Consequences of Social Isolation in Rats on Their Antioxidant Defense System and Erythrocyte Deformability

Sıçanlarda Sosyal İzolasyonun Oksidan Stres ve Eritrosit Deformabilitesi Üzerine Etkileri

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Abstract
Purpose: In this study, our purpose was to investigate effect of oxidative stress composing in isolated rats on erythrocyte deformability and antioxidant system, considering importance of mechanical, and biochemical properties of erythrocytes in efficiency of blood circulation
Material and Methods: Fourty Sprague-Dawley rats were divided into two groups according to their ages; where one of them consisted of 2 months old rats and the other 12 months old rats. Then, randomly selected ten rats in each group were isolated separately in a cage and hold 12/12 hour light-dark cycle lasting 21 days. Blood samples were collected at the end of the 21st day, and superoxide dismutase (SOD), catalase (CAT) activities and malondialdehyde (MDA) levels were measured spectrophotometrically. Changes in plasma nitric oxide level were determined by Griess method depending on total nitrite-nitrate. In addition, relative filtration rate (RFR), relative filtration time (RFT) and relative resistance (Rrel) of the erythrocytes were calculated as the indexes of erythrocyte deformability.
Results: The SOD and CAT levels were found significantly lower in both of young and adult isolated rats when compared to the young and adult control groups. The nitric oxide levels were also found significantly lower in the young and adult isolated rats when compared to their control groups. The peroxidation of the lipids were also decreased by social isolation, in the erythrocyte membrane of both young and adult isolated rats compared to the controls. The indexes of erythrocyte deformability, RFR was found significantly higher, whereas the RFT was significantly lower in young and adult isolated rats compared to the young and adult controls. However, the Rrel of the erythrocytes were not altered dramatically.
Conclusion: As a consequence, our data reveals that the social isolation causes a lack of plasma nitric oxide levels in the socially isolated rats and probably due to this decrease in one of the major oxidants for the erythrocyte membrane results in the decreased lipid peroxidation. Furthermore, our results reveal that the social isolation stress causes alterations in the antioxidant defense system and these alteration results in the changes in erythrocyte deformability reflecting that some tissue perfusion problems can occur with long term and repeated loneliness and especially in the early stages of the life span.
Keywords: **Social isolation, Erythrocyte deformability, Oxidative stress, Antioxidant enzymes, Nitric oxide**

