The Effect of Dipyrone Overdoses on the Levels of Lipid Peroxidation, Glutathione and Ceruloplasmin in Dogs.

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SUMMARY

The Effect of Dipyrone Overdoses on the Levels of Lipid Peroxidation, Glutathione and Ceruloplasmin in Dogs.

In this study, the effect of dipyrone at overdose on the levels of lipid peroxidation, glutathione and ceruloplasmin was investigated. Fourteen dogs (13-30 kg body weight, 1-3 years old) were used as research materials. Dogs were divided into two equal treatment groups. Blood samples were taken from each group before the experiment for control. Dipyrone was intravenously injected to the first experimental group at the dose of 0.4 g/kg, in 12 hour intervals for 12 days.. Same procedure was applied to the second experimental group intramuscularly. Blood samples drawn from animals at 1st, 3rd, 5th, 7th, 9th and 11th days of the experiment, were used for analyses. The concentrations of malondialdehyde were significantly high in both analysed groups (lowest level 2.97±0.18 and highest level 5.19±0.12 in the first group, lowest level 2.76±0.23, highest level 3.90±0.49 nmol/ml in the second group) as compare to the findings from control (first group control average level 2.39±0.12, second group average level 2.45±0.12 nmol/ml) ($p<0.001$). The changes in the levels of glutathione and ceruloplasmin concentrations were not statistically significant ($p>0.05$).

It was concluded that, overdoses of dipyrone does not cause severe lipid peroxidation . The reason of this could be the broad tolerans of the organism to the dipyrone.

Key Words: *Dipyrone, Lipid peroxidation, Glutathione, Ceruloplasmin.*

Köpeklerde Yüksek Dozda Dipironun Lipid Peroksidasyonu, Glutatyon ve Serüloplazmin Düzeyleri Üzerine Etkisi

ÖZET

Bu çalışmada köpeklerde yüksek dozda dipironun lipit peroksidasyonu , glutatyon ve serüloplazmin düzeyleri üzerine etkisi araştırıldı. Araştırmada 13-30 kg ağırlığında 1-3 yaşlarında sağlıklı 14 köpek kullanıldı. Köpekler eşit sayıda iki gruba ayrıldılar. Uygulama öncesi her iki grup köpeklerden alınan kanlar kontrol amacıyla kullanıldı. Birinci deneme grubu köpeklere 0.4 g/kg dipiron 12 saat aralıklarla toplam 12 gün süreyle intravenöz olarak uygulandı. İkinci deneme grubu köpeklere aynı miktar yine aynı süre içerisinde intramusküler olarak uygulandı. Denemenin 1., 3., 5., 7., 9. ve 11. günlerinde alınan kan örnekleri analizler için kullanıldı. Yapılan analizlerde malondialdehit miktarlarında her iki deneme grubunda anlamlı yükselmeler gözlendi (birinci grup en düşük konsantrasyon 2.97±0.18, en yüksek konsantrasyon 5.19±0.12, ikinci grup en düşük seviye 2.76±0.23, en yüksek seviye 3.90±0.49 nmol/ml) ve bu yükselmeler kontrol verilerine (birinci grup kontrol değeri 2.39±0.12, ikinci grup kontrol değeri 2.45±0.12 nmol/ml) göre istatistiksel açıdan anlamlı bulundu ($p<0.001$). Glutatyon ve serüloplazmin miktarlarındaki değişimler ise anlamlı bulunmadı ($p>0.05$).

Sonuç olarak, dipironun yüksek dozda verilmesine rağmen şiddetli lipit peroksidasyonuna neden olmadığı, bunun da dipironun organizmadaki geniş tolerans yeteneğiyle ilgili olduğu sonucuna varıldı.

Anahtar kelimeler: *Dipiron, Lipit peroksidasyonu, Glutatyon, Serüloplazmin.*

INTRODUCTION

Dipyrone (methamizole sodium) is a bitterish, thin crystallized and white colored powder, without smell. It is alcohol soluble and isoosmotic to the body fluids. When dipyrone stored, its color is converted to the yellow without losing its pharmacological activity. Dipyrone, structurally aminopyrine like and pyrazolone derivative, is one of the non-steroidal anti-inflamatory drugs (NSAIDs) and has analgesic, antipyretic, antirheumatic, spasmolitic and sedative effects (15). Dipyrone is an analgesic with its antinociceptive action, a spasmolitic by lowering peristaltic with stimulating autonomic centers, an antipyretic by peripheral vasodilatation with stimulating autonomic centers and an antirhumatic by changing the blood circulation of inflammation site (16, 19). It has been reported that dipyrone may exert its effects through inhibition of cyclooxigenase activity and prostaglandin synthesis(1, 6, 11).

