Effects of Immunosuppressive Acidic Protein on DMBA-Induced Pancreatic Cancer in Rats

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MATSUNO, S., EJIRI, T., KOBARI, M., YAMAUCHI, H. and SATO, T. Effects of Immunosuppressive Acidic Protein on DMBA-Induced Pancreatic Cancer in Rats. Tohoku J. exp. Med., 1984, 144 (2), 189-202 —— In order to determine the effect of immunosuppressive acidic protein (IAP) on the formation of pancreatic carcinoma, rats of the Sprague-Dawley strain were embedded with 7, 12-dimethylbenzanthracene (DMBA) in the pancreas with and without administration of IAP. In these animals, the growth of pancreatic cancer was studied both immunologically and histologically. Tumor was induced in 51 animals (85%) of 60 treated with embedding of 1 mg DMBA alone. Tumors began to appear from the 16th week after the embedding. Among animals in which tumor was induced, tubular adenocarcinoma and pleomorphic carcinoma accounted for 55% of the cases. When administration of IAP was combined, the period required for development of tumor was shortened. It became shorter with increases in the dosage and frequency of administration of IAP. In animals which received IAP in a mean dose of 75 mg/kg the area showing cancerous changes appeared as early as at the 8th week after the embedding of DMBA. A significant increase in the volume of tumors was seen in the group treated with IAP as compared to the group not treated with IAP. Animals which received IAP in increasing doses and frequencies showed an accelerated increase in the volume of the tumors which underwent cancerous changes. IAP was eliminated from the serum of rats within 72 hr after the administration, and acid protein was clearly recovered from the serum when tumors proliferated. These findings indicate that the acceleration of carcinogenesis in DMBA-induced pancreatic carcinoma may be attributable to the immunosuppressive effect of IAP administered and tend to be dependent on the dosage and frequency of its administration in the early phase of tumor induction.

As an approach to the diagnosis and treatment of pancreatic carcinoma, establishment of experimental models of pancreatic carcinoma has been attempted (Druckrey et al. 1968; Pour et al. 1975). Heretofore, few papers have been published describing the preparation of experimental models of pancreatic car-
cinoma by combined use of initiator and promoter. Only one paper has reported such combined use; Konishi et al. (1976) promoted induction of pancreatic carcinoma by injecting 4-hydroxyaminoquinoline-1-oxide (4-HAQO) at the period of pronounced DNA synthesis after administration of ethionin containing protein deficient diet and partial pancreatectomy. Using the method of Dissin et al. (1975) and Satake et al. (1975), the authors embedded 7,12-dimethylbenzanthracene (DMBA) directly into the pancreas of rats to induce experimental pancreatic carcinoma locally and then these animals were injected intravenously with immunosuppressive acidic protein (IAP), which had been found in increasing concentrations in ascites and serum of cancer bearing patients (Matsuda et al. 1978), as a promotive factor to increase carcinoma induction rate and to shorten the time required for the development of carcinoma. IAP is an acidic glycoprotein having immunosuppressive activity isolated in 1978 by Matsuda et al., and it is considered to be formed by incomplete sugar chains of \( \alpha_1 \)-acid glycoprotein (\( \alpha_1 \)-AG).

In vitro, it was confirmed that IAP depressed various mitogen reactions of human lymphocytes (Tamura et al. 1981). Moreover, experiments on mice demonstrated that i.v. injection of human IAP resulted in similar suppression of PPD reactions and mitogen reactions (Ishida et al. 1980). The aim of the present paper is to report the results of our study on the effect of IAP on the formation of pancreatic carcinoma induced by DMBA in rats in terms of its development, growth and induction rate.

**MATERIALS AND METHODS**

*Materilas.* Male rats of the Sprague-Dawley strain, aging 4 weeks and weighing about 80 g, were used. The carcinogenic chemical used was 7, 12-dimethylbenzanthracene (DMBA, Sigma Co., St. Louis). As the promotive factor, IAP extracted from ascites of cancer bearing patients was used. Animals were maintained with free access to solid diet (Oriental Yeast Co., Ltd., Tokyo) and tap water at room temperature.

