ADJUVANT IMMUNOTHERAPY WITH ANTI-TUMOR STR. PYOGENES PREPARATION (IMMUNOPOTENTIATOR OK-432) IN UROGENITAL CARCINOMA

Author(s): Nakagami, Yoshizo; Chin, Hansui; Kawai, Hiroshi; Tannowa, Kunio

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ADJUVANT IMMUNOTHERAPY WITH ANTI-TUMOR STR. PYOGENES PREPARATION (IMMUNOPOTENTIATOR OK-432) IN UROGENITAL CARCINOMA

Yoshizo Nakagami, Hansui Chin
and Hiroshi Kawai

Department of Urology, First Hospital of Nippon Medical School, Tokyo 102, Japan

Kunio Tannowa

Department of Urology, Kawakita General Hospital, Tokyo 166, Japan

As a curative means for urogenital carcinoma, non-specific immunotherapy is now standing in the spotlight, in addition to such measures as surgical operation, chemotherapy and radiation-therapy.

We applied an anti-tumor streptococcal pyogenes preparation (OK-432) as an adjuvant (immunopotentiator) to 54 cases principally with urogenital carcinoma through intracutaneous injection in order to examine its immunostimulative activity.

Items examined include counts of leucocyte and lymphocyte in the peripheral blood, delayed hypersensitivity skin tests to PPD and PHA, PHA and Con. A-induced blastogenesis, T. B. cell counting and IgG-FcR+ T cell.

As the results, this intracutaneous injection method was found to be simple and to have caused no side reaction, further preventing leukopenia which usually occurs after chemotherapy. It was found to be effective in preserving normal cellular immunity, thus working as an agent contributing to the prolongation of life of the host.

INTRODUCTION

The non-specific immunotherapy has recently been used more and more widely in the treatment of the carcinoma, and it is one of the anti-tumor therapeutic means which are expected to be effective. The immunotherapy is considered to exert its anti-tumor action by potentiating immunological functions of a host, by acting to suppress or reject the carcinoma in the host. This therapeutic method has been initiated by the BCG-therapy introduced by Mathé et al. (1969) and Morton et al. (1974). Since then, various immunopotentiators have been developed and clinically tested in almost all countries in the world.

In the present work, the non-specific immunotherapy with the use of an immunopotentiator, OK-432, was tested for the treatment of urogenital carcinoma as the fourth therapeutic means in addition to conventional surgical operation, chemotherapy and radiation-therapy. The effectiveness of the therapy was evaluated from the observed values of various immunoparameters. The results thus obtained from 54 cases are described below.

MATERIALS AND METHODS

The cases studied consisted of 54 cases including those with urogenital carcinoma and those for the controls, i.e., 8 cases with bladder cancer, 3 cases with ureteric cancer, 9 cases with renal cell carcinoma, 2 cases with renal pelvic carcinoma, 10 cases with prostatic cancer and 6 cases including cancers of the penis, testis and uterus, remaining 16 cases consisting of controls and non-carcinomatous patients (Table 1).

Immunopotentiator (OK-432, Chugai Pharm. Co. Ltd.)

The OK-432 is a lyophilized cell preparation of the low virulent Su-strain of strep-
Table 1. Materials

<table>
<thead>
<tr>
<th>Urogenital cancers and control groups; 54 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) bladder ca.</td>
</tr>
<tr>
<td>b) ureteral tumor</td>
</tr>
<tr>
<td>c) renal cell ca.</td>
</tr>
<tr>
<td>d) renal pelvic ca.</td>
</tr>
<tr>
<td>e) prostatic ca.</td>
</tr>
<tr>
<td>f) cancers of penis, testicle, uterus and others</td>
</tr>
<tr>
<td>g) control group and non-cancerous patient cases</td>
</tr>
</tbody>
</table>

Actinomycosis pyogenes (group A, type 3) treated with penicillin G, which is known to have anti-tumor effect. One K.E. as clinical unit of this preparation corresponds to 0.1 mg of the cell dry weight. The anti-tumor effect of OK-432 is exerted mainly by enhancing host immune responses, i.e.,

Table 2. Comparative results by intracutaneous and intramuscular administrations of OK-432.

