Effects of DNA Isolated, Limonin, α-Interferon, Interleukin-2 and Tamoxifen Citrate, Aqueous Extract of Eucalyptus Globulus Leaves on Amount of DNA in Cancerous Organs of Female Mice

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Summary: The aim of this study was to determine the effects of calf DNA, limonin, α-interferon, interleukin-2 and tamoxifen citrate, aqueous extract of *Eucalyptus globulus* on amount of DNA in cancerous organs of female mice. Tumor cells were carried out by injecting 0.5% ml/25g/day dimethylbenzanthracene (100 µg/ml DMBA) to the mice at average 48 days. DNA was isolated from six monthly calf thymus. *E. globulus* extract and limonin, α-interferon, interleukin-2 led to a significant lowering of organ and tissue DNA levels in experimental groups ($p<0.01$). Distinct injection practices of agents were exposed miscellaneous findings of DNA quantity related cancerous organs. DNA quantities of hepatocellular carcinoma, large cell carcinoma of lung, liposarcoma, renal cell carcinoma, adenocarcinoma of colon, craniopharyngioma, pineal parenchymal tumor, breast adenocarcinoma, meningioma, medulloblastoma, leukemia, thymoma, adenosarcoma of the uterus, rhabdomyom, sarcoma of heart, adenocarcinoma of stomach, hodgkin lymphoma and multiple myeloma cells were decreased significantly by agents treatmented ($p<0.01$). Particularly, 0.5 ml (50 mg/ml) *E. globulus* extract and limonin, α-interferon, interleukin-2 were significantly decreased DNA quantity than controls ($p<0.01$).

Introduction

There have been enormously experimental studies concerning DNA, limonoids, tamoxifen, interleukin and interferon, cancer cells [1-28]. Limonoids have been shown to inhibit breast cancer cellular proliferation in estrogen receptor negative (ER^-) and positive (ER^+) cells. Nude mice were given limonoids in both treatment and adjuvant-based models to observe their effects on the proliferation of established and resected tumours, respectively. It was found that limonoids, particularly limonin, slowed down tumour growth and was able to prevent or delay the regrowth of resected tumours in these models. Additionally, limonoids were detected in mammary tissue samples of mice from the treatment model experiment, indicating that these compounds are bioavailable and were responsible for the observed results. In the present studies, we tested deacetylnomilin, deoxylimonin, ichangin, isoobacunoic acid, limonol, limonin carboxyrnethoxime, methyl deoxylimonate, methylnomilinate, methyl deacetylnomilinate, methyl isolimonate, nomilin glucoside, nomilinic acid glucoside, 7a-obacunol, obacunone and obacunone glucoside for their ability to inhibit the proliferation of MDA-MB-435 ER^- and MCF-7 ER^+ human breast cancer cells [12-18]. *E. globulus* is a medium to very tall forest tree which may reach 70 metres in ideal conditions but is more commonly 15-25 metres in height. In very harsh and exposed conditions it can adopt a shrubby habit. The tree has a rough, greyish bark which is shed on the upper trunk and branches in long ribbons. The white flowers occur from winter to early summer. They are followed by greyish, 3 to 5-valved fruits. Leaves contain 70-80% eucalyptol [19,20], also includes terpineol, sesquiterpene, alcohols, aliphatic aldehydes, isoamyl alcohol, ethanol, terpenes, camphene, citronellal, fenchene, phellandrene and cineole. Tannin is not so copious in the leaves as of many other *Eucalyptus* species. The kino, containing 28.7% kino-tannin and 47.9% catechin contains the very antibiotic citriodorol [19,20].

Fresh leaves contain caffeic and gallic acids, however, dry leaves contain ferulic and gentisic, and quercetol, quercitrine, rutin, and a mixture of quercetol hyperoside and glaucoside. *N*-titriacontan-16,18-dione was identified as the compound responsible for antioxidant activity in the leaf wax

The primary chemical constituents of eucalyptus include essential oils (cineol, pinene), polyphenolic acids (caffeic, gallic), {http://www.viable-herbal.com/singles/herbs/s906.htm} (eucalyptin, hyperoside, rutin), tannins, aldehydes, and bitter resins. The properties of the oils from different species vary slightly, but all are very antiseptic.

