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THE EFFECT of FUROSEMIDE and ACETAZOLAMIDE-INDUCED ION TRAPPING PHENOMENA on RENAL EXCRETION of CHEMICALS

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Kimyasalların böbrekten atılımı üzerine furosemid ve asetazolamidle oluşturulan iyon tuzağının etkisi.

SUMMARY

This experimental study was conducted to determine the importance of diuretic drug selection on the account of pKa values of chemicals to accelerate the elimination from body by hard diuresis.

Fifteen healthy dogs weighing between 15-20 kg, were used as materials. Dogs were divided into three groups to be five dogs in each group (Group I, II, III). Animar® (Roche), which is containing 200mg sulfadoxin and 40 mg trimethoprim in 1 ml, was injected IM as an overdose to all dogs of experiment. Furosemide (Lasix®) was injected IM to the dogs of group II at the dose of 5 mg/kg body weight 2 hours after Animar® injection. Acetazolamide was given to the dogs of group III at the total dose of 300 mg by per oral at the 1st and 8th hours after Animar® injection. The concentrations of sulfadoxin and trimethoprim in all samples were measured by HPLC.

Furosemide was found effective on elimination of trimethoprim from the body, while acetazolamide was found effective on elimination of sulfadoxin. Acetazolamide significantly increased the urine pH and furosemide slightly decreased the urine pH.

The results of this study showed that the other factors rather than the potential effects of diuretic drug could be more effective on acceleration of renal excretion of chemicals

KEY WORDS: Diuretic, ion trapping phenomena, renal excretion, sulfadoxine, trimethoprim.

ÖZET

Bu deneysel çalışma, zorunlu diürezis ile vücuttan atılımı artırmak için kimyasalların pKa değerine göre diüretik ilaç seçiminin önemini ortaya koymak üzere gerçekleştirildi.

Denemede, ağırlıkları 15-20 kg arasında değişen 15 adet köpek kullanıldı. Köpekler Grup I, II ve III olmak üzere üç gruba ayrıldı ve her gruptaki köpeklere 1 ml'sinde 200 mg sülfadoksin ve 40 mg trimetoprim içeren Animar® (Roche)'dan İM yolla yüksek dozda injekte edildi. Grup II'deki hayvanlara Animar enjeksiyonundan 2 saat sonra İM yolla 5 mg/kg dozunda furosemid uygulandı. Üçüncü gruptaki hayvanlara ise Animar uygulamasını takibeden 1. ve 8. saatlerde toplam 200 mg dozunda asetazolamid ikiye bölünerek oral yolla verildi. Toplanan kan ve idrar numunelerindeki sülfadoksin ve trimetoprim konsantrasyonları HPLC yardımı ile belirlendi.

Asetazolamidin sülfadoksinin, furosemidin ise trimetoprimin böbrekten atılımı üzerinde daha etkili olduğu gözlendi. Asetazolamid idrar pH'sını önemli oranda artırırken, furosemid çok az derecede bir düşüşe neden oldu.

Bu çalışmanın sonuçları, kimyasalların böbrekten atılımı üzerine, diüretiklerin potansiyel etkilerinden daha çok diğer faktörlerin etkili olabileceğini ortaya koydu.

ANAHTAR KELİMELER: Diüretik, iyon tuzağı fenomeni, böbrekten atılım, sülfadoksin, trimetoprim.

INTRODUCTION

In the cases of acute toxicosis, the elimination of absorbed chemicals is accelerated by either increased renal excretion of chemicals or increased biotransformation of chemicals. It is generally accepted that the accelerated elimination of chemicals by induction of enzymes which play a main role in the biotransformation of chemicals is not practical in the clinical point of view (Klassen *et al*, 1986). Because biotransformation can be saturated, in addition, it is common that chemicals can be taken excessively in poisoning, and chemicals dissociate readily from the binding proteins and become free. However, it is believed that the acceleration of renal excretion of poisons is the most effective and practical approach in toxicosis (Bettinetti and Giordana, 1988; Laurence and Bennett, 1992).