<table>
<thead>
<tr>
<th>cases route</th>
<th>local findings</th>
<th>general condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before or after adm.</td>
<td>lymphocyte blastogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHA (20 µg)</td>
</tr>
<tr>
<td>i.c. adm.</td>
<td>pain</td>
<td>heat sensation</td>
</tr>
<tr>
<td>5 +</td>
<td>2 ~ 3 hours</td>
<td>±</td>
</tr>
<tr>
<td>i.m. adm.</td>
<td>2 ~ 3 hours</td>
<td>±</td>
</tr>
</tbody>
</table>

Intracutaneous administration of OK-432

Intracutaneous injection method of the immunopotentiator OK-432 has been reported to potentiate the reticuloendothelial system and to strongly stimulate the immunactivity, as suggested in intracutaneous vaccination of B.C.G. (Morton et al., 1974). Therefore, OK-432 was intracutaneously given to patients in this present work, the injection method employed being the modified intracutaneous vaccination.
method of Hoshino et al.3) (1976). Thus, OK-432, 1~2 KE, suspended in 1 ml of 1% procaine solution was slowly injected intracutaneously at the brachial deltoid muscle portion using a tuberculin injector. The dose for a cancer patient was selected to be 2.0 KE which was given 3 times a week as a rule.

The immunotherapy was commenced immediately after the termination of various laboratory tests for hospitalized patients. Imunochemotherapy, surgical therapy and radiation therapy were also performed, in principle, in parallel to the immunothersy with OK-432.

Parameters for immunoactivity

The following tests were performed in order to obtain the values of parameters which may reflect the immunoactivity of a host: the counts of leucocyte and lymphocyte in the peripheral blood, delayed hypersensitivity skin test to PPD and PHA (Welcome), and Su-polysaccharide skin test. PHA, Con. A-induced blastogenesis by means of the total blood method, countings of T. B. subpopulation and of IgG-FcR+ T cell etc. Also, the normal values of respective immunoparameters are as follows: counts of leucocyte (average, 6500), counts of lymphocyte (average 25%, 1600~2000), delayed hypersensitivity skin test to PPD (10 mm or over/48 hrs), to PHA (10 mm or over/24 hrs), Su-polysaccharide test (10 mm or over/24 hrs), induced blastogenesis of lymphocyte (S.I. or c.p.m.), T cell (70~85%), B cell (5~20%), IgG-FcR+ T cell (2~8%). These values were measured before and after the administration of OK-432. The side action of OK-432 was also examined.

As intramuscular injection is widely employed for the administration of OK-432, and the occurrence of side effects due to intramuscular administration including pyrexia was compared with that due to the intracutaneous injection which was used in the present work.

Cases studied were classified into the following groups: Group A: non-cancer patients, Group B: only surgical operation for cancer patients, Group C: cancer patients treated with chemotherapy, surgical operation and OK-432, Group D: cancer patients treated with multiple chemotherapies, surgical operation and OK-432, Group E: prostatic cancer patients surgically treated with castration and treated with anti-androgenic hormone and OK-432, and Group F: patients treated with OK-432 after the remission of the carcinoma. Two additional groups of non-cancer patients were also provided for the comparison of side effects of OK-432 due to intramuscular and intracutaneous injections. Representative cases were picked up from these patient groups taking the age factor into account and limiting the severity to the range of T2-T3, and variations of each of the parameters were graphically presented (Table 2).

RESULTS

Comparison of intramuscular injection with intracutaneous injection of OK-432 (Table 3)