*Experimental induction of pancreatic cancer.* Under anesthesia with ether, animals fasted overnight were disinfected of the lower abdominal area andlaparotomied by an upper abdominal median incision of about 1.5 cm. Only the splenic lobe of the pancreas was taken outside from the abdominal cavity and the pancreatic capsule was punctured with a 19 G elaster needle which was packed with 1 mg of DMBA crystals into the external syringe. The drug was embedded into the pancreatic parenchyma by pushing the drug using the internal syringe. The external syringe of the elaster needle was extracted and then the abdominal cavity was closed after confirming the absence of leakage of the drug or bleeding. For prevention of infection, each animal was injected subcutaneously with 5 mg of penicillin. Animals were divided into IAP group and non-IAP group. IAP group was further subdivided into groups according to the administration method and dosage of IAP:

- **Group I:** Embedded with 1 mg of DMBA without IAP.
- **Group II:** Embedded with 1 mg of DMBA into the pancreas similarly to Group I, and received 4 mg of IAP intravenously into the tail vein as one shot injection on the previous day of the operation and then at 1 day, 1 week and 2 weeks after the operation.
- **Group III:** Embedded with DMBA in the same manner as described for Group I and then received IAP intravenously in a dose of 4 mg on the previous day and the following
day of the operation and at 8 mg at 1 and 2 weeks after the operation.

Group IV: Embedded with DMBA in the same manner as described for Group I, and received intravenously IAP in a dose of 4 mg on the previous day and the following day of, and then at 1 and 2 weeks after the operation. In this group an additional dose of 4 mg each of IAP was also given intravenously at 3 and 4 weeks after the operation.

Group V: Embedded with DMBA similar to all of the above described groups and received IAP, 6 mg on the previous day and following day of the operation, 8 mg at 1 week, 10 mg at 2 weeks, 12 mg at 3 weeks and 14 mg at 4 weeks, totalling to about 75 mg/kg, intravenously into the tail vein.

Controls: animals undergone sham operation were used as the control animals. Five control animals each were prepared for Groups I, II and IV, and 3 animals each for groups III and V. The control animals for Groups II, III, IV and V received IAP in corresponding doses of each group.

Measurement of serum levels of human IAP injected intravenously. For determination of changes with time of serum IAP levels following its i.v. injection, blood samples were collected from control rats of Group V immediately after and at 3, 10, 24, 48 and 72 hr after the injection. IAP levels were determined by the single radial immunodiffusion method (SRID) of Mancini et al. (1965) using the plate for determination of IAP prepared by Tamura et al. (1981).

Determination of serum acidic protein in rats. From all animals blood samples were collected immediately before they were sacrificed and separated of serum by centrifugation at 3,000 rpm. The serum thus obtained was stored at −20°C.

For examination of serum, plate isoelectric phoresis was performed on an ampholyte plate, pH 2.5 to 6, with an apparatus for electrophoresis (LKB). The plates were stained with Coomassie brilliant blue (R-250).

Pathological examination of rats. From the 8th week after the operation, experimental animals were sacrificed 6 rats each for Groups I, II and IV and 4 rats for Group V at 4 weeks

Fig. 1. Histology of DMBA-induced pancreatic tumor in rat. Ductal proliferation: The tumorous area is occupied by ductal structure which can be clearly differentiated from the normal pancreatic parenchyma. H-E, ×160.
Experimental animals of Group III were sacrificed 6 rats each at 4 weeks intervals from the 16th week. At the time of sacrifice, the rats were laparotomied and thoracotomied and resected of tumors together with, as indicated, liver, regional lymph node and metastatic lesions in the abdominal cavity. After the specimens obtained were fixed in formalin, the primary lesion was measured for diameters using slide calipers and prepared for histological examination by use of various stainings such as hematoxylin-eosin, elastica-van Gieson and silver. According to the histomorphological characteristics, tumors were classified into the following histologic types.

No development type: Either only scar was seen with no tumor, or, despite the fact that tumor formation was observed, microscopic findings revealed only necrotic tissue and proliferation of connective tissue associated with infiltration of inflammatory cells around it in the central part (to be abbreviated as no dev. type hereinafter).