Alterations in renal excretion are related to diuresis and urinary pH. The basic principle of diuresis are ion-trapping and increasing urine flow. The ionisation of substance can be affected by changing of urine pH and blood pH. It is pointed out that changing of urine pH is an effective method of being ionized of poisons. Alkalinization of urine can result in a tenfold increase in the renal excretion of acidic drugs (Laurence and Bennett, 1992). The

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result of an experimental research showed that the accumulation rate of drugs in milk could be altered by changing pH of milk (Traş *et al*, 1994).

Diuretic drugs are not used to change urine pH. However, acetazolamide is a diuretic drug which elevates urine pH because it increases urinary bicarbonate concentration, but it is rarely used in veterinary medicine (Itoh *et al*, 1992). Although furosemide is frequently used for hard diuresis in poisoning, it is slightly decreased the urine pH (Freestone et al. 1988, 1989, Levine 1989). Freestone et al. (1988) stated that furosemide decreased urine pH. Diuretic potent of acetazolamide is less than that of furosemide. Young et al. (1990) determined that furosemide significantly decreased the plasma concentration of codein but not markedly altered plasma concentration of theophyline, phenylbutazone, pentazocine, guaifenesin and flunixin meglumine. Same research workers also informed that furosemide significantly reduced the urinary concentration of of guaifenesin, acepromazin, clenbutorol, phenylbutazone, flunixin meglumine, fentanyl and pentazocine.

Sulfadoxin is an antibacterial drug and its pKa value is 5.6. Trimethoprim is an alkalic substance and its pKa value is 7.6 . Both of the drugs are excreted in urine. There is no pharmacokinetic interaction between sulfadoxine and trimethoprim (Bettinetti and Giordana, 1988).

This experimental study was conducted to determine the importance of diuretic drugs selection on the account of pKa values of poison to accelerate the elimination of chemicals from the body by hard diuresis.

MATERIAL and METHOD

Animals: Fifteen healthy dogs, weighing between 15-20 kg, were used as material. Dogs were divided into three groups to be five dogs in each group (Group I, II and III). All dogs were fed with standard diet once in a day during the experiment.

Drug Administration and Sampling: A total of 4 ml Animar® (Roche), which is containing 200mg sulfadoxin and 40 mg trimethoprim in 1 ml, was injected IM as an overdose to all dogs of experiment. Group I was used as a control group. Furosemide (Lasix®) was injected IM to the dogs of group II at the dose of 5 mg/kg body weight after 2 hours Animar® injection. Acetazolamide was given to the dogs of group III at the total dose of 300 mg by per oral at the 1^{st} and 8^{th} hours after Animar® injection. Blood samples were taken into tubes with EDTA from all dogs at the 2^{nd}, 4^{th}, 8^{th}, 12^{th} and 24^{th} hours after Animar® injection. Ultrasound guided urine samples were collected from all dogs just after Animar® injection and the 4^{th}, 8^{th}, 12^{th} and 24^{th} hours after Animar® injection. Blood samples were immediately centrifuged at 3000 rpm for 5 minutes and then, plasma samples were separated. Plasma and urine samples were stored at 4 °C until the samples were extracted.

Quantification of Drugs and pH: Extraction of sulfadoxin and trimethoprim from plasma samples were performed by the method Löscher *et al.* (1990). 0.5 ml plasma, 0.2 ml 1 M phosphate buffer (pH 6.3) and 6 ml ethylacetate were added in a test tube and mixed by a wortex for 15 minutes. And then, centrifuged at 1200 rpm for 5 minutes. After removing the supernatant into another test tube, it was evaporated at 50 °C until drying. Residues were dissolved in 100 µl methanol and 20 µl of which were injected into HPLC system. The same extraction method was used for urine samples.

Concentrations of the drugs in samples were determined by HPLC (Schimadzu). A Shim-pack CLC-ODS column (150 x 6 mm) was used with a mobile phase of 0.067 M KH_2PO_4 and 0.067 M Na_2HPO_4 (97 + 3) and methanol (65:35) at a flow rate of 1 ml/min. Detection (UV-VIS Spectrophotometric) was at 270 nm for sulphadoxine and 225 nm for trimethoprim. The limit of detection for both drugs was 0.02 µg/ml.