Özet
Amaç: Bu çalışmada amacımız, eritrositlerin biyokimyasal ve mekanik özelliklerinin dolaşımın etkinliğinin belirlenmesindeki önemini dikkate alarak, sosyal izolasyon uygulanan sıçanlarda oluşan oksidatif stresin eritrositlerin deformabilite özellikleri ve antioksidan sistem üzerine etkilerini incelemektir.
Gereç ve Yöntemler: Çalışmada 40 adet Sprague Dawley sıçan, 2 aylık (genç) ve 12 aylık (erişkin) olmak üzere iki gruba ayrıldı. Daha sonra her gruptan 10'ar sıçan, 12/12 saat aydınlık/karanlık siklusunda, 21 gün boyunca tek başlarına ayrı kafeslere alınarak, sosyal izolasyon stresi yaratıldı. 21. gün sonunda alınan kan örneklerinde, eritrositlerde antioksidan enzimlerden SOD, CAT ve lipid peroksidasyonun bir göstergesi olarak MDA düzeyleri spektrofotometrik olarak ölçüldü. Plazma nitrik oksit değişiklikleri total nitrit-nitrat ölçümüne dayalı Griess yöntemi ile tayin edildi. Ayrıca hazırlanan eritrosit süspansiyonları %5 hematokrite ayarlanarak, filtrasyon yöntemi ile, relatif filtrasyon hızı (RFR), relatif filtrasyon zamanı (RFT) ve relatif direnç (Rrel) değerleri ölçüldü.
Bulgular: SOD ve CAT düzeyleri hem genç hemde erişkin olan sosyal olarak izole edilmiş sıçanlarda istatistiksel açıdan anlamlı derecede düşük bulunmuştur. Sosyal izolasyon uygulanan sıçanlarda ,nitrik oksit düzeyleri de erişkinlerde daha belirgin olmak üzere hem genç hem de erişkinlerde daha düşüktür.Lipid peroksidasyon ürünü MDA düzeyleri de her iki grupta kontrollere göre azalmıştır.Eritrosit deformabilite indekslerinden RFR değerleri izole sıçanlarda önemli derecede yüksek bulunurken, RFT hem genç hem de erişkinlerde kontrollere göre düşük bulunmuştur.Ancak eritrositlerin Rrel değerlerinde önemli derecede bir değişiklik olmamıştır.
Sonuç: Verilerimiz sosyal izolasyonun sıçanlarda plazma nitrik oksit düzeylerini azalttığı ve muhtemelen bu azalmayla bağlantılı olarak eritrosit membranında lipid peroksidasyonun da azaldığına işaret etmektedir.Ayrıca, sosyal izolasyon stresi antioksidan enzim aktivitelerini de değiştirmiş ve buda muhtemelen daha uzun süreli ve tekrarlanan yalnızlık durumlarında, özellikle yaşamın ilk dönemlerinde doku perfüzyonu problemlerine yol açabilecek deformabilite değişiklikleri ile sonuçlanabilecektir.
Anahtar kelimeler: **Sosyal izolasyon; Eritrosit deformabilitesi; Oksidatif stress; Antioksidan enzimler; Nitrik oksit**

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Introduction

Social relationships are significant for health, and both social networks and social support influence mortality and morbidity (1). Psychosocial stress in form of intermittent maternal separation and social isolation during early postnatal life has been repeatedly shown to induce profound and irreversible alterations in neuroendocrine and behavioral mechanisms of adaptation (2,3), however little evidence exist in the mechanisms of these alterations. The multidisciplinary approach of Kanitz et al (2) was designed to investigate the influence of a repeated social isolation in very young on piglets on important behavioral, neuroendocrine and immunological measures of their stress response.

It is well known that biochemical and physiological mechanisms play an important role in the deformability of erythrocytes which is crucial for the tissue perfusion under stress. In the microcirculation, especially in the narrow capillaries, the passage of RBCs and other blood formed elements are very sensitive to perfusion pressure as well as to their deformability and aggregability. The better they deform, the better they perfuse the tissue with oxygen. So it is crucial for a normal cell to be in a good physiological pattern under in vivo circulation and experimental conditions. The erythrocyte deformability, is an important hemorheological parameter contributing to the exchange of metabolic products with the tissue environment, is thus attributed to the various constituents of the membrane and hemoglobin.

Any variation in these may result in the impaired functioning of erythrocytes. One of the mechanisms underlying in impaired deformability is the oxidative stress caused by reactive oxygen and nitrogen species. Lipid peroxidation induced by oxidative stress is a consequence of excess free radicals and provides marked damage to the structure and function of the cell membranes. Since the erythrocyte is susceptible to free radical insults because of the high concentration of the oxidizible polyunsaturated fatty acids, they are enriched in protective antioxidant enzymes (4).

The nitric oxide pathway has also been implicated in the regulation of anxiety, at least in adulthood. Although most psychopharmacological research examining NOS inhibitors has assessed adult animals, there are reports that inhibiting NO synthesis can have behavioural consequences early in life as well (5-7). The NO pathway has been implicated in learning process, especially in young animals (7), however it not yet known whether NO is involved in neuronal process regulating anxiety. Huong et al (8) has shown the brain NO metabolites increase by 6-8 weeks social isolation besides the increase in the TBARS (tihobarbituric acid reactive substances). There is also evidence that the NO plays a regulatory role for the deformability of erythrocytes. The excessive amounts of NO as well as the reactive oxygen species are shown to cause impaired deformability under different physiological and pathophysiological conditions (4). However, no information is, so far, available if exposure of laboratory animals to socially isolation stress, another kind of psychological stress produces oxidative damage in the erythrocytes and this damage causes a lack in deformability of erythrocytes. This study was performed to clarify if the 3-weeks socially isolation stres causes any oxidative damage to the erythrocyte membrane, especially via NO and if these changes are related to the erythrocyte deformability.