Extensive studies had been carried on steroid hormones and DNA, however, no more studies are evaluated the effects of DNA purified, limonin, α-interferon, interleukın-2 and tamoxıfen cıtrate, aqueous extract of *E. globulus* on amount of DNA in cancerous organs of female mice. Therefore, this study was conducted on female mice.

Results and Discussion

Findings related experiments were shown in Table 1-4. DNA purified from healthy calf was injected as I.V., I.P. and I.M. to the mice. Exactly like, DNA was carried out as DNA therapy. According to table 1, differnt injection methods of DNA were decreased significantly the DNA quantity in all cancerous organs than control ($p<0.01$). Also, in Table 2, aqueous extract of *Eucalyptus globulus* leaves was significantly decreased DNA quantity than control ($p < 0.01$) but was increased ($p<0.01$). On the other hand, in Table 3, limonin led to decreasing in DNA quantitiy in experimental groups than control ($p<0.01$). In Table 4, α-interferon, interleukin-2 and tamoxifen citrate were caused the considerble reduce in DNA quantities ($p<0.01$).

Various injection methods of agents were put forward different reults. Intra venous injection of agents was shown that I.V. applications were more effected. DNA quantities of cancerous organ or tissues were decresed significanly in effects of aqueous extract of *E. globulus* leaves, limonin, α-interferon, interleukin-2 and tamoxifen ($p<0.01$). These reults showed that, agents have destructed cancer cells or their DNA. In this way, it appears a concept with concerning DNA chelating but it is not become clear. Quantities of DNA isolated from hepatocellular carcinoma, large cell carcinoma of lung, liposarcoma, renal cell carcinoma, adenocarcinoma of colon, craniopharyngioma, pineal parenchymal tumor, breast adenocarcinoma, meningioma, medulloblastoma, leukemia, thymoma, adenosarcoma of the uterus, rhabdomyom, sarcoma of heart, adenocarcinoma of stomach, hodgkin lymphoma and multiple myeloma cells have decreased more than control groups ($p<0.01$). These results shown that cancer cells analyzed have been influenced by agents tested. However, reduction in DNA quantity of cancerous organ or tissues is considerable fix. Also, tamoxifen citrate, α-

Table 1: Effects of distinct injection practices of calf DNA purified on amount of DNA in cancerous organs, cell and glands of female mice.

Cancerous organ and gland	DNA Quantity (µg) Injection of 0.5 ml thymus DNA isolated (50 µg/ml) per 25 g weight and three times in a week and during 60 days			
	Intra venous (I.V.), N: 10	Intra periotonal (I.P.), N:10	Intra muscular (I.M.), N:10	Control , N:10 (without DNA injection)
	After 60 daily treatment			
Hepatocellular carcinoma	785± 2.27[a]*	515±1.90[b]	410±0.19[c]	1298±3.76[d]
Large cell carcinoma of lung	764±3.35[a]	526±4.82[b]	398±2.76[c]	1302±1.00[d]
Liposarcoma	753±4.87[a]	520±2.93[b]	404±3.19[c]	1313±4.21[d]
Renal cell carcinoma	749±1.11[a]	500±3.44[b]	396±2.73[c]	1300±3.67[d]
Adenocarcinoma of colon	673±4.98[a]	356±4.21[b]	256±5.27[c]	1152±2.20[d]
Craniopharyngioma	285±2.00[a]	202±3.01[b]	192±4.42[c]	1119±3.09[d]
Pineal parenchymal tumor	274±4.97[a]	265±6.21[a]	266±3.33[a]	1123±1.04[b]
Infiltrating ductal carcinoma of breast	657±3.00[a]	668±1.06[a]	667±2.20[a]	1283±2.04[b]
Meningioma	873±5.52[a]	612±2.01[b]	489±4.90[c]	1316±3.80[d]
Medulloblastoma	902±4.98[a]	710±1.11[b]	602±2.04[c]	1412±5.66[d]
Leukemia	982±2.00[a]	978±3.90[a]	712±4.55[b]	1345±3.78[c]
Thymoma	903±5.99[a]	900±2.21[a]	734±6.10[b]	1713±2.01[d]
Adenosarcoma of the uterus	975±6.27[a]	982±5.52[a]	672±3.86[b]	1442±2.49[c]
Rhabdomyom	799±6.77[a]	789±3.92[a]	734±2.05[b]	1663±1.11[c]
Sarcoma of heart	523±2.89[a]	534±5.91[a]	543±6.91[b]	1621±2.89[c]
Adenocarcinoma of stomach	452±6.77[a]	461±1.03[a]	472±3.41[a]	1291±1.89[b]
Hodgkin lymphoma	526±3.33[a]	529±5.51[a]	310±3.07[b]	1276±5.91[c]
Multiple myeloma	779±6.92[a]	768±7.82[a]	957±7.20[b]	1429±5.01[c]