The administration of OK-432 is usually carried out by means of intramuscular injection and it is known to result in unpleasant pyrexia as a side effect of this substance. As shown in Table 3, the results obtained with non-cancer patients indicated that pyrexia and local pain were not induced by intracutaneous injection of OK-432. In addition, no formation of an ulcer was demonstrated at the locus of the injection. It was also shown that OK-432 was absorbed within 2~3 hrs into the body. The immuno-parameters were not affected, of course, by either the intracutaneous or intramuscular injection of OK-432 into non-cancer patients, while the intracutaneous injection of OK-432 into cancer patients stimulated the reticuloendothelial system, thus enhanced the immunoactivity of the host, in a manner similar to that due to intramuscular injection of OK-432. Therefore, it may be concluded that intracutaneous injection of OK-432 is superior to conventional intramuscular injection on the basis of the lack of any apparent side effect in intracutaneous injection of this substance.
### Table 3. Classification of groups and their representative cases.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Diagnosis</th>
<th>T-classification</th>
<th>Metastasis</th>
<th>Surgical operation</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
<th>Immunotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> non-cancerous patients group</td>
<td>B.P.H prostatectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong> cancer op. group</td>
<td>testicular tumor</td>
<td>$T_2$</td>
<td>$(-)$</td>
<td>orchietomy</td>
<td>$(-)$</td>
<td>$(-)$</td>
<td>$(-)$</td>
</tr>
<tr>
<td><strong>C</strong> cancer patients chemotherapy+op+OK-432, group</td>
<td>bladder tumor</td>
<td>$T_1$</td>
<td>$(-)$</td>
<td>T.U.R.Bt Blomycin</td>
<td>$(-)$</td>
<td>OK-432</td>
<td></td>
</tr>
<tr>
<td><strong>D</strong> cancer patients multi-drug combination+op+OK-432, group</td>
<td>renal cell ca.</td>
<td>$T_3$</td>
<td>$(-)$</td>
<td>nephrectomy</td>
<td>FAMT Adriacin</td>
<td>$(-)$</td>
<td>OK-432</td>
</tr>
<tr>
<td><strong>E</strong> prostatic cancer group castration+antiadrogenic therapy +OK-432, group</td>
<td>prostatic cancer</td>
<td>$T_3$</td>
<td>$(-)$</td>
<td>castration</td>
<td>$(-)$ antiandrogenic therapy (+)</td>
<td>$(-)$</td>
<td>OK-432</td>
</tr>
<tr>
<td><strong>F</strong> OK-432 administered group after remission of cancer</td>
<td>ureteral tumor</td>
<td>$T_3$</td>
<td>$(-)$</td>
<td>nephro-ureterectomy</td>
<td>$(-)$</td>
<td>$(-)$</td>
<td>OK-432</td>
</tr>
</tbody>
</table>

Therapeutic effect of OK-432 for cancer patients
Various immunoparameters were obtained before and after the commencement of the therapy at two-week-intervals from all the patients investigated. The comparison of variations of the parameters were carried out with representative cases selected from each of the groups.

a) The counts of leucocyte and lymphocyte in peripheral blood
The counts of both leucocyte and lymphocyte were found to increase or to be around normal levels in all cases treated with OK-432, whereas the group D which was treated with multiple chemotherapeutic drugs demonstrated decreased levels of these cells, and groups A and B to which OK-432 was not administered also showed low levels of both leucocytes and lymphocytes (Fig. 1).

b) Skin tests
Results of PPD skin test (delayed hyper-

![Leucocyte](image1)
![Lymphocyte](image2)

Fig. 1. Variation of leucocytes and lymphocytes on pre-post therapy with OK-432.
sensitivity reaction) showed low values for the group A. This might be due to the age factor for this group. Other group showed higher values except for the group D which exhibited the declining curve.

In PHA skin test, abnormally high values for the group A and declining curves for the group D were most distinguished. No correlation was observed between the results of PPD and PHA skin tests.

As Su-polysaccharide test is specific to OK-432, all cases treated with OK-432 showed positive reaction (Fig. 2).

c) Induced blastogenesis of lymphocyte (PHA, Con.A)

Although the induced blastogenesis of lymphocyte is usually examined by measuring the responses to PHA and Con.A, it is suggested by other investigators that these values vary considerably and that it is often improper to express the blastogenesis in terms of the stimulation index (S.I.) obtained from these values. In order to avoid such uncertainty, the graph was drawn at levels of 20/μg PHA (c.p.m.) and 10/μg Con.A (c.p.m.). The results thus obtained showed a common pattern in curves for the PHA and Con.A. Groups treated with OK-432 showed increasing patterns, while the group D exhibited decreasing curves. The decrease observed in the group A might be attributed to the influence from surgical operation and that from the age of the patients.

A correlation was suggested between the results of PHA skin test and the induced blastogenesis of lymphocyte obtained by PHA measurement (Fig. 3).

Fig. 2. Variation of PPD skin test and PHA skin test and Su-polysaccharide test on pre-post therapy with OK-432.