Ductal proliferation type: The tumorous area was occupied by ductal structure which could be clearly differentiated from the normal pancreatic parenchyma and which was associated with small tubular structure of relatively uniformed size, with lobular structure retained without atypism either in structure or in cells (abbreviated as duct. type) (Fig. 1)

Atypical ductal proliferation type: Within tumorous node there were ductal tissues of varying sizes with partially multilayered area. Nucleus showed heavy staining, irregularity in size and swollen nucleoles (to be abbreviated as atp. type) (Fig. 2).

Tubular adenocarcinoma type: Nucleus showed irregularity in size and was rich in chromatin. Tubular structure with clear cellular and structural atypism was noted (abbreviated as tub. type) (Fig. 3).

Pleomorphic carcinoma type: Pleomorphism characterized by presence of large cells of varying sizes, multinucleated cells and atypical cells resembling to spindle cells were observed. Although it was difficult to differentiate whether the carcinoma originated from the epithelium or not, arrangement of epithelium was seen partially (to be abbreviated as pleo. type) (Fig. 4).

Osteosarcoma type: Formed through cellular atypical proliferation of cells resembling to osteocytes. Partially atypical proliferation of spindle cells was seen (to be abbreviated as ost. type) (Fig. 5).

The volume of tumor was calculated as an ellipsoid from measurements of longer and shorter diameters and thickness of the mass after fixed with formaline. For calculation of the volume of cancerated portion, the preparate with the longest diameter of tumor was traced using a projector (Nippon Kogaku, Tokyo) and the percentage of the area showing cancerous changes was obtained by the point counting method (Weibel 1966). The percentage obtained was multiplied by the volume of mass.

Significance of difference of measurements was analyzed by Student's t-test. Difference was taken to be statistically significant when p values were less than 0.05.

**Results**

**Histologic findings**

1) Control rats of each group

In all of these animals blood samples were collected at the end of the study period when they were sacrificed. None of these animals died before completion of the study period. Macroscopic findings at laparotomy revealed no tumor in any of these animals, although slight adhesion was observed. Microscopically also the pancreatic parenchyma showed almost normal picture with only slight proliferation of fibrous tissue and lymphocytic infiltration.
Fig. 2 (upper). Atypical ductal proliferation. Within tumorous node there are ductal tissues of varying sizes with partially multilayered area. Nucleus shows heavy staining, irregularity in size and swollen nucleoles. H-E, ×200.

Fig. 3 (lower). Tubular adenocarcinoma. Nucleus shows irregularity in size and is rich in chromatin. Tubular structure with clear cellular and structural atypism is noted. H-E, ×200.
Fig. 4 (upper). Plemorphic carcinoma. plemorphism characterized by presence of large cells of varying sizes, multinucleated cells and atypical cells resembling to spindle cell are observed. H-E, ×200.

Fig. 5 (lower). Osteosarcoma. Formed through cellular atypical proliferation of cells resembling to osteocytes. Partially atypical proliferation of spindle cells is seen. H-E, ×200.
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Table 1. Classification of the histologic type of DMBA-induced pancreatic tumors in rats and its incidence (%)

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>G-I (n=60)</th>
<th>G-II (n=48)</th>
<th>G-III (n=18)</th>
<th>G-IV (n=30)</th>
<th>G-V (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No development</td>
<td>15.0</td>
<td>6.2</td>
<td>5.6</td>
<td>6.8</td>
<td>8.3</td>
</tr>
<tr>
<td>Ductal proliferation</td>
<td>10.0</td>
<td>12.5</td>
<td>5.6</td>
<td>13.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Atypical ductal proliferation</td>
<td>20.0</td>
<td>16.7</td>
<td>11.0</td>
<td>13.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Tubular adenocarcinoma</td>
<td>51.7</td>
<td>56.3</td>
<td>72.2</td>
<td>63.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Pleomorphic carcinoma</td>
<td>3.3</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>5.6</td>
<td>3.3</td>
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2) Rats embedded with DMBA

i) In Group I, 6 animals each were sacrificed at 4 weeks intervals from the 8th week. During the observation period up to 8 weeks, death occurred in 5 animals. The causes for death were tumor in 2 cases, ileus due to adhesion in 2 cases and bleeding within the abdominal cavity in 1 case. Histologically, no dev. accounted for 15%, duct. 10% and atp. for 20%, with carcinoma formation in 55.0% of the Group I animals (Table 1).

