A \times B$. Tumour shrinkage rate of Adp53-injected tumours after treatment was counted by comparison to pre-treatment tumour size. And the tumour shrinkage rate was used to evaluate complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) according to WHO's evaluation standard of solid tumour treatment. Low-density area (LDA) on CT images which appeared and expanded within the tumour after hyperthermia consisted of coagulation necrosis histologically. A greater extent of LDA indicated the improvement of local control and survival of patients. So LDA's percentage area in the tumour may be an index for the prognosis of a patient treated by hyperthermia [8, 9].

Results

After treatment by Adp53-injection combined with hyperthermia, the patients achieved CR in two cases, PR in four cases, SD in eight cases (tumour shrinkage rate was 43%, 40%, 28%, 24%, 15%, 3%, 0% and 0%, respectively) and PD in one case. After the treatment, tumours of two cases disappeared and seven of the other 13 cases (54%) had LDA of more than 50% in tumours (Table I). The 15 patients with advanced cancer received multiple intra-tumoural injection of Adp53. No dose-limiting toxicity and adverse events were noted, except transient fever after Adp53 administration, which was the most common finding. Most fever events occurred within a few hours after the injection. All events were grade 1 (less than 38

°C) and grade 2 (range 38~40°C) self-limited fever. Data showed that the 15 patients all kept normal status in the blood, urine and stool examination, liver and renal function and lung and heart function before and after the treatment.

Discussion

The p53 gene is one of the factors determining cellular thermosensitivity and wtp53 contributes to thermosensitization resulting in enhancement of heat-induced cell cycle arrest and apoptosis [1–3]. About 50% of all human tumours contain p53 mutation. A high incidence of local-regional failure and distant metastasis contributes to the poor overall survival rate of ~40% for patients with cancer. Thus, replacement of wtp53 gene into tumour cells which have p53 abnormality becomes a new strategy in cancer gene therapy [6, 7].

From October 2001–May 2003, a multi-centre randomized controlled clinical trial (phaseII/III) was completed of head and neck squamous carcinoma treated by Adp53 combined with radiation and it was proved that the complete response rate of the gene therapy combined with radiotherapy group at 2 months after treatment was 1.68 times higher than that of the radiotherapy alone group [10]. The Adp53 was approved to market by FDA China on 16 October 2003. Meanwhile, this clinical study was also conducted on Adp53 combined with hyperthermia at Beijing Cancer Hospital.

Table I. Characteristics of the 15 patients and tumours after treatment of p53 gene-hyperthermia.

Patient no	Sex	Age (years)	Histology ^a	Injected tumour site	Baseline tumour (cm ²)	Adp53 dose (vp)	Hyper ^b (time)	Chemo ^c (time)	RT ^d (dose)	Tumour shrinkage rate (%)	LDA's area (%) ^e
1	F	69	Thyroid adeno	Front neck	5.0 × 4.5	8 × 10 ¹²	8 × H ^f	No	No	50	<50
2	F	68	Lipo	Post-peri ^f	6.3 × 4.6	8 × 10 ¹²	7 × H ^d	No	No	40	>50
3	M	36	NPC squam	Right neck	7.0 × 3.8	6 × 10 ¹²	8 × H ^f	No	No	28	>50
4	M	55	Cheek adeno	Back neck	9.4 × 8.7	10 × 10 ¹²	18 × H ^f	No	No	3	>70
5	F	69	Lipo	Abdo-wall ^g	9.5 × 6.0	8 × 10 ¹²	8 × H ^f	No	No	24	<50
6*	M	45	NPC squam	Nasop harynx	2.8 × 1.9	4 × 10 ¹²	4 × H ^f	No	No	77	>50
7	M	46	Larynx squam	Front neck	5.5 × 3.3	5 × 10 ¹²	4 × H ^f	No	No	Progress	
8*	M	50	Lipo	Pelvic cavity	13.7 × 9.7	8 × 2 × 10 ¹²	22 × H ^d	No	60 Gy	0	>50
9*	M	73	Mali-neuri	Retro-tongue	4.0 × 2.6	8 × 10 ¹²	4 × H ^f	No	70 Gy	75	
10	F	69	Ovarian adeno	Pelvic cavity	10.5 × 9.0	8 × 10 ¹²	8 × H ^d	No	60 Gy	88	>50
11*	M	68	Squam	Peri-neum	9.0 × 8.0	11 × 10 ¹²	4 × H ^f	No	70 Gy	100	
12	M	44	NPC squam	Right neck	4.1 × 2.5	8 × 10 ¹²	9 × H ^f	No	50 Gy	43	>50
13	M	71	Squam	Hypop harynx	4.6 × 3.0	6 × 10 ¹²	9 × H ^f	4	No	100	
14*	M	62	Squam	Right lung	7.8 × 5.8	7 × 10 ¹²	8 × H ^d	8	No	15	>50
15	M	52	Larynx squam	Hypop harynx	3.3 × 1.8	4 × 10 ¹²	4 × H ^f	3	No	0	

