Determination Of The Protective Effect Of The Shark Cartilage And The Shark Liver Oil (SLO) Against Formaldehyde And Dmh Application Causing To Cancer And Dna Damage On Genetic Bases

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Abstract— It is known that DMH, a potent strong carcinogen and formaldehyde (FA) cause to epithelial cell damage. It has been stated that cartilage can be used to treat malignancies and shark liver oils are very rich in 1-O-alkylglycerols with the ability to strengthen the immune system. 80 rats have been classified as follows: 40 DMH Group: 4 control - 6; given DMH but not cured - 15; given DMH and cured with SC - 15; given DMH and cured with SLO 40 Formaldehyde group: 4 control - 6; Given FA but not cured - 15; given FA and cured with SC - 15; given FA and cured with SLO. The cartilage (SC) administration have a curative effect by increasing the activities of both Tnf-α and p53 genes, against to DMH application, reason of cancer formation and increasing of p53 genes FA application, reason of lung damage. In both application; the SLO administration showed pronounced curative effect mainly in Tnf-α activity. Against to DMH application, reason of cancer formation; the cartilage (SC) was more effective via increasing the p53 gene activities. Liver oil (SLO) application has an better effect on mainly Tnf-α gene. SC showed better effect in p53 activity; and SLO was more effective on Tnf-α gene for lung damage treatment, caused by FA administration.

Index Terms— DMH, FA, Colon cancer, Lung damage, Shark cartilage, Shark liver oil.

1 INTRODUCTION

Cancer, is one of the main mortality factors in our country and in the world. There are several factors to be cancer. Some times it can results from mutations cause in the regulatory mechanism of normal cell growth or can be from deragments of cell differentiation. There are a lot of different control mechanism on the growth of normal cells. Some specific proteins, produced from oncogens, take part in these mechanism responding to growth factors[1].

The five most common cancer types in both sexes are; stomach, lung, breast, colon-rectum and cervical cancers. The frequency of cancer types can show differences on the bases of age, organs, developed by the cancer, sex, and environmental factors [2].

It is known that DMH, a potent strong carcinogen and mimicking the histopathology of human colonic epithelial neoplasms, induces rat colon carcinogenesis. DMH is widely used to stimulate colon cancer formation in rodents [3]. 1,2 Dimethyldihydrazine (DMH) is a potent colon carcinogen and appears to be used extensively in colon cancer studies, especially in relation to diet effects [4].

The FA from the aldehyde group is well soluble in water, colorless, sharp-smelling and has the chemical formula CH2O. Formaldehyde reacts with amino acids in living organisms to form toxic intermediates that cause epithelial cell damage [5-9]. FA is not stored in the body, it is transformed into formic acid by urine and feces (gaita) or carbon dioxide is excreted by respiration as oxides [10]. Formaldehyde is a chemical substance that is also used in the natural structure of the organism, which is widely used because of its chemical properties. It is used for construction of contra plaques, chipboards, insulation materials, paints and plastic materials in industrial area. In medicine, it is used as a fixation solution in anatomy, histology and pathology laboratories and in disinfection clinics.

CAM techniques have been used widely especially within cancer patients in nowadays. The minimal success of traditional treatment or the increasing interest to the natural treatments can be the reason to the CAM techniques by the cancer patients[11]. Recently ginseng, echinacea, ginko, centaurine and aloe vera etc. herbal drugs are found in large quantities in our pharmacy cupboards. WHO has published reports that more than 20,000 plants in use for medical treatment in more than 90 countries. The datas show that the medicinal plants are widely used for the treatment of diseases naming as complementary medicine, in the world and the interest into these plants is increasing every day [2].

The chemical structure of shark cartilage (SC) formulation contains some specific molecules. These are thrombospondin-1, chondromodulin-1, type XVIII-derived endostatin, SPARC (acidic and cysteine rich secretory protein) and type II collagenous derivative N-terminal propeptide (PIIBNP). These specific molecules have been used for antiangiogenic or antioxidant properties. For example, thrombospondin-1, endostatin and shark-cartilage derived Neovastatin preparation have been tested for different types of cancer, but still need to be
improved as cancer preventive agents [12].

Shark Liver Oil (SLO) and Protective Effect

Alkylglycerols and squalene are come up as the most important material in the fight against infection and cancer. High levels of alkylglycerols and squalene, and -3 EFA representing are found in shark liver oil. Therefore, it is seen as an important alternative source for use against to cancers and infections.

It is known that fish oils contain different active compounds which regulate cell activity and perform various functions of the human body. Shark liver oils are very rich in 1-O-alkylglycerols with the ability to strengthen the immune system. In one study, it was found that 1-O-alkylglycerols derived from fish oils were effective in the combination therapy of different types of cancer and antitumor content [13]. With this study, we aimed to investigate the toxic effects of systemically applied formaldehyde on the lungs and the relationship between tumor development in colon and DMH application and the protective effect of SC (shark cartilage) and SLO (shark liver oil) against these toxic effects; at genetic level.