ii) In Group II, proliferation classified as tub. and pleo. began to be observed from 12th week. Histologically, 6.2% of the animals of this group were classified as having tumors of no dev., 12.5% as duct., 16.7% as atp., 56.3% as tub., and 8.3% as pleo., with cancerous changes seen in 64.6% of the animals. The tumor classified as pleo at 20th week was as large as 73×42 mm (Table 1).

iii) In Group III, histologic type classified as tub. was seen in 72.2% and ost. in 5.6%, with malignancy detected in 77.8% of the animals. No difference was found in the incidence of histologic types of tumors of this group when compared to that at corresponding period in Group II (Table 1).

iv) In Group IV, tumor developed in all animals, with histologic type classified as no dev. in 6.8%, duct in 13.3%, atp. in 13.3%, tub. in 63.3% and ost. in 3.3%. Malignancy was found in 66.6% of the animals (Table 1).

v) Animals (n=12) of group IV received IAP at higher dosage and frequency. Out of 4 animals sacrificed at early stage of 8 weeks after the operation, findings indicative of malignancy were found in 2 animals. In this group the incidence of malignancy was 66.7% (Table 1).

Tumor size

Fig. 6 shows change with time in the volume of tumors observed in rats sacrificed at 4 weeks intervals from the 8th week.

i) In Group I the volume recorded at the 8th week was $18.1 \pm 4.5 \text{ mm}^3$ (mean $\pm$ s.d.), which increased relatively slowly to reach $126.9 \pm 24.7 \text{ mm}^3$ at the 44th week.
ii) Tumor grew at higher rate in Group II as compared to Group I. The difference was small and reached a significant level only at the 8th week ($p < 0.01$) and 32nd week ($p < 0.05$).

iii) In Group III, similar tendency as observed for Group II was seen. At the 24th week slightly higher value was obtained, but the difference from Group I was not significant.

iv) In Group IV, tumor grew rapidly to $70.4 \pm 20.9 \text{ mm}^3$ ($p < 0.001$) at 8th week and to $142.9 \pm 17.6 \text{ mm}^3$ at 12th week ($p < 0.001$), with significant inter week differences ($p < 0.01$).

v) In Group V which received IAP at increased dosage and increased frequency, tumor showed stronger growing tendency. At any of the periods, significant difference was seen in tumor size at $p < 0.001$ when compared to Group I. There was also significant difference in growth curve of tumors between Group IV and Group V, with significantly more rapid growth recorded for Group V ($p < 0.05$).

Volume of cancerated area in tumors

i) In Group I cancertated area was confirmed from the 16th week after the operation. The cancertated area accounted for $5.60 \pm 2.10\%$ ($n = 4$) of the whole tumor, with mean volume of $24.0 \pm 1.06 \text{ mm}^3$. Subsequently, the cancertated area increased with time to reach a volume of $16.83 \pm 9.96 \text{ mm}^3$ at 44th week.

ii) In animals of Group II, cancertated area appeared from the 12th week after the operation. At the 16th week the cancertated area accounted for $8.44 \pm$
Fig. 7. Changes in volume of cancerated area in DMBA-induced pancreatic tumors in rats (mean value).

- Group I (n=60); - Group II (n=48); - Group III (n=18);
- Group IV (n=30); - Group V (n=12).

Fig. 8. Changes in serum level of human IAP injected intravenously in rats. White rings show precipitation ring in SRID method which indicate IAP levels of serum. No. 1 and No. 6 are standard solutions containing IAP at 250 μg/ml and 1,000 μg/ml, respectively. The levels immediately after the administration are shown in No. 3 and No. 8, after 3 hr in No. 4 and No. 9, 10 hr in No. 5 and No. 10, 24 hr in No. 2 and No. 7, respectively.
3.44% of the whole tumor, with mean volume of 9.33±3.07 mm³. The difference from Group I was significant (p < 0.01) (Fig. 7).

iii) In Group III, similar tendency as observed for group II was seen.

iv) In Group IV the volume of the cancerated area recorded at 12th week was 19.84±5.59 mm³. From 16th week thereafter cancer grew more rapidly than in Group I with significant difference (p < 0.01).

v) In Group V mean volume as high as 23.76±8.10 mm³ was obtained already at the 8th week.