* Values shown with different letters in the same column are significant ($p<0.01$) ; but values shown with same letter are not significant ($p>0.05$).

Table 2: Effects of distinct injection practices of aqueous extract of *Eucalyptus globulus* leaves on amount of DNA in cancerous organs, cell and glands of female mice.

Cancerous organ and gland	DNA Quantity (µg) Injection of 0.5 ml (50 mg/ml) *Eucalyptus globulus* leave extract per average 25 g weight and three times in a week and during 60 days			
	Intra venous (I.V.), N:10	Intra periotonal (I.P.), N: 10	Intra muscular (I.M.), N: 10	Control, I.V. without cancer, N: 10
	After 60 daily treatment			
Hepatocellular carcinoma	610± 1.02[a]	400±4.23[b]	367±2.74[b]	1201±4.98[c]
Large cell carcinoma of lung	563±6.63[a]	423±5.03[b]	275±1.01[c]	1771±7.20[d]
Liposarcoma	613±2.20[a]	497±4.82[b]	386±4.08[c]	1973±3.00[d]
Renal cell carcinoma	521±2.22[a]	428±0.93[b]	423±5.02[b]	1289±7.34[c]
Adenocarcinoma of colon	545±6.66[a]	400±6.00[b]	298±3.77[c]	1163±3.84[d]
Craniopharyngioma	126±1.19[a]	134±7.31[a]	137±0.29[a]	973±4.55[b]
Pineal parenchymal tumor	117±4.55[a]	120±4.90[a]	125±3.02[a]	974±5.67[b]
Breast carcinoma	146±2.11[a]	386±5.90[b]	392±2.75[b]	938±4.02[a]
Meningioma	635±8.67[a]	498±8.98[b]	301±5.90[c]	921±5.38[d]
Medulloblastoma	733±7.88[a]	602±5.50[b]	499±8.83[c]	1409±7.74[d]
Leukemia	730±3.02[a]	739±6.63[a]	605±6.90[b]	1257±4.45[c]
Thymoma	1120±6.97[a]	924±1.00[b]	598±7.28[c]	1926±8.03[d]
Adenosarcoma of the uterus	702±4.87[a]	707±3.99[a]	525±3.94[b]	1245±3.01[c]
Rhabdomyom	503±7.93[a]	526±2.45[a]	511±6.77[b]	1431±5.52[c]
Sarcoma of heart	410±6.63[a]	419±1.11[a]	634±2.20[b]	1420±5.47[c]
Adenocarcinoma of stomach	334±4.00[a]	342±3.33[a]	376±7.35[a]	1173±4.98[b]
Hodgkin lymphoma	410±7.73[a]	427±6.91[a]	303±1.03[b]	1153±1.04[c]
Multiple myeloma	498±3.03[a]	504±6.09[a]	519±0.98[b]	1349±4.20[c]

* Values shown with different letters in the same column are significant ($p<0.01$) ; but values shown with same letter are not significant ($p>0.05$).

Table 3: Effects of distinct injection practices of limonin on amount of DNA in cancerous organs, cell and glands of female mice.