2 MATERIALS AND METHOD:

All the applications has been carried out by taking the report of the Local Ethics Committee of the Animal Experiments in Afyon Kocatepe University.

Animal Material

The 80 rats for this study were grouped as follows;

40 DMH groups:
4 of them: The control group
6: DMH given but untreated group
15 animals: group treated with DMH and shark cartilage
15 animals: group treated with DMH and shark liver oil

40 FA groups:
4 of them: The control group
6: Formaldehyde treated but untreated group
15 animals: Group treated with formaldehyde and shark cartilage
15 of them: Group treated with formaldehyde and shark liver oil

The RNA Isolation from tissue

30 mg of tissue were weighed into RNAse free ependorfs. 300 µl Lysis Buffer Solution was added, pressed and crushed and vortexed for 10 sec. 600 µl Proteinase K solution was added and incubated at 24.5 °C for 10 min. 10 min. Centrifuged at 12,000 g at +4 [deg.] C. at 12,000 g. The lower part of the emerging tube was poured, and the remaining solution was transferred into the upper part. And centrifuged at +4 [deg.] C. at 12,000 g.

700 µl Wash Buffer 1 solution was added. 1 min. Centrifuged at 12,000 g at +4 [deg.] C., the lower part was poured. 600 µl Wash Buffer 2 solution was added. 1 min. Centrifuged at 12,000 g at +4 [deg.] C., the lower part was poured. 250 µl Wash Buffer 2 solution was added. 2 minutes. Centrifuged at 12,000 g at +4 [deg.] C., the lower portions were replaced with new RNAase free ependorfs. 100 µl Nuclease-free water was added, 2 min. centrifuged at +4 [deg.] C. at 12,000 g. Then again 1 min. centrifuged at +4 [deg.] C. at 12,000 g. The isolated RNAs were stored at -70 °C. All phases of this work were done on ice.

Lysis Buffer Solution: 1200 µl Lysis Buffer Solution
25 µL of Mercaptoethanol
Proteinase K solution: 10 µl Proteinase K
590 µl Water
Wash Buffer 1 solution: 8 µl Wash Buffer 1
2 µl ethanol
Wash Buffer 2 solution: 4600 µl Wash Buffer 2
7800 µl ethanol

The Data analysis

The analysis was carried out using the LightCycler 480 instrument using 465-510 channels. Using the values obtained by relative quantitation analysis (Target gene / reference gene), the rates of change of the mRNA expression levels of the target genes were calculated by 2 -ΔΔCt method [14]. In the calculation, these formula was used as: ∆ΔCt = (Ct target gene - Ct App) test group - control group (Ct target gene - Ct App).

The Primers:

β-actin: NC¬- 005111.4 Rattus norvegicus
F 5’: GAGGGAAATCGTGCGTGACAT 3’
R 5’: ACATCTGCTGGAAGGTGGACA 3’

P53: NC¬- 005109.4 Rattus norvegicus
F 5’: CGGAGGTCGTGAGACGCTG 3’
R 5’: CACATGTACTTGTAGTGGATGGTGG 3’

Tnf-α: NC¬- 005119.4 Rattus norvegicus
F 5’: AGCCAGGCAGGTTCCGTCCCTC 3’
R 5’: TTACTGTGCCCACCAGCCGAC 3’

β-actin: Actin is the most abundant protein in eukaryotic cells and has a highly conserved structure. It is expressed that it participates in cellular mechanisms such as cell organization and differentiation of nerve cells. In genetically based studies with mice (house-keeping gene), it is widely preferred as control gene. Because it is stated that it expresses similarly in all cell types and tissues.

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P53: NC¬- 005109.4 Rattus norvegicus
F 5’: CGGAGGTCGTGAGACGCTG 3’
R 5’: CACATGTACTTGTAGTGGATGGTGG 3’

Tnf-α: NC¬- 005119.4 Rattus norvegicus
F 5’: AGCCAGGCAGGTTCCGTCCCTC 3’
R 5’: TTACTGTGCCCACCAGCCGAC 3’
Tnf alpha gene: Tumor Necrosis Factor family polypeptides activate apoptosis by activating apoptosis-stimulating receptors in the immune system. Tnf-α is required for the induction of cytokine cascade, which will produce immune response. Tnf-α also functions in inflammation, wound healing and tissue repair. The TNF molecule has 2 receptors that mediate these effects. These; Type I (CD120a) at a molecular weight of 55 kDa and Type II (CD120b) receptors at a molecular weight of 75 kDa. The TNF gene is located in the 6p21.3 region of the MHC locus on chromosome 6 in human. The Tnf-α is an effective anti-cancer effector produced by immune cells and it has been determined as both of which are useful and a demolition effective proinflammatory cytokine. It has also been expressed that Tnf-α changes mitochondrial complex-I activity, decreases ATP levels and increases levels of reactive oxygen species, mediates tumor progression by stimulating metastasis in proliferation and spread in tumor cells.