The pH values of all urine samples were immediately measured after collection with pH-meter.

Statistical Analysis: Mean values are expressed ± standard errors of the mean (SEM). Concentrations of sulfadoxin and trimethoprim in plasma and urine at same sampling time in all groups were compared using two way t test by a computer program (Minitab Release 9.2, 1993). Similarly, differences between initial and other sampling time were determined.

RESULTS

Plasma sulfadoxin concentrations of the dogs in group III were lower than those of group I and group II (Table I) and the difference significant ($p<0.05$). The sulfadoxin concentrations of plasma of the dogs in group II were lower than those of group I at the 4^{th}, 8^{th} and 12^{th} hours of the experiment (Table I). However, the difference was not statistically significant ($p>0.05$). Contrary to this, plasma sulfadoxin concentrations of the dog in group II were higher than those of group I at the 2^{nd} and 24^{th} hours of the experiment. The difference at the 24^{th} hours was significant ($p<0.05$).

Plasma trimethoprim concentrations of the dogs in group II were lower than those of group III (Table I), but the difference was not significant ($p>0.05$).The trimethoprim concentrations in plasma of the dogs in group II were lower than those of group I at the 4^{th}, 8^{th} and 12^{th} hours of the experiment (Table I). The difference at the 12^{th} hours of the experiment was statistically significant ($p<0.05$). Plasma trimethoprim concentrations of the dogs in group I were lower than those of group II and III at the 2^{nd} and 24^{th} hours of the experiment (Table I) and the difference at the 24^{th} hours of experiment was significant ($p<0.05$). Plasma trimethoprim concentration of the dogs of group III were lower than those of group I at the 4^{th}, 8^{th} and 12^{th} hours of the experiment, but the difference was not significant.

Urine sulfadoxin concentrations of the dogs in group III were higher than those of group I and II at

the 4th, 8th and 12th hours of the experiment (Table II) and differences was significant ($p<0.05$). Urine sulfadoxin concentrations of the dogs in the group II were higher than those of group I at the 4th, 8th, 12th and 24th hours of the experiment (Table II) and the difference at 8th and 12th hours of experiment was significant ($p<0.05$). Urine trimethoprim concentrations of the dogs in group II were higher than those of group I and III (Table II) and the difference between group II and III was significant ($p<0.05$) only at the 8th hours.

When the pH value of urine samples at other sampling time in each group compared with the pH value of initials (Table III), acetazolamide increased significantly urine pH ($p< 0.05$), but furosemide had no significant effect ($p> 0.05$).

DISCUSSION

Elimination of absorbed chemicals is accelerated by either diuresis or changing urine pH with the objective to prevent tubular reabsorbtion, which are an important approach in the clinical point of view (Baggot, 1988; Hoppe *et al*, 1988; Klassen *et al*, 1986; Laurence and Bennett, 1992; Levine, 1989).

In this study, the effects of acetazolamide which has a weak diuretic effect and can change urine pH, and furosemide which has a strong diuretic effect and frequently used in poisoning, were compared on account of renal excretion of acidic and basic drugs and their plasma concentration. In this study, sulfadoxin and trimethoprim have been used to represent acidic and basic chemicals, respectively. Although furosemide has been offered for hard diuresis in the treatment of acute poisoning, there is no enough information in this respect.