Fig. 3. Variation of lymphocyte blastogenesis (PHA, Con-A) on pre-post therapy with OK-432.
d) T. B. subpopulation

It was found that T. B. subpopulation was low in cases of highly advanced carcinoma (metastasis cases) which belonged to the grade T₃ or T₄ according to the T-classification. It was also observed, however, that the case which had shown a low T cell level improved or returned to the normal level by the treatment with OK-432, and the case which had an abnormally high level also approached to the normal level in the course of the therapy. The group D did not give the curve lower than the normal value.

Level of B cell were generally lower than the normal level in the patients examined and thus seemed to be in a relation opposite to the T cell. Although there were two groups before the therapy, the one with high B cell level and the another with low B cell level, there was a tendency that these B cell levels approached to the normal level in association with the restoration of the T cell level during the therapy with OK-432.

IgG-FcR⁺ T cell is known to give a pattern significant in reflecting the host immune response. It was demonstrated that the administration of OK-432 clearly restored the reactivity of the host according to the curves obtained from IgG-FcR⁺ T cell. The curves for groups A and B which were not given OK-432, however, were at lower levels than those of other groups; this might be due to factors of the age and surgical operation. The administration of OK-432 certainly restored the level of IgG-FcR⁺ T cell, the subset of T cell, after the performance of surgical operation or chemotherapeutic treatment, as indicated by the increasing curves given (Fig. 4).

According to the results obtained in this study, it may be concluded that the non-specific immunotherapy with intracutaneous administration of OK-432 is relatively easy in application, only with minor side effects and might be effective in preventing, to some extent, the side effects due to the chemotherapy, such as leukopenia and depilation. It was also demonstrated in the present work that the therapy with OK-432 given by intracutaneous injection method may enhance the immunoactivity of the host, thus leading to the prolonged life of the host.

It was shown that the immunoactivity of the cancer-bearing host may well reflect the host immunoactivity, which is sensitive to drastic surgical operation procedures, radiation-therapy, chemotherapy with the anti-cancer agent and the age factor. In the case of multiple administrations of large amounts of anti-cancer drugs, however, the lowering of the host immunoactivity seems not to be completely prevented by OK-432 (Table 4).

According to the immunoparameters measured in this work, levels of T cell and mitogen (values of PHA and Con.A) were found to become lower with the progression
Table 4. General results.

<table>
<thead>
<tr>
<th>Group</th>
<th>T classification</th>
<th>skin test</th>
<th>lymphocyte blastogenesis</th>
<th>T.B. subpopulation</th>
<th>side effects and others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;a&lt;/sub&gt;, T&lt;sub&gt;b&lt;/sub&gt;, T&lt;sub&gt;c&lt;/sub&gt;, T&lt;sub&gt;d&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6 0 0 0 0</td>
<td>(+)</td>
<td>normal or lower</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>after operation</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>B</td>
<td>4 1 2 1 0</td>
<td>(+)</td>
<td>(−)</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>after operation</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>C</td>
<td>11 2 6 3 0</td>
<td>(−)</td>
<td>elevated</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)</td>
<td>elevated</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td>D</td>
<td>6 0 1 4 0</td>
<td>became negative</td>
<td>lower during chemotherapy</td>
<td>recovered subsequently</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>10 0 3 6 1</td>
<td>(−)</td>
<td>elevated</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)</td>
<td>elevated</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>F</td>
<td>7 0 5 2 0</td>
<td>(−)</td>
<td>elevated</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)</td>
<td>elevated</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

of the carcinoma. The level of IgG-FcR<sup>+</sup>
T cell also decreased with the severity of
the carcinoma and was also affected by
surgical procedures and the age factor.
The administration of OK-432 restored the
reactivity of the T cell and consequently
improved systemic conditions of the patients.

**DISCUSSION**

The immunotherapy for carcinoma has
recently become one of therapeutic methods
which are attracting world-wide attention.
This method, however, is basically the non-
specific immunotherapeutic means, thus it
is to be employed supplementally to surgical
operation, radiation-therapy or chemo-
therapy<sup>10</sup>.

The immunotherapy for cancer which is
non-specific in nature, has been initiated
by the application of B.C.G. to the leukemia
by Mathé et al. (1969)<sup>11</sup> and to the melano-
mma by Morton et al. (1972)<sup>23</sup>, and since
then numbers of immunopotentiators have
been developed and clinically tested.