P53, or other known tumor protein 53 (TP53), is a transcription factor that regulates cell cycle. In many organisms it is a very important protein to suppress cancer. TP53 protects genomic stability by preventing mutation in the genome. P53 is functionally quadruplicated (tetramer) in the cell. It has been reported that in cell cycle control points, it plays a crucial role in regulation of apoptosis, genetic stability and DNA repair mechanism. P53 translation is activated in response to intracellular and extracellular stress signals, including DNA damage, inadequate nutrition, hypoxia and oncogen activation, leading to increased levels of p53 protein in the cell and accumulation. It has been reported that in response to this stimulus, p53 has been shown to facilitate DNA repair and inhibit tumor cell proliferation [16]. DMH administration has adversely affected particularly p53 gene activity, compared to the control. The same effect is seen at a lower level in the TNF alpha gene. The cartilage application to the adverse effect of DMH can be said to have a curative effect by slightly increasing the activities in both genes compared to the control. SLO administration did not cause a significant change in p53, but Tnf-α also showed curative effect by increasing its activity.

**Fig.1:** In terms of the pathways followed by Tnf-α, it causes to apoptosis via kaspaz or if it goes to seramids and MEKK which leads to life(yaşamsal) activities by gene expression, (via p38) [15].

**Fig.2:** The patways of P53 gene

**Fig.3:** The working profile of Rattus Beta actin control gene

### 3 RESULT
4 DISCUSSION

In studies conducted by other researchers, the DMH administration changes determined as; varied between 10 and 30 mg/kg in the doses of, while ranged from 1 week to 22 weeks in duration, and DMH was administered subcutaneously or intraperitoneally to the animals. In almost all of these studies histopathologic changes have been reported from the colon mucosa [17-23].

In a study by Karaca and colleagues on colon cancer induction, it was observed that repeated doses of DMH resulted in the development of colonic tumors after a long silence period in mice [4].

In a study by Blum et al., they investigated the antiproliferative potential of these substances by giving morine and/or esculetin to the mouse column where they proliferated by giving DMH. As a result, when the oncogenes in the column of rats given DMH were supplemented with morine and/or esculetin, the antitumor effect of these phytochemicals was shown to inhibit cell proliferation [24].

In an experimental study of isolated rat hepatocytes, even low concentrations of formaldehyde have been reported to cause oxidative damage [25].

Thrasher and Kilburn reported that embryo deaths and fetuseanomalies such as cryptorchidism and aberrant ossification venters has increased, the ascorbic acid concentration has reduced and the abnormalities were observed in the endoplasmic reticulum, lysosomes and mitochondry enzymes particularly in the fourth month after birth, when the pregnant rats exposed to formaldehyde before mating, during mating and during pregnancy [26].

In another study, it was determined that formaldehyde application cause to oxidative damage in rat hippocampus inhibited via ω-3 fatty acid application [27].

Kuş and et al. (2004) found that male rats received melatonin at a dose of 25 mg / kg, as well as 10% formaldehyde, which they applied daily for 14 days via i.p appliaction and that formaldehyde exposure resulted in prefrontal cortex oxidative damage and this injury was prevented by melatonin administration [28].

In one study, it was determined that when the cartilage, which was taken from a rabbit, was placed next to the tumor in the experimental animals, the development of the tumor was completely inhibited [29]. This study is compatible with the anti-angiogenesis of cartilage as it is supported by our study.

Leeand Langer (1983) investigated the inhibitory effect of shark cartilage on tumor angiogenesis. They stated that because of the absence of vessel formation in cartilage, they prevent tumor growth and that neoplasm is seen very rarely in shark [30].

In one study; it was stated that SLO is a natural source of alkylglycerols and can be used at doses of 100 mg 3 times a day without side effects. In the same study it was stated that alkylglycerols could be used as an adjunct to the immune system as an alternative therapy [31]. This study, in which SLO can be used as an antioxidant, has reached the same conclusion as our experimental model.

SLO is include a low level polyunsaturated fatty acid (EFA), which has been reported to play a role in increasing of immunity against to bacterial and fungal infections and cancer [32].

The effect of SLO on the angiogenesis of mice injected with tumor cells into the skin was investigated in an experiment. It has been reported that the component of SLO, called Ecomer, suppresses the formation of new blood vessels and increases the number of blood granulocytes in mice containing sarcoma L-1 type tumor [33]. In this study, we can understand that the component called Ecomer enhances the immune system by increasing the number of blood granulocytes, acting in parallel...
with the SLO in our study.

REFERENCES


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