The results of this study showed that the other factors rather than the potential effects of diuretic drug could be more effective on the account of acceleration of chemicals elimination from the body. The result was confirmed by increased sulfadoxin elimination when acetazolamide was used, despite furosemide has a more potent diuretic effect than acetazolamide. Young *et al* (1990) also stated that furosemide did not markedly alter the plasma concentration of acidic drugs such as acepromazine, phenylbutazone and flunixin meglumine. Some factors which are effective on the elimination of chemicals from the body can be evaluated when the some properties of sulfadoxin, trimethoprim, furosemide and acetazolamide which has been informed at introduction, were taken into consideration. Increased elimination of sulfadoxin by acetazolamide could be the result of changing urine pH. Acetazolamide increased the urine pH in this study (Table III). It has been informed that the reabsorbtion of substances could be prevented by changing urine pH which lets the substance more ionized. Ionized substances are not reabsorbed by passive diffusion and a small changes in body fluid pH effects the chemicals distribution in the body. The weak effect of acetazolamide on the acceleration of trimethoprim elimination is though to be related with alkali properties of trimethoprim. Because alkali substances exist in nonionized form in elevated urine pH, and nonionized substances are reabsorbed by tubular passive diffusion.

In this study, elimitaion of trimethoprim from body was increased by furosemide. This could be the result of non-change or a small decreaments in urine pH. Because it is though that alkalic trimethoprim is ionized in acidic urine and its tubular reabsorbtion is decreased. So, its urine concentration increase. Freestone *et al* (1988) stated that furosemide decreased urine pH.

In conclusion, if a diuretic drug is wanted to use to accelerate elimination of poison from the body, the pKa value of poison should be taken into consideration in the selection of diuretic drug. In the case of poisoning with basic substances, furosemide should be used either alone or in combination with substances causing decrease in urine pH such as NH_4Cl. Acetazolamide might be more convenient to induce diuresis in poisoning with acid substance. Because acetazolamide increased urine pH and let acidic substances more ionized. So, tubular reabsorbtion of acid substance was decreased and elimination of substance from the body was accelerated.

Table I.- The effects of furosemide and acetazolamide on plasma sulfadoxin and trimethoprim concentrations in dogs injected Animar®

Time (hour)	Sulfadoxin (μg/ml)			Trimethoprim (μg/ml)		
	Group I	Group II	Group III	Group I	Group II	Group III
2	40.493±8.421 a	43.085±10.747 a	16.992±2.88 b	1.328±0.042 a	1.652±0.352 a	2.591±1.413 a
4	30.985±4.891 a	23.945±2.113 a	8.100±1.059 b	3.211±1.413 a	1.655±0.397 a	1.607±0.122 a
8	37.070±17.490 ab	30.842±2.708 a	7.927±0.820 b	1.329±0.551 a	0.433±0.205 a	0.522±0.068 a
12	26.447±14.329 a	12.159±17.519 a	3.276±0.787 b	1.420±0.016 a	0.438±0.152 b	0.920±0.413 ab
24	11.081±0.231 b	19.515±0.659 a	4.792±0.621 c	0.175±0.009 b	0.359±0.317 ba	0.861±0.233 a

*Different letters in the line indicate the significant differences ($P<0.05$)

Table II.- The effects of furosemide and acetazolamide on excretion of sulfadoxin and trimethoprim in dogs injected Animar®

Time (hour)	Sulfadoxin (µg/ml)			Trimethoprim (µg/ml)		
	Group I	Group II	Group III	Group I	Group II	Group III
4	34.001±8.329 b	37.735±2.901 b	142.985±30.577 a	11.870±15.0.96	8.041±1.870	10.787±1.265
8	35.507±9.547 c	72.784±12.800 b	117.482±10.311 a	32.381±14.827 ab	53.133±11.854 a	18.317±3.778 b
12	22.191±8.75 c	44.156±1.236 b	79.344±19.144 a	20.172±12.162 a	24.159±8.547 a	14.594±5.659 a
24	68.350±23.325 a	86.547±48.833 a	15.873±2.416 a	18.398±3.787 a	19.488±4.688 a	17.320±0.572 a

*Different letters in the line indicate the significant differences (P <0.05)

Table III.- The effects of furosemide and acetazolamide on urine pH

Time (hour)	Group II	Group III
Initial (0)	6.2±0.3 a	6.5±0.2 b
4	6.1± 0.4 a	9.2±0.8 a
8	6.5± 0.6 a	8.2±0.1 a
12	5.9± 0.3 a	8.0±0.1 a
24	6.3± 0.2 a	6.7±0.5 bc

*Different letters in the column indicate the significant differences (P <0.05)

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