Comparison of systemic and local effects of nitric acid and hydrochloric acid: an experimental study in a rat model

Nitrik ve hidroklorik asidin sistemik ve lokal etkileri: Sıçan modelinde deneysel çalışma

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BACKGROUND

We aimed to determine the local and systemic effects of widely available household cleaners, namely 45% nitric acid (NHO_3), and 18% hydrochloric acid (H C l), in a rat model.

METHODS

This prospective, experimental, placebo-controlled trial was carried out in the Animal Research Laboratory of Akdeniz University hospital. Commonly available solution of 45% NHO_3 and 18% HCl were tested against normal saline. Each solution was administrated orally to groups consisting of ten rats. The metabolic changes were determined by measuring the pH and calcium (Ca) levels before and after the administration of solutions. In addition, the pathological changes and mortality rates were determined for each group.

RESULTS

There was a statistically significant increase in the post-ingestion (30 minutes later) Ca levels and a decrease in the post-ingestion pH levels after the administration of test solution in the NHO_3 (p=0.006 for Ca increase, p=0.001 for pH decrease) and HCl (p=0.007 for Ca increase, p=0.023 for pH decrease) groups. There was also a statistically significant difference between groups for Ca increase (p=0.000) and pH decrease (p=0.006). In post hoc analysis, the difference between the groups was found to be originated from the placebo group. In the pathological evaluation of esophagus and stomach, there was a statistically significant difference between groups (p=0.009 (E) and p=0.016 (S)) and the difference was found to be originated from the control group (p=0.543 (E), p=0.244 (S) for NHO_3 and HCl). The 30-minute mortality rates were 0,2 in the NHO_3 group, 0,6 in the HCl group and 0 in the control group.

CONCLUSION

Serious metabolic and mild local pathological changes can occur after the ingestion of household NHO_3 and HCl solutions. Further studies should be performed to elucidate the causes of death following oral ingestion of these compounds and appropriate public health warnings should be taken.

Key Words: Burns, chemical; experimental study/rats; nitric acid/adverse effects; hydrochloric acid/adverse effects; rats, Wistar.

AMAÇ

Bu çalışmada temizlik amaçlı yaygın olarak kullanılan %45 nitrik asit (NHO_3) ve %18'lik hidroklorik asidin (HCl) lokal ve sistemik etkileri belirlemeyi amaçladık.

GEREÇ VE YÖNTEM

Bu ileriye dönük, deneysel ve plasebo kontrollü çalışma Akdeniz Üniversitesi hayvan laboratuvarında gerçekleştirildi. %45'lik N H O_3 ile %18'lik H C l plasebo ile karşılaştırıldı. Solüsyonlar her biri 10 sıçandan oluşan 3 gruba ağızdan verildi. Solüsyonlar verilmeden önceki ve sonraki pH ve kalsiyum değerleri ölçülerek, meydana gelen metabolik değişiklikler izlendi. Ayrıca patolojik değişiklikler ile ölüm oranları da kaydedildi.

BULGULAR

Solüsyonların verilmesinden 30 dakika sonra yapılan ölçümlerde, HCl (Ca artışı için p=0.007, pH'deki azalma için p=0.023) ve NHO_3 (Ca artışı için p=0.006, pH'deki azalma için p=0.001) grubunda kalsiyum seviyelerinde anlamlı olarak artış, pH seviyelerinde ise azalma saptandı. Ayrıca, Ca ve pH değerlerinde değişiklikler açısından üç grup ANOVA ile karşılaştırıldığında istatiksel olarak anlamlı fark bulundu. Yapılan çoklu karşılaştırmada farkın kontrol grubundan kaynaklandığı saptandı. Mide ve özofagusun patolojik incelemesinde gruplar arasında anlamlı fark bulunurken (p=0.009 (Ö) ve p=0.016 (M)), farkın yine kontrol grubundan kaynaklandığı saptandı (p=0.543 (Ö), p=0.244 (M) NHO_3 vs HCL). Otuzuncu dakikadaki ölüm oranları NHO_3 grubunda %20, HCl grubunda %60 ve kontrol grubunda ise %0 idi.

SONUÇ

NHO_3 ve HCl alımları sonrası ciddi metabolik ve hafif lokal patolojik değişiklikler olabilir. Bu maddelerin alımları sonrası oluşan ölümleri engellemek için yeni çalışmalara ihtiyaç vardır; halk sağlığı önlemlerinin arttırılması gerekir.

