LIPID PEROXIDATION and ANTIOXIDANT LEVELS IN THE LUNGS and ASSOCIATED LYMPH NODES OF CATTLE WİTH ANTHRACOSİS

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Summary: The aim of the present study was to examine lipid peroxidation and antioxidant levels in the lungs, mediastinal and bronchial lymph nodes of cattle with anthracosis. In the study, the lungs and associated lymph nodes of 600 cattle of various species and ages, brought from different villages in the Kars region and slaughtered in two local abattoirs, were examined for anthracosis. Upon gross examination, anthracosis were detected in the lungs and their associated lymph nodes of 20 (3.3 %) cattle and specimens were taken for laboratory analysis. Additionally, samples from the lungs and the lymph nodes with no pigmentation of 20 cattle were used as control. The levels of thiobarbituric acid reactive substance (TBARS), vitamin E, A, b-carotene, glutathione (GSH) and the activity of glutathione peroxidase (GSH-Px; EC 1.11.1.9) in the lungs and the lymph nodes were determined.

TBARS levels in the lungs and the lymph nodes of cattle with anthracosis were significantly ($p<0.001$) increased as compared to the controls. Vitamin E, A and b-carotene levels in the lungs and the lymph nodes of the animals with anthracosis were also significantly ($p<0.05$, $p<0.01$, respectively) decreased. GSH-Px activity and GSH levels in the lungs and the lymph nodes with anthracosis were also significantly ($p<0.05$) decreased as compared to the controls.

These results suggested that increase in lipid peroxidation of the lungs and the lymph nodes were most likely produced by the suppression of antioxidant defence abilities in cattle with anthracosis.

Key words: Anthracosis, lipid peroxidation, antioxidant defense system, lungs, lymph nodes, cattle.

Antrakozisli Sığırların Akciğer ve İlgili Lenf Düğümlerinde Lipid Peroksidasyonu ve Antioksidan Düzeyleri

Özet: Sunulan çalışmada, antrakozisli sığırların akciğer, mediastinal ve bronşial lenf düğümlerinde lipid peroksidasyonu ve antioksidan düzeyleri belirlendi. Çalışmada, Kars yöresinin değişik köylerinden getirilen ve bölgedeki iki mezbahada kesilen farklı ırk ve yaşlardaki 600 sığıra ait akciğer ve ilgili lenf düğümleri antrakozis yönünden incelendi. Makroskobik muayenede siyah pigmentasyon gözlenen 20 akciğer ve bunlara ait lenf düğümlerinden laboratuar analizleri için doku örnekleri alındı. Ayrıca, makroskobik olarak pigmentasyon bulunmayan 20 sığıra ait akciğer ve ilgili lenf düğümleri ise kontrol olarak kullanıldı. Akciğer ve lenf düğümlerinde glutatyon peroksidaz (GSH-Px; EC 1.11.1.9) aktivitesi ile glutatyon (GSH), A ve E vitaminleri, b-karoten ve tiyobarbiturik asit substrat (TBARS) düzeyleri belirlendi.

Antrakozisli sığırların akciğer ve lenf düğümlerinde TBARS düzeylerinin kontrollere göre istatistiksel olarak yüksek ($p<0.001$) olduğu gözlendi. Yine, antrakozisli akciğer ve lenf düğümlerinin A ve E vitaminleri ile b-karoten düzeyleri de önemli derecede düşük (sırasıyla $p<0.05$, $p<0.01$) bulundu. Ayrıca, antrakozisli akciğer ve lenf düğümlerinde GSH-Px aktivitesi ve GSH düzeyleri de kontrollere göre düşüktü ($p<0.05$).

Bu sonuçlar, antrakozisli sığırların akciğer ve lenf düğümlerindeki lipid peroksidasyon artışının muhtemelen antioksidan savunma yeteneğinin zayıflamasına bağlı olabileceğini göstermektedir.

INTRODUCTION

The lungs and mediastinal lymph nodes are the primary organs at risk from the effects of inhaled environmental agents, including carbon particles and asbest dust. Carbon particles have been implicated as a factor to the contributing and exacerbation of various respiratory diseases including cancer of the lungs and pneumoconiosis as well as anthracosis[1]. In addition to morphological lesions[2,3], carbon particles have been shown to alter a number of biochemical parameters, including the activities of antioxidant enzymes and endogenous antioxidants in animal lungs[4].