Cancerous organ and gland	DNA Quantity (µg) Injection of 0.5 ml (50 mg/ml) limonin per average 25 g weight and three times in a week and during 60 days			
	Intra venous (I.V.), N:10	Intra periotonal (I.P.), N: 10	Intra muscular (I.M.), N: 10	Control, I.V. without cancer, N: 10
	After 60 daily treatment			
Hepatocellular carcinoma	610± 1.02[a]	400±4.23[b]	367±2.74[b]	801±4.98[c]
Large cell carcinoma of lung	563±6.63[a]	423±5.03[b]	275±1.01[c]	971±7.20[d]
Liposarcoma	613±2.20[a]	497±4.82[b]	386±4.08[c]	973±3.00[d]
Renal cell carcinoma	521±2.22[a]	428±0.93[b]	423±5.02[b]	989±7.34[c]
Adenocarcinoma of colon	545±6.66[a]	400±6.00[b]	298±3.77[c]	963±3.84[d]
Craniopharyngioma	126±1.19[a]	134±7.31[a]	137±0.29[a]	673±4.55[b]
Pineal parenchymal tumor	117±4.55[a]	120±4.90[a]	125±3.02[a]	774±5.67[b]
Breast carcinoma	151±2.11[a]	386±5.90[b]	392±2.75[b]	1148±4.02[a]
Meningioma	635±8.67[a]	498±8.98[b]	301±5.90[c]	1221±5.38[d]
Medulloblastoma	733±7.88[a]	602±5.50[b]	499±8.83[c]	1409±7.74[d]
Leukemia	730±3.02[a]	739±6.63[a]	605±6.90[b]	1257±4.45[c]
Thymoma	1120±6.97[a]	924±1.00[b]	598±7.28[c]	1904±8.03[d]
Adenosarcoma of the uterus	702±4.87[a]	707±3.99[a]	525±3.94[b]	1245±3.01[c]
Rhabdomyom	1503±7.93[a]	1526±2.45[a]	1101±6.77[b]	1943±5.52[c]
Sarcoma of heart	1410±6.63[a]	1419±1.11[a]	634±2.20[b]	1956±5.47[c]
Adenocarcinoma of stomach	334±4.00[a]	342±3.33[a]	376±7.35[a]	973±4.98[b]
Hodgkin lymphoma	410±7.73[a]	427±6.91[a]	303±1.03[b]	953±1.04[c]
Multiple myeloma	498±3.03[a]	504±6.09[a]	519±0.98[b]	1539±4.20[c]

* Values shown with different letters in the same column are significant ($p<0.01$) ; but values shown with same letter are not significant ($p>0.05$).

interferon, interleukin-2 and aqueous extract of *E. globulus* leaves have effected on cancer cells tested. I.V, I.P. and I.M. injections of agents have caused the enormusly increasing in control groups. DNA quantities in control groups have been influenced as affirmative. Thymus DNA isolated from helathy calf may have ceased increasings in these cancer cells and apparently, DNA of cancerous cell has destroyed. According to our study, healthy DNA protects the control cells and increases their quantity of DNA. Limonin, *E. globulus* extract, tamoxifen citrate, α-interferon and interleukin-2 have effected similarly to

Table 4: Effects of *distinct* injection practices of α-interferon, interleukin –2 and tamoxifen citrate on amount of DNA in cancerous organs of female mice.

Cancerous organ and gland	DNA Quantity (μg) α-Interferon , Interleukin –2 and Tamoxifen citrate were treatmented per average 25 g weight and three times in a week and during 60 days			
	α-Interferon (α-INF) (Alferon 25 mcg, 10μl I.V.) N:10	Interleukin-2 (IL-2) (Aldesleukin 25 mcg, 10μl I.V.) N: 10	Tamoxifen citrate (Nolvadex, 10μl oral) N: 10	Control without cancer N: 10
	After 60 daily treatment			
Hepatocellular carcinoma	210± 3.52[a]	200±1.04[a]	197±3.90[a]	401±4.98[b]
Large cell carcinoma of lung	263±6.53[a]	257±7.03[a]	275±1.93[a]	371±7.20[b]
Liposarcoma	316±2.20[a]	156±4.09[b]	164±3.87[b]	415±7.32[c]
Renal cell carcinoma	335±4.87[a]	327±4.62[a]	231±6.65[b]	410±5.35[c]
Adenocarcinoma of colon	210±5.08[a]	219±4.90[a]	147±4.09[b]	356±1.19[c]
Craniopharyngioma	187±0.26[a]	190±1.99[a]	116±3.98[b]	442±6.06[c]
Pineal parenchymal tumor	220±7.34[a]	223±3.65[a]	201±3.00[a]	389±3.21[b]
Breast carcinoma	324±5.35[a]	339±7.95[a]	126±1.09[b]	342±3.90[a]
Meningioma	289±6.98[a]	295±4.56[a]	472±5.90[b]	579±2.98[c]
Medulloblastoma	378±8.23[a]	386±7.39[a]	245±2.00[b]	556±4.23[c]
Leukemia	210±5.29[a]	228±3.79[a]	119±3.89[b]	300±7.70[c]
Thymoma	354±2.20[a]	368±4.54[a]	173±2.09[b]	499±7.33[c]
Adenosarcoma of the uterus	229±8.30[a]	237±5.20[a]	104±4.40[b]	398±4.06[c]
Rhabdomyom	235±4.02[a]	241±1.09[a]	103±2.75[b]	387±3.65[c]
Sarcoma of heart	245±2.89[a]	254±3.21[a]	109±5.45[b]	445±5.66[c]
Adenocarcinoma of stomach	290±3.01[a]	301±5.02[a]	105±5.30[b]	400±1.11[c]
Hodgkin lymphoma	198±7.73[a]	202±7.36[a]	214±4.00[a]	665±6.22[b]
Multiple myeloma	488±5.55[a]	505±6.09[a]	234±5.99[b]	897±6.77[c]

