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Research for Using Nanoferric Tetroxide as a Strain for Biopsy of Sentinel Node in the Patients with CNo Oral Squamous Cell Carcinoma (Stage Ⅰ, Ⅱ)

So Hyon Il, Kim Kwang Ho, Song Chun Hui, Ri Nam Gil and *Jang Pyong Chol*

Abstract We studied endostability of nanoferric tetroxide in mice and rats and lymph tropism of nanoferric tetroxide in rabbits. Based on it, we injected about 4mL of nanoferric tetroxide of 30 mg/mL to the submucous layer around local lesion 1 day before operation for 32 patients of oral squamous carcinoma. We examined lymph node, which is pigmented black on eyes, pathologically for extracted tissue after operation. As a result, nanoferric tetroxide had no toxicity, irritability, and good blood safety and doesn't give minus influence on heart and liver function. Nanoferric tetroxide, of which size is 20 to 120 nm, has clear lymph tropism, and when we assessed presence metastasis of cervical node by biopsy of sentinel node for using nanoferric tetroxide in 32 patients with oral squamous carcinoma (stage I, II), it has 90.9% of hyper-affectivity, 100% of specificity, 96.6% of accuracy and 9.0% of actual extract rate.

Key words nanoferric tetroxide, oral squamous carcinoma, sentinel node, lymph node excision

Introduction

The great leader Comrade **Kim Jong Il** said.

"**The most important factor in the current development of medical science and technology is to concentrate on solving the pressing scientific and technological problems in the health service.**"("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 18)

It is the most important problem to stop metastasis of tumor cell in the treatment of malignant tumors.

Especially, the stream of vessels and lymph is very complicated in the oral and maxillofacial region and the patients of oral squamous carcinoma (stage I, II) have high metastasis rate of lymph nodes by cancer cell from the beginning, so cervical nodes excision for stopping metastasis of cancer cell has great significance clinically. The most important problem of them is to confirm the sentinel node and its presence of metastasis of cancer cell and the presence of success of lymph nodes dissection depends on it.

In 1977, Cabanas described lymph nodes, which cancer cell of mother tumor arrived at first through the lymph duct, sentinel node for the first time [1, 3, 4, 11]. Usually lymph nodes of human organ are connected with rounded nodes and lymph stream has regular and order property. If the first lymph nodes don't occur metastasis, there is little possibility to occur metastasis at the

next nodes. So many researchers have been widely studying for biopsy of the sentinel node in the treatment of cancer patients. A researcher studied activated charcoal strain for getting the most suitable biopsy effect of the sentinel node [2, 5, 8, 10]. Generally, good lymph strains must be quickly absorbed at the lymph tissue, correctly indicate lymph duct and node and stay for a long time to concentrate at the sentinel node. And they must have no toxicity and cheap price.

Recently researchers have found out that organic and inorganic nanograin, whose size are 100 to 600nm, have lymph tropism and studied for biopsy of the sentinel node as a strain [6, 7, 9, 10]. However, we could not find data for using black nanoferric tetroxide as a strain of the sentinel node until now.

We have studied for using nanoferric tetroxide as a strain for biopsy of the sentinel node in the patients of oral squamous carcinoma (stage Ⅰ, Ⅱ).

1. Materials and Objects

1.1. Materials

We made the nanoferric tetroxide of which grain size is 20 to 120nm and used it.

1.2. Objects

1.2.1. Experimental animals

We used mice, which weigh 18 to 20g, rats, which weigh 180 to 220g, and rabbits, which weigh 1.8 to 2kg without distinction of sex.

1.2.2. Clinical research objects

They were 32 patients, who were diagnosed with CNo oral squamous cell carcinoma (stage Ⅰ, Ⅱ) at Dental General Hospital of Public Health Ministry of DPR Korea during the period of January 2011 to July 2014.

2. Research Methods

2.1. Examination method for endostability of nanoferric tetroxide

2.1.1. Toxicity examination

2.1.1.1. Acute toxicity examination

We observed mice, which injected nanoferric tetroxide solution, exactly 600, 1 200, 1 800, 2 400 and 3 000mg/kg of Fe into oral, and 300, 600, 900 and 1 200mg/kg of Fe into abdominal cavity, and 210, 420, 630 and 840mg/kg of Fe into caudal vein, for 72 hours.

2.1.1.2. Subacute toxicity examination

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of mice and observed changes of general state, motion and weights of mice between before and after injection for 40 days.

2.1.2. Irritability examination

2.1.2.1. Irritability examination of ocular-mucous membrane

We dropped 0.1mL of nanoferric tetroxide solution (25mg/mL), on one eye of rabbits and observed change states of iris, cornea and conjunctiva for 48 hours.

2.1.2.2. Irritability examination of ear vein

We injected 0.1mL of nanoferric tetroxide solution (25mg/mL) into ear vein once a day for 5 days and observed presence of erythema, eschar and necrosis of ear vein after 48 hours after the last injection.

2.1.2.3. Irritability examination of skin

We cut hair of rabbit's back, dropped 0.1mL of nanoferric tetroxide solution (25mg/mL) on the skin, and observed presence of erythema, eschar and formation of water vacuole 1 day later.

2.1.3. Examination of blood safety

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of rats, and observed change states of platelets, erythrocytes and leukocytes 1 hour later.

2.1.4. Influence on the other organ

2.1.4.1. Influence on cardiomotility

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of rats, and observed R-R interval and R wave height before and after injection.