Carbon particles or smoke contain a large variety of compounds including oxidants and free radicals[5]. The aqueous phase of the smoke extract has been shown to be capable of initiating autooxidation of the unsaturated lipids of alveolar macrophages (AM) in vitro[6]. In a study by Janoff et al.[7], the reduction of the elestase inhibiting capability of a_1-antitrypsin in the lung lavage fluid of rats affected by cigarette smoke was shown to be preventable by a reducing agent.

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These findings suggest that an oxidative damage mechanism may be involved in the adverse effects of smoke or carbon particles. However, antioxidant defence systems exist to ameliorate free-radical-mediated peroxidation of cellular components. These include a-tocopherol, a major membrane-band lipophilic antioxidant in humans that is crucial to cellular defence systems[8]. The cytosolic enzymes glutathione peroxidase (GSH-Px; EC 1.11.1.9), superoxide dismutase (SOD; EC 1.15.1.1) catalase (CAT; EC 1.11.1.6), glutathione (GSH) and b-carotene contribute to the antioxidant defence mechanisms in living cells[9].

A literature review failed to find any information on the antioxidant response of the respiratory tract to disease caused by exogen pigmentation in cattle lungs and associated lymph nodes. In anthracosis, a disease caused by exogen pigmentation, AM release increased amounts of superoxide and hydrogen peroxide, which have been linked to the pathogenesis of the disease[10]. The present study was designed to investigate whether antioxidants and lipid peroxidation (LPO) increase in response to disease caused by exogen pigmentation in the lungs and associated lymph nodes of cattle with anthracosis.

MATERIAL and METHODS

Animals and Collection of Samples: In the present study, the lungs, mediastinal and bronchial lymph nodes of 600 cattle of various species and ages, brought from villages in the Kars region and slaughtered in two local abattoirs, were examined for anthracosis throughout the year 2002. Upon gross examination, the lungs and the associated lymph nodes of 20 (3.3 %) cattle were found to have varying degrees of black pigmentation and were taken for laboratory analyses. It was confirmed histopathologically that the tissues were anthracotic. However, as the animals were brought from the animal trade market it was not possible to determine their precise local origin. Additionally, samples from the lungs and associated lymph nodes taken of 20 cattle with no pigmentation were also used as control. Tissue samples were stored for < 3 month pending measurent of reduced GSH and enzymatic activity. The remaining tissue were used for immediate LPO and vitamin assay.

Tissue Preparation: The tissues were weighed,

rinsed with ice-cold deionized water, cut into small pieces and then dried on a filter paper. The tissues were homogenized using the appropriate buffer, depending upon the variable to be measured. The homogenates were centrifuged at 20 000 g for 10 min at 4 °C.

Thiobarbituric Acid Reactive Substance Analyses in the Tissues: LPO contents were measured with the thiobarbituric acid reaction as the thiobarbituric-acid reactive substances (TBARS) in tissues using the method of Placer et al.[11], modified by Matkovics et al.[12]. The values of the malondialdehyde (MDA) reactive material were expressed in terms of TBARS (nmol/g tissue). To prevent artefuctual LPO during the boiling step samples analysed for MDA contained 1.0 mM butylated hydroxytoluene (BHT).

Glutathione Peroxidase and Reduced Glutathione Assay: GSH-Px activity was determined using cumene hydroperoxide and reduced GSH as co-substrates and the loss of GSH following enzymic reaction was measured spectrophotometrically with Ellman's reagent at 37 °C and 412 nm according to Matkovics et al.[12] The reduced GSH levels of the tissue homogenates were measured spectro-photometrically using Ellman's reagent[13].

Vitamin E, Vitamin A and b-carotene Assays: The tocopherol content of the tissues was determined spectrophotometrically according to Tsen[14] and Martinek[15]. Retinol and b-carotene in tissue homogenates were determinated according to the method of Suzuki and Katoh[16].

Protein Determination: The protein content in the tissue homogenate was measured by the method of Lowry et al.[17] with bovine serum albumin as the Standard.

Statistical Analysis: All results were expressed as the mean ± standard error (SE). Statistical analysis (Mann-Whitney U test) was performed using the SPSS software program and the values of group with anthracosis were compared to those of the control group. Differences at a level of 5 % were considered statistically significant.

RESULTS

The levels of LPO in animals with anthracosis, as

measured by TBARS assay (MDA), increased to 77 % and 93 % above the control levels in the lungs and lymph nodes, respectively (p<0.001) (Tables I and II).