Fig. 9 (upper). Plate isoelectrophoretic patterns of serum in rats. No. 1 and No. 15 indicate the marker of pH 3.0. No. 2 is the serum of control rat. From No. 3 to No. 8 and from No. 10 to No. 13 are serum of rats in which tumor grew within 500 mm³ in volume. No. 9 and No. 14 are serum of rats in which tumor grew over 500 mm³ in volume.

Fig. 10 (lower). Plate isoelectrophoretic pattern of serum in rats induced pancreatic tumors over 500 mm³ in volume. No. 1 and No. 9 indicate the marker of pH 3.0. No. 2 is the serum of the control rat. Volume of the tumors are 1,473.2 mm³ in No. 3, 1,603.5 mm³ in No. 4, 95,793.4 mm³ in No. 5, 506.8 mm³ in No. 6, 623.2 mm³ in No. 7 and 78,695.2 mm³ in No. 8, respectively.
As described above, in the group received IAP at higher frequency and dosage the growth of tumor and the increase in the volume of cancerated tumor area was accelerated.

**Changes in serum level of human IAP injected intravenously**

As to the serum level of IAP rats injected the drug intravenously into the tail vein, the level immediately after the administration of IAP at 6 mg was 556.7 ± 12.5 \( \mu g/ml \) \((n = 3)\), which decreased to about one half \((260 ± 21.6 \mu g/ml)\) at 3 hr later. Subsequently the serum IAP level further lowered to 163.3 ± 20.5 \( \mu g/ml \) at 10 hr and 80.0 ± 10.5 \( \mu g/ml \) at 24 hr. No detectable amount of IAP was observed at 48 hr, and precipitation ring was not found at the 72nd hr (Fig. 8). Similar elimination curve was obtained for IAP given additionally at 1 and 2 weeks later.

**Serum acidic protein in rats**

With sera from control animals and from animals developed of tumor of less than 500 mm\(^3\) in volume no clear band appeared in the acidic area in electrophoresis. When taken out from the staining solution and removed of excessive staining, the sera became almost free from IAP. No. 1 and No. 15 in Fig. 9 showing electrophoretic patterns of human IAP used as the control marker gave clearly one band. Specimens of No. 9 and No. 14 from rats with tumor volume of 623.2 and 506.8 mm\(^3\), respectively, gave several bands when subjected to electrophoresis. On the other hand, sera of No. 2 from control rats and those from No. 3 through No. 8 as well as from No. 10 through No. 13 from rats with tumor less than 500 mm\(^3\) in volume showed no clear band in the acidic area (Fig. 9). Sera of rats in which tumor greater than 500 mm\(^3\) in volume developed showed several bands in the acidic area as shown in Fig. 10, with heavier bands for greater tumors. The histologic types of tumors of rats, whose sera contained acidic protein as evidenced by appearance of bands as plate isoelectric focusing, were classified as **tub.** type for 2 cases, **pleo.** type for 4 cases and **ost.** type for 2 cases.

**DISCUSSION**

Carcinogenic agents can be classified into biological, chemical and physical agents. Chemical agents have been chiefly used for experimental induction of pancreatic carcinoma. Following intraperitoneal injection of azaserine in Wistar rats, only adenoma was seen up to 8 months, but carcinoma associated with metastatic lesions in other organs such as the liver developed in 34.1% of rats observed more that 1 year after the injection (Longneker and Crawford 1974). However, carcinoma induced with the method was of acinar cell type and developed more frequently in organs other than the pancreas.

The method described by Pour et al. (1974) and Nakazawa et al. (1977), in which Syrian-Golden hamsters were treated with di-n-propylnitrosamine (DHPN) and di-isopropanolnitrosamine (DIPN), gave more favorable results in terms of the
incidence of carcinoma formation and the period required for development of carcinoma. Moreover, the method was superior to other methods in that ductal type pancreatic carcinoma could be induced. However, it was associated with drawbacks of high mortality before induction of carcinoma and high incidence of tumors found in other organs: Denda, Inui and Konishi (1977) injected 4-HAQO intravenously on the 3rd day after partial pancreatectomy when pronounced DNA synthesis was seen and succeeded in inducing carcinoma in all of the animals thus treated. Most of the carcinomas they obtained with their method, however, were of acinar cell type.