Free radicals, occuring as a result of staying under chemical substance, drug toxication, radiation, antineoplastic preparation, cause undesirable reactions in the organisms when they reach passing beyond the positions off cell defense mechanisms (14, 18, 23). Biomolocular lipids are mostly negative affected by occurred free radicals. Lipid peroxidation is oxidative destruction of polyunsatured fatty acids and a guite harmful chain reaction. Lipid radicals (malondialdehyde, lipid peroxyl radical, lipid alchoxyl radical), resulted as a concequence of lipid peroxidation, are structurally hydrofobic and most of the reactions are occured in structural lipids of the membranes. Serious interactions are observed in the membrane permeability and viscosity. Malondialdehyde, occuring result of peroxidation, cause cross bonding and polymerization of membrane components. This also changes the characteristics of deformation, ion transportation, enzyme activity and cell surface compounds (3, 4).

Glutathione is a tripeptide compound that consist of glutamic acid, cystine and cysteine amino acids. Glutathione is a very important antioxidant and protects the cells againts oxidative injury by interacting with free radicals and

peroxides. It prevents enzymes and proteins inactivation by keeping reduced of the proteins' –SH groups. At the same time it provides foreign compounds detoxification and membrane transportation of amino acids (3, 7).

Ceruloplasmin is a naturally existing antioxidant in the cell. Ceruloplasmin has an effect that breaks the chains of free radicals and binds them itself, thus prevents their functions. In addition, ceruloplasmin inhibits fenton reaction by oxidizing ferro iron (Fe^{2+}) to the ferri iron (Fe^{3+}) and thus the occurence of free radicals (3).

Unwanted complications can be occured in permanent use of pyrazolone derivatives and this kind of complications can be observed espacially in man. In contrast to this, there is not enough information on about those general side effects of pyrazolone derivatives are observed clearly in animals (19). To our knowledge, there is no reported study on the reletionship of dipyrone and lipid peroxidation.

The present study was conducted to investigate the changes of lipid peroxidation, glutathione and ceruloplasmin levels related to overdose of pyrazolone-derived dipyrone.

MATERIAL AND METHOD

In this study as materials, fourteen dogs in different sexes were used weighing 13-30 kg and aging 1-3. Dogs were divided into two equal groups. The animals were kept for one week under control prior to the experiment in clinical boxes of Veterinary Medicine Faculty of Yuzuncu Yil University. Healthy dogs were clinically examined and administered with antiparasiter drugs (Mansonil-Bayer, Dectomax-Pfizer).

The both two group animals' control blood samples were drawn before the experiment. After blood drawn the dogs were prepared for the experiment. First group were administered intravenously 0.4 g/kg dipyrone per day in 12 hour intervals for 12 days. The second group were administered intramuscularly same dose dipyrone in the same period.

The normal doses of dipyrone for the dogs were recommended as 0.025-0.1 g/kg per day (20).

Blood samples were drawn into the normal tubes and the tubes with EDTA from the both two groups for analyses in the 1st, 3rd, 5th, 7th, 9th and 11th days of experimental period. The collected blood samples with EDTA were used for reduced glutathione and malondialdehyde analyses.

Malondialdehyde concentration was determined using the method thiobarbutüric acid reactivity (3). Glutathione content was determined using the method previously described by Beutler et al. (5). Serum ceruloplasmin activities were determined spectrophotometrically using the modified method of Ravin (24). All analyses were conducted on the same day of the collection for preventing the activity loses.

Data were statistically analysed by SPSS Tukey test (2, 10).

RESULTS

The concentrations of malondialdehyde, glutathione and ceruloplasmin of groups were shown in Table1 and 2.

Malondialdehyde concentrations in first group were found statistically higher ($p<0.05$) in the 1st day and found significantly higher ($p<0.001$) in the 3rd, 5th, 7th, 9th and 11th days compared with control values. The increase of malondialdehyde concentrations in the second group versus control values were not significant ($p>0.05$) in the 1st day and significant ($p<0.01$) in the 3rd day and highly significant ($p<0.001$) in the 5th, 7th, 9th and 11th days.

The undulations of glutathione and ceruloplasmin concentrations in the both two experimental groups were not significant ($p>0.05$) versus control values.