Materials and Methods

40 Sprague-Dawley rats were used in 4 different groups comprising of 10 in each. Firstly, the 40 rats were divided into two groups due to their ages; where one of them consisted of 2 months old rats and the other 12 months old rats. Then, these two groups were divided into two separate subgroups in which the first group was the young control, the second was the young-isolated, the third was the adult control and the last one the adultisolated group. The isolation of the young and adult rats was performed by replacing them to individual houses for 21 days. Briefly, after being removed from the 25x40x15cage, social isolation subjects were placed in an unfamiliar cage (length x height x depth: 10x9x9) made of opaque plastic material to prevent visual contact to environment) in a separate room (held at a constant temperature of 24 C and %55 humidity) and left undisturbed. Standard rat chow and water were available ad libitum. The nursery was maintained on a 12/12 h light/dark cycle.

Blood samples collected at the end of the 21st day were used to measure the superoxide dismutase (SOD) and catalase (CAT) activities as the reflectors of the antioxidant defense system as previously described (9,10). Further more, the nitric oxide levels were measured by Griess method (11) and the Malondialdehyde (MDA) levels were measured for lipid peroxidation(12). In addition, Relative Filtration Rate (RFR), Relative Filtration Time (RFT) and Relative Resistance (Rrel) of the erythrocytes were calculated as the indexes of erythrocyte deformability.

Bulk filtration method was used to evaluate the filterability of erythrocytes, in which the RFR, RFT and Rrel of the erythrocytes were determined as the indexes of deformability while passing through a polycarbonate filter with a diameter of 20nm and pore diameter of 5 μm at a constant pressure of 10cmH_2O (13).

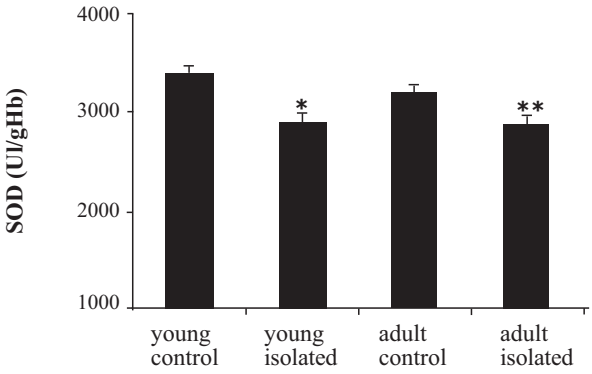
The data obtained were analysed by the help of Kruskal-Wallis and Man-Whitney U test for the possible significant differences between the groups. The data were analysed with the help of a computer software, SPSS 10.0 and all the data were expressed as the mean ± SD and the number of subjects are indicated by n.

Results

As the reflector of antioxidant defense system, the SOD levels were found significantly lower in both the young (2885.215 ±104.6 U/gHb) and adult (2876.861 ± 87.1 U/gHb) isolated rats when compared to the young (3394.32 ± 87.8 U/gHb) and adult (3209.14 ± 58.06) ($p<0.001$) control groups (Figure 1). In addition, the CAT levels were also significantly lower in both young (20.30 ± 3.5 U/gHb) and adult (28.47 ± 7.5 U/gHb) isolated groups than those of the control groups (41.08 ± 5.6 U/gHb, 36.8 ± 4.2 U/gHb) ($p<0.001$) (Figure 2)