Reduced GSH contents were significantly lower in the lungs and lymph nodes of animals with anthracosis than in the control group (respectively p<0.01, p<0.05). Similarly, the GSH-Px activity of the lung and lymph nodes was also significantly (p<0.05) lower in the group with anthracosis than in the control group.

Table 1: Levels of the parameeters investigated in the lungs of cattle in the controls and group with anthracosis.
Tablo 1: Kontrol ve antrakozlu sığırların akciğerlerinde araştırılan bazı parametrelerin düzeyleri.

	Groups	
	Control	Group with Anthracosis
Vitamin E (mg/g dry tissue)	15.98±2.65	11.65±1.69*
Retinol (mg/g dry tissue)	48.12±3.21	36.38±2.15*
b-carotene (mg/g dry tissue)	225.33±2.56	165.41±2.08*
TBARS (nmol/g dry tissue)	2.91±0.18	5.15±0.02***
GSH-Px (IU/g protein)	13.46±0.23	10.78±1.96*
GSH (nmol/g dry tissue)	1.66±0.23	0.86±0.09*

Note: Values are expressed as mean ± SE
*: p<0.05, **: p<0.01, ***: p<0.001
*, **, ***: Statistically significant according to control group.

Table 2: Levels of parameeters investigated in the lymph nodes of cattle in the controls and group with anthracosis.
Tablo 2: Kontrol ve antrakozlu sığırların lenf düğümlerinde araştırılan bazı parametrelerin düzeyleri.

	Groups	
	Control	Group with Anthracosis
Vitamin E (mg/g dry tissue)	12.65±1.28	7.46±1.09**
Retinol (mg/g dry tissue)	35.26±2.19	22.78±1.15**
b-carotene (mg/g dry tissue)	214.00±1.45	143.0±1.05**
TBARS (nmol/g dry tissue)	1.99±0.28	3.88±0.12***
GSH-Px (IU/g protein)	11.78±0.31	8.98±0.46*
GSH (nmol/g dry tissue)	1.23±0.34	0.66±0.09*

Note: Values are expressed as mean ± SE
*: p<0.05, **: p<0.01, ***: p<0.001
*, **, *** : Statistically significant according to control group.

As the levels of vitamin E were slightly decreased (p<0.05) in the lung tissues of the group with anthracosis compared with the control group, the levels of retinol and b-carotene were significantly decreased (p<0.05) in the same group (Table I). Similarly, vitamin A, E and b-carotene levels of lymph nodes were also significantly decreased (p<0.01) in the group with anthracosis as compared to control.

DISCUSSION

Anthracosis is recognized to be related to the inhalation and deposition of carbon particles and smoke in the lungs[18]. However, reports of anthracosis in animals are scarce[19-22]. Probably, this is because these animals are rarely exposed to air pollutants in comparison to humans[23] and to other animal species living in urban areas. It is well know that smoke is the most common cause of oxidative stress in daily life and that it may affect the antioxidant capacity of humans and animals. On the other hand, smoke increases the levels of TBARS in the lungs[24]. In the our study, the TBARS levels of the lungs and lymph nodes increased in cattle with anthracosis resulting from chronic smoke inhalation. Similar to the results of the present study, Yoshimura et al.,[25] reported that MDA concentration increased in the lungs of hamsters which had inhaled cigarette smoke. However, to our knowledge, these has been no previous study on TBARS levels and antioxidant status in the lungs and lymph nodes of cattle with anthracosis resulting from chronic smoke inhalation. Interestingly, neither coal mining nor industrial activities causing heavy atmospheric pollution are present in the Kars region of Turkey and therefore they may be discounted as cause of anthracosis. However, in the region, animal barns are simple constructions and are generally built adjacent to houses. Both houses and barns are simple stone and mud constructions which deteriorate quickly. However, stoves and clay ovens for heating, cooking and making bread etc. are generally built in that part of the house adjacent to the barn. Thus, smoke may easily permeate the eroded walls of houses, entering barns and potentially resulting in anthracosis in the animals exposed to it. It was our direct experience that both sheep and cattle in some barns in villages are directly exposed to the heavy smoke emitting from ovens etc. for about 1-2 hours daily, at least 2 days in a week. Thus, it has been speculated that the lungs and lymph nodes of cattle which have inhaled smoke may tend to produce reactive oxygens and lipid peroxides easily.