* Values shown with different letters in the same column are significant ($p<0.01$); but values shown with same letter are not significant ($p>0.05$).

that of DNA isolated. Tamoxifen citrate found to be more toxic than interferon and interleukin-2 and limonin. Therefore, injection of DNA isolated may use in therapy in cancerous organ or tissues.

The medication α-interferon (IFN) is a synthetic reproduction of the naturally produced interferon. Interferons are natural substances produced by cells in the body to help fight against infections and tumors. There are synthetic versions of these substances. α-Interferons are used to treat hairy cell leukemia, malignant melanoma, and AIDS-related Kaposi's sarcoma. They are also used to treat laryngeal papillomatosis in children, genital warts, and some kinds of hepatitis. α-Interferons may cause some people to become unusually tired or dizzy, or less alert than they are normally. IFN therapy also showed efficacy in viral tumors and chronic hepatitis B and C. The antitumoral action of α-IFN mainly involves its capacity to inhibit cell proliferation, partly *via* antagonistic effects on growth factors. The elucidation of α-IFN signalling pathway(s) leading to gene activation, a better understanding of the interactions between α-IFN and cytokine network, and the development of combination therapy with other biological treatments or chemotherapy should greatly improve the clinical use of IFNs [21-28].

Aldesleukin is in a class of drugs known as cytokines. It is similar to a chemical substance as the body produces. Aldesleukin increases the body's ability to fight against cancer. In addition, aldesleukin stimulates the body to produce other chemical substances which enhance the body's ability to fight against cancer.

A number of chemical constituents were isolated from *Eucalyptus* species **3**-penten-2-ol, 1,8-cineole, γ-terpinene, β-ocimene, 3-hydroxy-2-butanone, 2,5-dihydrofuran, 5-hexen-2-ol, linalool, terpinen-4-ol, α-terpinol, 3,4-pentadienal, nerolidol, thymol and globulol. *Eucalyptus globulus* is a fine stimulating expectorant for bronchopulmonary catarrhal disorders, when no very active inflammation is present. It restrains discharges, facilitates expectoration, and deodorizes and antisepticizes the sputum. Chronic bronchitis, bronchorrhea, and the debility, with difficult expectoration, lingering in the wake of bronchopneumonia and lobar pneumonia are conditions in which it has very great value. Agents of this type, which may be compared to the turpentines, and which influence the respiratory membranes, usually are valuable for similar uses in the urinogenital tract. Thus we find eucalyptus as an alterative and antipyic in pyelitis and in catarrhal and

purulent cystitis, particularly in the aged. Being eliminated by all the mucous surfaces, it exerts its antiseptic influence upon them in all parts of the body. *Eucalyptus* is a stimulating antiseptic for the angina of scarlatina, for which it is administered internally. This should be done with great care, however, for the drug is liable to produce congestion of the kidneys, one of the dreaded complications which are easily provoked in the acute exanthemata. If acute desquamative nephritis is present it should not be employed; in the advanced stage of chronic nephritis with very marked fetor in the urine, and scanty secretion of urine, very small doses of eucalyptus may be cautiously tried. Eucalyptol is especially much employed in subacute inflammations and chronic diseases of the bronchopulmonic tract, with fetor, relaxation and abundant secretions. Used upon cancerous surfaces they mask the fetid odor and give some relief from pain [19,20].