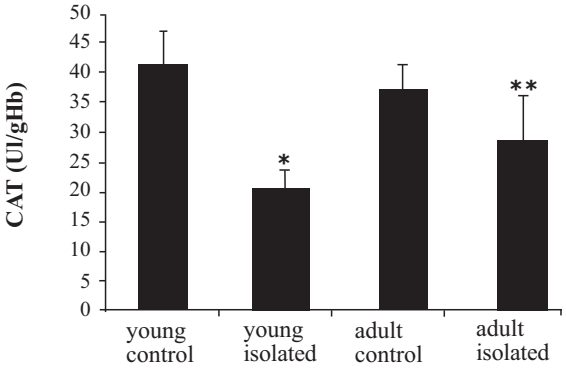
The nitric oxide levels are found significantly ($p<0.05$) lower in the young (0.09±0.01 nmol/ml) and adult (0.09±0.01 nmol/ml) isolated rats when compared to the young control group (0.116±0.02 nmol/ml) and this decrease in the adult isolated rats was more significant ($p<0.001$) compared to the adult control (0.121±0.02) (Figure 3) The peroxidation of the lipids were also decreased ($p<0.05$) by social isolation in the erythrocyte membrane of both young (28.07±7.7) and adult (24.39±9.6) isolated rats compared to the young control (39.8±14.1) (Figure 4) The indexes of erythrocyte deformability, RFR was found significantly higher, whereas the RFT was significantly lower in young and adult isolated rats compared to those of control groups. However, the Rrel of the erythrocytes were not altered dramatically (Table 1).

Figure 1. Superoxide Dismutase Activity.



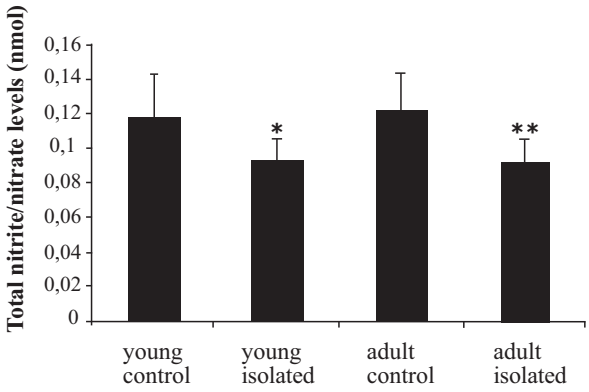
*compared to young control $p<0.001$;
**compared to adult control $p<0.001$.

Figure 2. Catalase Activity.



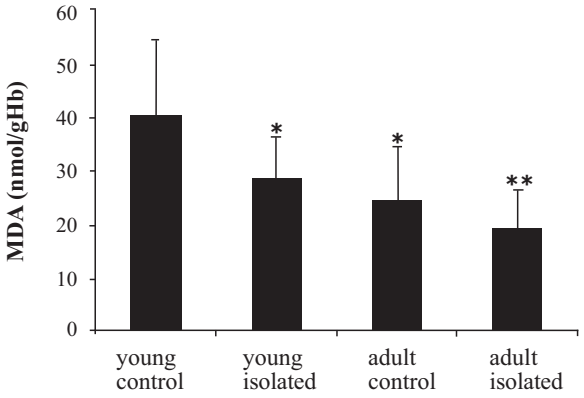
*compared to young control $p<0.001$;
**compared to adult control $p<0.001$.

Figure 3. Total nitrite and nitrate levels.



*compared to young control $p<0.05$;
**compared to adult control $p<0.001$.

Figure 4. Malondialdehyde levels



*compared to young control p<0.05;
**compared to young control p<0.001.