2.1.4.2. Influence on BP

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of rats, and observed BP and R wave height before and after injection.

2.1.4.3. Influence on breathing

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of rats, and observed R wave height and respiration rate before and after injection.

2.1.4.4. Influence on liver function

We injected nanoferric tetroxide solution, exactly 840mg/kg of Fe into caudal vein of mice, and observed GPT and GOT before and after injection.

2.2. Examination for lymph tropism of nanoferric tetroxide

We made 7 groups of 5 rabbits and observed cervical node macroscopically after injecting several molarity of 0.1mL of nanoferric tetroxide to the submucous layer of the right side of tongue and the same amount of physiological sodium chloride solution to the one of the left side. Table 1 shows assessment norm.

Table 1. Assessment norm of lymph pigmentation

Score	Assessment norm
0	No pigmentation
0.5	Partial gray or black pigmentation
1.0	Fully black pigmentation

2.3. Clinical methods

We injected about 4mL of nanoferric tetroxide of 30mg/mL to the submucous layer around local lesion 1 day before operation for 32 patients of oral squamous carcinoma (stage I, II). We examined lymph node, which

is pigmented black on eyes pathologically for extracted tissue after operation.

We assessed presence metastasis of cervical node by biopsy of sentinel node for using nanoferric tetroxide in the patients of oral squamous carcinoma with hyperaffectivity, specificity, accuracy and actual extract rate.

We analyzed all the statistic data by using SPSS (Statistical Package for the Social Sciences) 16.0.

3. Result

3.1. Examination results for endostability of nanoferric tetroxide

3.1.1. Toxicity examination

3.1.1.1. Acute toxicity examination

At the examination of acute toxicity, there were no dead animals and no changein normal state.

3.1.1.2. Subacute toxicity examination

At the examination of sub-acute toxicity, there was no clear change in normal state, motion and weight of mice.

3.1.2. Irritability examination

3.1.2.1. Irritability examination of ocular-mucous membrane

At the irritability examination of ocular-mucous membrane, there was no pathologic change such as clouding, nebula and inflammation in the eye of rabbits.

3.1.2.2. Irritability examination of ear vein

At the irritability examination of ear vein, there was no pathologic change.

3.1.2.3. Irritability examination of skin

At the irritability examination of skin, there was no pathologic change.

3.1.3. Examination of blood safety

Table 2. Influence of Fe_3O_4 on peripheral blood

Group	Erythrocytes/($G\cdot L^{-1}$)	Leukocytes/($T\cdot L^{-1}$)	Platelets/($G\cdot L^{-1}$)
Control group	8.32±0.20	1.34±0.08	0.85±0.16
Study group	8.45±0.27	1.27±0.07	0.80±0.14

n=7

As seen in table 2, there were no significant differences between control and study groups in erythrocytes, leukocytes and platelets (p>0.05).

3.1.4. Influence on the other organ

3.1.4.1. Influence on cardiomotility

As seen in table 3, there were no significant differences between before and after injection in R wave height and R-R interval (p>0.05).

Table 3. Influence of Fe_3O_4 on heart function

Case	R wave height/mm	R-R interval/mm
Before injection	9.1±0.12	6.2±0.15
After injection	9.5±0.14	6.4±0.16

n=7

3.1.4.2. Influence on BP

As seen in table 4, there were no significant differences between before and after injection in R wave height and BP. (p>0.05)

Table 4. Influence of Fe_3O_4 on BP

Case	R wave height/mm	BP/kPa
Before injection	10.3±0.25	15.2±0.31
After injection	10.5±0.24	15.1±0.34

n=7

3.1.4.3. Influence on breathing

As seen in table 5, there were no significant differences between before and after injection in R wave height and respiration rate. (p>0.05)

3.1.4.4. Influence on liver function

As seen in table 6, there were no significant differences between before and after injection in GPT and GOT. (p>0.05)

Table 5. Influence of Fe_3O_4 on respiration

Case	R wave height/mm	Respiration/min^{-1}
Before injection	8.5±0.51	94.2±1.34
After injection	8.4±0.56	95.3±1.45

n=7

Table 6. Influence of Fe_3O_4 on liver function

Group	GPT/unit	GOT/ unit
Control group	24.0±6	14.5±1.1
Study group	22.4±1.9	13.8±1.1

n=7

3.2. Examination for lymph tropism of nanoferric tetroxide

As seen in table 7, lymph nodes were well pigmented on eyes according to molarity of nanoferric tetroxide, but not 20 mg/mL.

Table 7. Pigment degree according to molarity of nanoferric tetroxide

Nano Fe_3O_4 concentration /(mg·mL^{-1})	5	10	15	20	25	30	50
Pigment degree	0	0.38	0.74	1.0	1.0	1.0	1.0

3.3. Clinical results

In all of 32 patients, all the cervical nodes were pigmented by nanoferric tetroxide.

The assessment result of presence of metastasis of cervical nodes by the pigmented nodes was same as table 8.

Table 8. Assessment of presence of metastasis of cervical nodes by biopsy of the sentinel nodes

Index	Hyper-affectivity	Specificity	Accuracy	Actual extract rate
Percent/%	90.9(10/11)	100(21/21)	96.6(31/32)	9.0(1/11)

Conclusion

The nanoferric tetroxide (20 to 120nm) we made has a good endostability and a clear lymph tropism, so we think we can use it as a strain for biopsy of sentinel node in the patients of CNo oral squamous cell carcinoma (stage I, II).

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