It has been pointed out by Kimura et al.<sup>4</sup>,
as to clinical application of immunoche-
motherapy, that the efficacy of the non-
specific immunotherapeutics to the carci-
noma naturally has a certain limitation
when the agent is used singly. The activity
of such drugs is usually slow-developing and
is largely influenced by the number, size,
developmental stage and extent of the
spread, of a cancer cell at the stage of
therapeutic treatment. Therefore, the
agent should be used as one of immuno-
chemotherapeutic means in order to obtain
the highest effectiveness.

The immunotherapeutics seem to prevent
development of side effects of chemothe-
rapeutics, such as the decrease of leucocyte
numbers, this being one of the merits of
this method. As the immunotherapy is
thus supplemental to the chemotherapy
as to the side effect and the influence to
the host, it is convenient to clinically apply
the immunotherapy as a supplemental
therapeutic means.

OK-432 has been widely used clinically
as the immunotherapeutics for the carci-
noma in association with the chemothera-
pedics resulting in respective curative
effect on host immune response and also to have the activating effect on macrophages and lymphocytes.

In order to evaluate the effectiveness of the immunotherapeutics with certainty, the examination should be carried out with the cases which are therapeutically treated on a common basis and by the randomized trial method, and it is also necessary to observe various immunological parameters. Clinically speaking, however, the parameter which may most faithfully indicate the immuno-condition of a patient and may give information as to therapeutic effect and the post-treatment condition is not defined yet.

In the present study, the parameters which have been most widely and most frequently employed and investigated, such as the induced blastogenesis of lymphocyte (PHA, Con. A), T. B. subpopulation, the delayed hypersensitivity skin reaction (PPD), PHA skin test and the leucocyte count in peripheral blood and lymphocyte count were tentatively examined. Yata et al. has reported that the measurement of IgG-FcR+ T cell level in association with that of T. B. subpopulation may well reflect the host reactivity as well as the observation on mitogens. We also agree with his remark. These parameters are important in indicating the relation between in vitro parameters and the extent of progression of the disease or post-treatment condition of a patient. The induced blastogenesis of lymphocyte, T. B. subpopulation and IgG-FcR+ T cell are also considered to be suitable as a means to indicate a non-specific cellular immunity. From the point of view against cancer immunity, it is considered that the cellular immunoactivity mainly consisted of the function of the T cell is important as a diagnostic parameter for the non-specific anti-cancer immunotherapy.

The intracutaneous injection method of the immunopotentiator OK-432 into patients with urogenital carcinoma was performed, as the immunochemotherapy, in the present work; however, there seemed to be no dose-response relation. Side effects of the chemotherapeutics used as an immuno-suppressor were possibly prevented by the co-administration of OK-432.

Since the immunoactivity of the host has changed by the administration of OK-432, it is a drug to be clinically tried because of possibility to prolong the host’s life.

In view of the present progress in clinical application of the non-specific immunotherapy throughout the world, the establishment of the specific immunotherapy should be expected in the future.

REFERENCE


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和文抄録

尿路性器癌に対する Immunopotentiator OK-432 による免疫療法

日本医科大学附属第一病院泌尿器科
中神 義三，陳 泽水，川井 博

河北総合病院泌尿器科
淡 輔 邦 夫

尿路性器癌に対する治療法として非特異的免疫療法は、今日、外科的療法、化学療法、放射線療法に加えて非常に難光を浴びている。われわれは、尿路性器癌54例に対し immunopotentiator OK-432 を皮内投与し、その担荷宿主の免疫賦活作用を検討した。

検討項目は、末梢血の白血球数、リンパ球数、遅延型皮膚反応として PPD skin test および PHA skin test, mitogen PHA, Con-A によるリンパ球増殖率、T, B 細胞測定および IgG-FcR⁺ T cell の測定を施行した。結果として、OK-432 の皮内投与法は容易に施行でき、発熱などの副作用もなく、抗癌剤などの投与時における白血球数減少もある程度防止できることを知った。

そして、その細胞性免疫能は OK-432 の投与により回復することが認められ、宿主の延命効果に役立っているように思われた。

訂正：Table 4. Group B の PPD 標の $\frac{1}{4}$ は $\frac{1}{4}$ の誤りです。