Citrus fruits are popular and have established nutritional and medicinal values. Juices from most of the citrus varieties are facing commercial problems due to bitterness, caused by limonoids, a group of chemical compounds which highly oxygenated tetracyclic triterpenoids. Among different citrus limonoids, limonin and nomilin are the primary causes of bitterness. The limonoid content in citrus fruits reportedly decreases during the process of ripening. This decrease is considered to be due to conversion of limonoid to a corresponding glucoside. In citrus juice, the glucoside levels are much higher than the free limonoids. The free limonoids cause a bitterness problem after juice preparation. Some reports have suggested that limonoids and their glucosides have many health benefits, including prevention of cancer. For this reason, researchers are putting emphasis on alternative ways to remove bitterness without affecting the levels of limonoids or their metabolites. Several classes of bitter citrus compounds have looked promising as anticancer agents in laboratory tests. A study indicates that long-term consumption of orange juice, a sourcc of such chemicals reduces the risk of cancer in rats. In test tube studies, one class of these bitter compounds: flavonoids has inhibited the growth of breast cancer cells [20]. Similar studies showed that bitter citrus limonoids, protect against cancer in animals. Orange juice drinking would have a beneficial effect and data showed that orange juice helps to protect against cancer. This study might also apply to breast, prostate, and lung cancers [12,15,16,17,19]

Our data show that citrus limonin protect against chemically induced cancerous organs in lab animals. These agents have inhibited tumors in the cancerous tissues of mice with agents tested. Indeed, there is no doubt that these anticancer compounds are bioavailable in animals to the site of a cancer. The question remains whether they are similarly available in human being. To find out this, we will soon begin measuring the human body's uptake of present agents.

Experimental

Limonin, α-interferon, interleukin-2 were obtained from Sigma Chemical Co. (Poole, Dorset, UK). The purity of the limonoids was evaluated at >99%. Dimethylbenzanthracene was purchased from E. Merck (Darmstadt, Germany). The *E. globulus* seeds and leaves were collected from Mersin (Karacailyas) in Turkey in the months of March, April and May in year 2004. Aqueous extract of *Eucalyptus globulus* was prepared from fresh leaves. All animals were selected from Experimental Animal House of Department of Biology, Faculty of Sciences and Arts, Dumlupınar University, Kutahya, Turkey. Albino mice were divided into sixteen treatment groups, four control and twelve experimental groups. Animals were used in a completely randomized design experiment. Treatments were arranged in 160 rats and there were 10 mice (*Mus musculus*) in each group. Mice were fed with pellet food contained 17% animal protein ration.

Experiment 1

Extraction of total DNA from healthy calf thymus gland (2-10):

Total DNA was isolated from thymus of six month old calf. No more than 30 minutes after the glands had been removed from the animal and they were frozen by dry ice. When so preserved, the glands could be stored at -30 °C for several days prior to processing; if the glands were frozen later than 2 h after removal, the final product may already be damaged as tested by its stability to heat. Fifty grams of frozen glands were thawed, freed from fat, cut into small pieces, and washed with 5x100 ml ice-cold portions of 0.1 *M* aqueous sodium citrate (pH 7.4) or 0.1 ethylenediaminetetraacetate (EDTA) (pH 7.35).

The mixture was then homogenized in a cooled glass tissue grinder with addition of 250 ml of