It is known that more than 90% of pancreatic carcinomas found in humans are adenocarcinoma originated from the pancreatic duct. Therefore, the ideal experimental model for pancreatic carcinoma should provide tumors of histologic type resembling human pancreatic carcinoma. As to induction of carcinoma originated from the pancreatic duct, Pour et al. (1977) reported favorable results obtained by giving N-nitrosobis (2-oxopropyl) amine (BOP) to Syrian Golden hamsters. Dissin et al. (1975) and Satake et al. (1975) who embedded DMBA directly into the pancreas of SD rats also reported relatively favorable results. None of these experimental models were not completely satisfying conditions required for experimental models since they were associated with advantages, as well as drawbacks in terms of the induction rate of carcinoma, specificity for the pancreas, resemblance to human pancreatic carcinoma and the time required for induction. It has been shown experimentally that induction of carcinoma consists of initiation and promotion. The present authors used DMBA, which has been proven to induce adenocarcinoma with high incidences, as the carcinogenic substance and IAP prepared and purified from ascites of cancer bearing humans as the promotive factor in order to induce pancreatic carcinoma experimentally.

In 1977 Matsuda et al. found the presence of IAP in the serum of cancer bearing mice. This acidic protein was also detected in the serum of cancer bearing humans and shown to be a glycoprotein having almost similar isoelectric point and molecular weight. In the present study, acidic protein which had isoelectric point almost corresponding to that of human IAP and which was stained by Coomassie brilliant blue was detected in the serum of rats in which large tumor developed. It could not be made clear from the results of the present study whether the acidic protein isolated from these rats has immunosuppressive activity or which one of the several bands appeared in the acidic area in electrophoresis corresponds to IAP from rats. Little or no acidic protein, however, was detected in control rats, whereas the amount of acidic protein in rats developed of tumor tended to increase with growth of tumors. These findings indicate that in pancreatic carcinoma in rats also special acidic protein has close relation to its formation.

As to the relation between immunity against tumor and immunosuppressive factor, Urushizaki and Ishitani (1980) pointed out that suppression of immunor-
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Reaction itself contributes to acceleration of progression in cancer, in turn, extension of cancer promotes aberration in immune response. Human IAP injected intravenously into the tail vein in rats was eliminated from the serum within 72 hr. However, it is highly possible that human IAP exerted immunosuppressive activity in vivo while it is metabolized, which produced environment favorable for development of cancer in rats with induction of new appearance of immunosuppressive substance intrinsic for rats. Shibata et al. (1983) treated mice with human IAP and found that serum level of human IAP decreased to one half at 3 hr later and no detectable amount of IAP was found at 48 hr. They reported, however, that mouse IAP began to increase from 4 to 6 hr after the injection of human IAP to reach 3 times as high as normal amount at 10 hr later. Moreover, IAP was reported to enhance a taking rate of methylcholanthrene induced sarcoma implanted in mice and to promote its growth. Also in the present study, in the group treated with human IAP in addition to embedding of DMBA the period required for development of carcinoma was shortened to 8 weeks and the volume of cancerated area at the 16th week was increased to 8 to 9 times the volume recorded for the group untreated with IAP (treated with DMBA alone). These results indicate that human IAP has a promotive effect in carcinogenesis also in rats.

In the experimental model described here, tumors resembling human pancreatic carcinoma could be induced with high incidences and within a period clearly shorter as compared to that of conventional experimental models. Therefore, this model is considered to be useful since it provides experimental carcinoma assuredly and efficiently. In these experiments formation of pancreatic carcinoma after embedding of DMBA was promoted by human IAP, which was assumed to be attributable to immunosuppressive effect of human IAP. The efficacy of human IAP in promotion of experimental carcinoma became greater with an increase in dosage and frequency of administration in the early phase of induction.

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References

sive acidic protein (IAP) and its diagnostic evaluation. *Igaku no Ayumi*, **115**, 423–433. (Japanese)