Table 1. Erythrocyte deformability indexes

Groups (n=10)	RFR	RFT	Rrel
Young Control	0.6415±0.0917	1.5882±0.2353	1.4374±0.1249
Young Isolated	0.7645±0.1171*	1.3361±0.2059*	1.3260±0.1145
Adult Control	0.6903±0.1059	1.4798±0.2228	1.4434±0.2755
Adult Isolated	0.7427±0.1590**	1.4075±0.3258**	1.5039±0.2925

RFR: Relative Filtration Rate, RFT: Relative Filtration Time, Rrel: Relative resistance.
*Compared to young control p<0.05;
**Compared to adult control p<0.05.

Discussion

In this study, we have demonstrated that social isolation stress causes alterations in the deformability of erythrocytes via altered antioxidant enzyme activities. Moreover, we evaluated the levels of NO metabolites to figure out the relation between the NO levels and lipid peroxidation in the erythrocyte membrane, which is one of the common factors affecting the deformability of erythrocytes.

Previous behavioral and pharmacological studies have demonstrated that social isolation of mice produce oxidative damage to brain membrane damage in a manner related to the duration of the social isolation stress and that a significant increase in the brain content of TBARS occurred in the mice exposed to social isolation stress for 6-8 weeks. And further more they have found an increase in NO production in the brain, where these results were significantly revealed after 6-8 weeks of stress exposure, while no changes were observed 2 week or 4 week isolation stressed mice. In brief, Huong et al (8) has shown the brain NO metabolites increase by 6-8 weeks social isolation besides the increase in the TBARS (tihobarbituric acid reactive substances). However, controversial to these findings, we demonstrated that the NO levels in the erythrocytes were decreased significantly following the 21 days of isolation stress exposure. These results may be due to the synthesis of the NO in brain and the circulatory system depends on different kinds of NOS (nitric oxide synthase) enzymes. Moreover, the decrease occurred partly in association with the changes in the MDA levels in the erythrocyte membrane. Thus, our data reveal that the social isolation causes a lack of nitric oxide levels by 21 days of social isolation stress and probably due to this decrease in one of the major oxidants for the erythrocyte membrane results in the decreased lipid peroxidation.

In this study, the reduction in erythrocyte MDA content in 21 days socially isolated animals was observed in association with a reduction in the erythrocyte antioxidant enzymes, SOD and CAT. Particularly the CAT activity was more significantly reduced in the young isolation group showing that this system is more sensitive to the psychological stress in the early stages of the life span. To clarify the exact mechanisms underlying the decrease of NO levels and antioxidant enzyme activities resulting from social isolation requires further investigation.

It is of interest to note that the alterations in the NO levels are directly related to the deformability of erythrocytes. There are evidences that the low levels of NO plays a regulatory role for the deformability of erythrocytes (14). However, the excessive amounts cause a significant damage in the membrane structure leading to the impaired deformability (4). In this study our results support the low levels of NO can protect the erythrocyte membrane from the impaired deformability. And this low levels of NO plays a significant role in the regulation of the normal cell function for the erythrocytes. As the indexes of erythrocyte deformability, Rrel is assign for the cells if they have the ability to pass through the pores which has a similar diameter of capillary pores in the microcirculation throughout the circulatory system. Rrel shows the resistance of the cells passing through narrow capillary pores and is calculated by the RFT and RFR of the cells. RFR is the rate and RFT is the time of this process. In this study, the

RFT and RFR of the cells were found significantly lower in isolated young and adult rats compared to their controls. However, the Rrel of the erythrocytes were not altered dramatically. Furthermore, it has shown by the previous studies that the inhibition of nitric oxide (NO) production selectively impairs learning and memory (15) and if this inhibition is in the early stages of the life span its role is more important forthe development of the mental status.

Our results showing that the NO levels in the socially isolated rats especially in the young groups shows that the social activities can be more important and crucial than it is thought to be, for the mental development of the learning children (16). Since the newborns and particularly pre term infants are very susceptible to oxidative damage induced by ROS and their antioxidant defense systems are imbalanced in favor of oxidants, one important point that should be taken into account for further studies is that, social isolation may have more significant effects on long term especially in the early stages.

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