ice-cold sodium citrate or EDTA as above. The homogenate was centrifuged for 30 minutes at 1800 x g and the supernatant discarded. The residue was then stirred in 250 ml of sodium citrate or EDTA and centrifuged as before. This operation was repeated two more times and the final residue was extracted with 1000 ml of 2 *M* aqueous NaCl solution. The extraction was best accomplished by resuspending small portions of the residue in 2 *M* NaCl homogenizing in a cooled glass tissue grinder for 3 minutes and gradually adding more 2 *M* NaCl solution until the total volume for this portion had been used up and the residue was uniformly distributed. The mixture was left at 4 °C for one day and then centrifuged for 1 h at 1900 x g. To the opalesecent supernatant, 2 vol of absolute ethanol was added at a rate of 150 ml/h with constant slow swirling of the recipient flask; the resulting fibers were lifted, washed with 75% aqueous ethanol, drained, and redissolved, as described before, in a cooled tissue grinder in 1000 ml of 0.14 *M* aqueous NaCl solution made 0.015 *M* with respect to sodium citrate (pH 7.1), thereafter called "standard buffer." To this solution was added 1/9 vol of 5% duponol in 45% aqueous ethanol and the mixture was stirred for 1 h at room temperature. Solid NaCl was then added, to obtain a final concentration of 5% NaCl. The mixture was stirred for anoher half an hour at room temperature and then left overnight at 4 °C. The mixture which contain precipitated duponol was then centrifuged for 1 h at 31.000 x g; to the clear supernatant, 2 vol of ethanol redissolved in 1000 ml of standard buffer with 1/9 vol of 5% duponol, and the mixture was stirred for 1 h. Solid NaCl was then added to obtain 5% NaCl solution, and the mixture was stirred, stored, and centrifuged, as described before. To the final clear supernatant, 2 vol of absolute ethanol was slowly added, as described before, and the fibers were lifted, washed with 75% ethanol, and redissolved in standard buffer to yield a clear, very viscous solution, containing 1 to 1.5 mg of DNA/ml. The product was stored in the frozen state at -15 °C. When so stored, it does not undergo any demonstrable change for at least one year. Storage in nonbuffered of low ionic strength as well as drying should be avoided, as they invariably lead to a degraded product and drying with absolute ethanol and ether is particularly harmfull. All the steps were performed at 0 to 4 °C, with glass, rubber, or plastic tools and vessels. In particular minute traces of rust ($Fe^{++} \rightarrow Fe^{+++}$) cause rapid degradation, for this reason the use of a blender shold be avoided.

All the steps from thawing the gland until the first alcohol precipitation were performed as fast as possible; otherwise, owing to the action of thymus DNAse, a product is obtained which can be shown to be degraded. The above described precautions refer to DNA from all sources investigated thus far. DNA obtained from organs was in 87% purified form. Also, double stranded (ds) calf thymus DNA (D-1501), was purchased from Sigma.

Experiment 2.

First of all, pre-experimental studies were realized sufficiently on another 13 mices. After pre-studies, tumor cells were carried out by injecting 0.5 ml/25g/day dimethylbenzanthracene (100 DMBA µg/ml) at average of 48 days. To obtain tumor, DMBA was injected as 0.5 ml/25 g/day within each organ per day during 60 days period. Between 40 and 48 days, eyes of three mice were happen cross-eyed and diarrhoae. After 40 to 48 daily injection of DMBA, hepatocellular carcinoma, large cell carcinoma of lung, liposarcoma, renal cell carcinoma, adenocarcinoma of colon, craniopharyngioma, pineal parenchymal tumor, breast adenocarcinoma, meningioma, medulloblastoma, leukemia, thymoma, adenosarcoma of the uterus, rhabdomyom, sarcoma of heart, adenocarcinoma of stomach, hodgkin lymphoma and multiple myeloma cells were obtain.

Experiment 3.

Agents were applied to the cancerous mice. Treatments were arranged as 0.5 ml (50 µg/ml) DNA isolated from calf thymus, 0.5 ml (50 mg/ml) leave extract of *Eucalyptus globulus*, 0.5 ml (25 mg/ml) limonin, 10µl α-interferon (Alferon 25 mcg), 100µl (100 µg/ml) interleukin-2 (Aldesleukin, 25 mcg) and tamoxifen citrate per average 25 g weight and three times in a week during 60 days. Tamoxifen was given orally but other agents were adminstrated i.v., i.m. and i.v. to mice.

Experiment 4.

Mice in each group were sacrificed under the sodiumpentobarbital anesthezied and cancerous organs and tissues were removed immadiately and DNA quantities of these cancerous organ or tissues were determined by extracting in the similar way of thymus process.

Statistical analysis.

ANOVA followed by Dunkan test was used to see differences amongst various groups; and means ± SEM of percent values were calculated [29].

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