Protection against oxidative damage is provided by enzymatic and non-enzymatic antioxidant defences. The antioxidant defences in the lungs are widely distributed and include both enzymatic and non-enzymatic systems. GSH-Px responsible for the catalytic reduction of H_2O_2, provides a biochemical basis for the protective role of Se in ameliorating or

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preventing peroxidative tissue damage[26]. The literature on GSH-Px activity and GSH levels in smoke-affected animals and humans is rather limited and contradictory. In our study, increase in LPO was associated with reductions in the activity of the selenoenzyme GSH-Px in the lungs and lymph nodes. Similar to our results, Baskaran et al.[27] observed that GSH-Px activity in the lungs were lower in rats which had inhaled smoke than in control rats. It is con-ceivable that GSH-Px may be induced in response to the greater release of lipid hydroperoxide from phospholipids by phospholipase A_2 in the cells of animals which have inhaled smoke.

One of the most important components of the defence mechanisms is GSH[28]. In this study, we found a decrease in the GSH levels of tissues with anthracosis. In accordance with our results, in a study by Baskaran et al.[27], it has been demonstrated that a reduction in GSH levels in the lungs occurred when rats exposed to smoke. It is conceivable that the decline in GSH in association with increase in tissue MDA concentration (Table I) demonstrated in cattle with anthracosis may indicate an increased utilization of GSH in the reductive regeneration of MDA from the hydroxyl radical. GSH depletion studies in animals demonstrate that the rate of decline of GSH in tissues reflects its rate of utilization[29].

The vitamins might protect cellular components against peroxidative damage via the free-radical scavenging mechanism or as a constituent of the membrane[30]. According to antioxidant theory[31,32], when the concentration of antioxidant vitamins decreases, LPO increases in the tissues leading to the damage of cell membranes. Vitamin A can function as an effective radical-trapping antioxidant[32]. Vitamin A represents a previously unknown class of biological antioxidants. There is ample evidence that vitamin A is a very effective quencher of singlet oxygen[31,33]. Wang et al.[34] reported that the concent-ration of vitamin A in the lungs decreases after exposure to tobacco smoke. Similarly, McDowell[31] indicated that smoke causes a reduction in the conversion of carotene to vitamin A. Also, in the present study the concentration of vitamin A in the lungs and lymph nodes affected by anthracosis decreased. Consequently, these results confirm the thesis that chronic smoke inhalation and vitamin A concentration in the lungs are correlated positively[34,35].

b-carotene, an effective quencher of singlet oxy-

gen[36], has a mildly effective, radical-trapping antioxidant capability[8] and has recently received considerable attention because of its possible contribution to preventing cancer[37]. It has been reported that cigarette smoking decreases the concentration of b-carotene in serum and tissue in humans[38]. In the present study, anthracosis resulted from chronic smoke inhalation caused a decrease in b-carotene levels in the lungs and lymph nodes of the animals (Tables I and II, respectively). It is known that smoke causes an increase in LPO in tissues, thus decreasing protects the cell membrane against cellular changes caused by peroxidation, and that b-carotene plays a role essential in reducing peroxidative damage to the cellular membrane[39].

Vitamin E is well accepted as the first line of defence against LPO. By its free-radical quenching activity, vitamin E breaks chain propa-gation and thus terminates free-radical attack at an early stage[31]. The present study documented a signi-ficant reduction in vitamin E levels in the lungs and lymph nodes of smoke-exposed animals. Pocker[40] proposed that smoke inhalation is responsible for LPO and that vitamin E is able to modulate the modification caused by LPO. In the light of the current literature, our finding of vitamin E depletion in the anthracotic lungs and lymph nodes resulting from chronic smoke inhalation indicates an increased potential for cellular damage due to unsca-venged reactive oxygen radicals in smoke affected animals, suggesting a possible increased requirement for vitamin E in cattle with anthracosis. Similar to our results, Chow et al.[41] reported that vitamin E in the lungs has severely depleted 7 days after smoke inhalation, suggesting a possible increased require-ment for vitamin E in animals exposed to smoke.

The results suggest that increase in TBARS levels were possibly produced by the suppression of anti-oxidant defence abilities and the significant induction of oxidative activity in the lungs and lymph nodes with anthracosis resulted from chronic smoke inhalation. In conclusion, we speculate that oxidative stress caused by smoke may play an important pathophysiological role in the development of anthracosis in smoke-affected animals, and may possibly increase their risk of future lung cancer.

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