^aAll tumours histologically confirmed as squam, squamous cell carcinoma; lipo, liposarcoma; adeno, adenocarcinoma; mali-neuri, malignant neurinoma; ^bHyperthermia, H^f mean that superficial tumour heating at 43~44°C using 915 MHz microwave machine for 1 h or H^d mean that heating at 42~43°C using 41 MHz radiofrequency machine for deep-seated tumour for 1 h; ^cChemotherapy with cisplatin 50mg + 5-fluorouracil 1.25g once a week; ^dRadiotherapy; ^eLDA's area (%), LDA's percentage area in the tumour after treatment; ^fPost-peritoneum; ^gAbdominal wall.

*Patient 6 received nasopharyngeal intracavity hyperthermia at 43~44°C using 915 MHz microwave machine for 1 h. Patient 8's great tumour was completely resected and weighed 8kg 1 month later after the treatment. Patients 9 and 11 had primary tumours, while the other patients all had recurrent tumours. Patient 14's right lung tumour was totally resected 2 weeks after the treatment.

A previous study showed that the growth of subcutaneous tumour of two human gastric carcinoma cell lines with different p53 status was significantly inhibited by hyperthermia combined with Adp53 as compared with tumours receiving either treatment alone [11]. The clinical trial (phase II) proved that 11 patients with late-stage head and neck

squamous cell carcinoma received injection of Adp53 alone; only PR in two cases, SD in seven cases and PD in two cases. The results proved that sole Adp53 treatment is negligible for late-stage cancers. Up to now, hyperthermia is not considered as a unitary treatment method for cancer, because sole hyperthermia is negligible for late-stage cancer. So, a combination of Adp53 and hyperthermia was adopted in this non-randomized and non-control clinical trial.

Two patients had primary tumour, namely patient 9 who had malignant neurinoma resistant to radiotherapy and chemotherapy and patient 11 who had perineum squamous cell carcinoma invaded into bladder and rectum refractory to radiotherapy or chemotherapy. The other 13 patients with recurrent tumours failed in conventional treatments before the combination treatment. Of them, eight failed in operation, nine failed in radiotherapy and four failed in chemotherapy. Moreover, four patients had soft tissue carcinoma and three patients had adenocarcinoma, which are all resistant to radiotherapy and chemotherapy.

For example, patient 1, who had thyroid adenocarcinoma, developed recurrence 9 months after operation and the recurrent front-neck tumour failed in radiochemo-hyperthermia. One month after the failure, the recurrence was injected with Adp53 1×10^{12} vp once a week, over to 8 times and hyperthermia alone was given 3 days after each injection at $43 \sim 44^\circ\text{C}$ using a 915MHz microwave machine for 1 h, with a total of 8 times. After the treatment front-neck scab was exfoliated and severe dyspnea stopped. The neck tumour area decreased to 50% of the original (22.5 cm²). In this study, the results of Adp53tHTtRT or chemotherapy seemed better than that of Adp53tHT, because CR in two cases was only achieved in the Adp53tHTtRT or chemotherapy.

In conclusion, Adp53 combined with hyperthermia was safe and effective for patients with advanced cancer. p53 gene therapy has recently emerged as a potential thermosensitizer for advanced cancer.

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