Table 1. The values of control and first experimental group dogs.

		Parameters		
		Malondialdyd mol/ml	Glutathions mg/dl	Ceruloplasmin %mg
n		7	7	7
Control		2.39±0.12	18.49±3.4	10.23±0.3
Days	1.day	2.97±0.18a	16.48±1.80c	10.65±0.38c
	3.day	3.73±0.12b	15.41±1.4c	11.03±0.3c
	5.day	4.17±0.20b	17.71±1.33c	11.65±0.21c
	7.day	4.51±0.15b	16.60±1.0c	11.10±0.2c
	9.day	4.82±0.005b	16.16±1.27c	11.47±0.16c
	11.day	5.19±0.12b	17.65±1.7c	10.95±0.7c

Values are expressed as mean ±standart error (SE)
[a]$p<0.05$, [b]$p<0.001$, [c]$p>0.05$

Table 2. The values of control and second experimental group dogs.

		Parameters		
		Malondialdyd mol/ml	Glutathions mg/dl	Ceruloplasmin % mg
n		7	7	7
Control		2.45±0.12	17.46±2.87	10.57±0.29
Days	1.day	2.76±0.23a	16.40±1.5a	10.06±0.3a
	3.day	2.86±0.10b	17.10±0.7a	10.45±0.2a
	5.day	3.03±0.10c	16.80±0.6a	10.60±0.1a
	7.day	3.15±0.11c	17.00±0.63a	10.82±0.30a
	9.day	3.29±0.15c	17.11±0.5a	11.06±0.2a
	11.day	3.90±0.49c	17.20±0.5a	10.89±0.3a

Values are expressed as mean ±standart error (SE)
[a]$p>0.05$, [b]$p<0.01$, [c]$p<0.001$

DISCUSSION

Dipyrone is absorbed fast and undergone to biotransformation after its oral administration. Most important metabolites of dipyrone are 4-aminophenazone, 4-methilaminophenazone, 4-formilaminophenazone and 4-asetilaminophenazone. It is reported that the hazardous effect of dipyrone is result of its metabolites (15). 70 % of dipyrone metabolites are excreted within urine in 24 hours and metabolite plasma half-life is in between 3-10 hours (9).

Dipyrone has general unwanted side effects as other pyrazolone group members. For this reason, some complications such as peptic ulcer, ulcerative stomathitis, hepatitis, nephritis, aplastic anemia, leucopenia, agranulositosis and thrombositopenia can be observed as result of overdoses and long uses of dipyrone (12, 19). In addition, it causes side effects on fluid and electrolyte

balances. The necessity of patients' persecution against agent's complication is emphasized in different sources(9, 12).

In fact, free radical metabolites are absolute compounds of aerobic organisms and they are important in synthesis of a series enzyme and antibacterial defense of many organisms by its controlled use (22). But they are antioxidant factors that prevent them to come over a level by controlling radical reactions. The substances described as antioxidants prevent autooxidation and peroxidation by reacting fast with radicals. Preventing the attack of any occuring radical metabolite to biomoleculer and celluler buildings depending its reactive character is function of antioxidant defense system. If neutralizing antioxidant compenents are insufficient when organisms confront with radical attack then expose to irreversible damages (7, 13, 21).

In the present study, malondialdehyde levels in the dogs both in two groups given dipyrone at overdose for 12 days have been found higher than control data. The increases of malondialdehyde in the first group dogs have been observed higher than in the second group dogs. This may be result of the intravenous treatment of dipyrone to the first group dogs.

The effect of intravenously treated dipyrone solutions starts quite fast (15-30 seconds) because of the introduction to directly sistemic circulation. However in intramuscularly treatment, the solution enters directly into the extracelluler fluid and absorbed directly. The intramuscular absorbtion of the solution is slower because of the barriers such as epithelial and mucous layers and they generally reach to the plasma peak level in 30 minutes (19).

The increase of malondialdehyde concentration increases in two experimental groups was statistically significant. There were undulations in glutathione and ceruloplasmin levels but not significant.

As a result, overdose dipyrone can cause partial lipid peroxidation. Considerable increases in malondialdehyde concentrations are accepted as lipid peroxidation marker (8). In contrast to this, lack of the considerable changes in glutathione and ceruloplasmin, recollect that the level of this lipid peroxidation is not causative for cell and tissue damage. It can be concluded from this study that the overdose treatments of dipyrone does not cause severe lipid peroxidation and this may be result of broad tolerance of dipyrone.

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