Anahtar Sözcükler: Yanık, kimyasal; deneysel çalışma/sıçan; nitrik asit/yan etki; hidroklorik asit/yan etki; sıçan, Wistar cinsi.

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Accidental and intentional exposures to caustic agents represent a significant source of mortality.[1] The caustic agents involved in such cases in Turkey are usually household products such as fertilizers, pesticides, solvents, dyes, plastic and toilet cleaners. They are widely available in neighborhood markets throughout the country. The high mortality rates due to the ingestion of these products are thought to result from severe metabolic acidosis.[2]

Nitric acid (NHO_3), a strong oxidizing agent which ionizes readily, is a highly electrically conductive solution. It reacts with metals, oxides and hydroxides to form nitrate salts. Nitric acid is used mainly in the preparation of explosives and fertilizers such as ammonium nitrate. Forty-five percent compound of NHO_3 is commonly used as a cleaner in Turkey. Bleaching agents contain dilute (less than 10%) hydrochloric acid (HCl). Furthermore, concentrated solutions of HCl (36%) are used in dye and chemical synthesis, metal refining and plumbing industry.[3]

Many articles in the literature report injuries and deaths from ingestion of a variety of acids.[4-8] In a retrospective analysis of hydrofluoric acid ingestion by Kao and colleagues revealed 1772 exposures in a two-year-period; 135 involved ingestion. Ca concentrations of 29 patients were recorded and 4 patients had hypocalcaemia. Death occurred in two patients who ingested more than 3 ounces of hydrofluoric acid.[7] Deaths can occur as a result of severe metabolic acidosis and necrosis of esophagogastric and duedonopancreatic junctions due to the ingestion of HCl.[9,10] In our clinical practice, suicidal attempts with NHO_3 and HCl solutions are not uncommon. Despite their potential systemic and regional toxic effects, patients who ingest NHO_3 often present with only mild clinical symptoms. So far, no laboratory studies have been performed to investigate the pathological findings due to the ingestion of this acid. So, we designed this experimental study to determine and compare the systemic and regional pathological effects of nitric and HCl solutions in a rat model.

MATERIALS AND METHODS

This experimental study was carried out in the Animal Research laboratory of a university hospital and approved by the institutional Animal Laboratory Ethics Committee. Commonly available solutions of 45% NHO_3 (Porçöz®, Levent Kimya, Antalya, Turkey) and 18% HCl (Tuzruhu®, Joco Kimya, Turkey) were compared to normal saline. First, Urethane®, Sigma, USA, ethyl carbamate, was administered intraperitoneally as a 20% of saline solution at a dose of 1 mg/kg to all rats for anesthesia.

Groups of ten rats received one of the following solutions orally via an esophageal tube inserted following anesthesia: normal saline, 45% NHO_3 or 18% HCl. Each rat was catheterized using a femoral line. The rat groups, Wistar albino rats, were similar according to age, gender and strain. Blood samples were taken in order to measure pH and Ca levels before and 30 minutes after the administration of test solution in NOVA Stat Profile M analyzer (Nova Biomedical, Waltham, MA, USA). In the HCl group, the post-ingestion pH of three rats and the post-ingestion Ca levels of four rats were not measured due to the deaths. One of the test solutions was administered to each rat at a dose of 4 ml/kg orally through an esophageal tube. The respiration and heart rate of rats were observed and the deaths occurred within 30 minutes of solution administration were recorded. Distal esophagus and proximal stomach were removed in all rats at the end of the study for pathological evaluation.

Rats were sacrificed using ether inhalation. The samples were fixed in 10% buffered formaldehyde and all samples were evaluated by a blinded pathologist. Esophagus and stomach were evaluated separately. Blinded pathological examination scores were assigned as follows:

0=normal,
1=erosion in the superficial epithelium,
2=necrosis in the mucosa,
3=ulcer.

Statistical analysis

The mean values of weight, initial pH and calcium in each group were compared using 'one way ANOVA'. Kruskal-Wallis test was used to perform multi-group comparisons if the variances of groups are not homogenous and for the ordinal data. Post-hoc analysis was achieved by Mann-Whitney U test. Paired-T test was used for repeated measures of continuous variables. The comparison of deaths

Table 1. The mean values, initial pH and serum Ca levels of each group with the post exposure measurements of pH and Ca^{++}

	Control group	NHO_3 group	HCl group	*p*
Weight (g)	232±18.74	228±13.98	233±21.63	0.814
Initial pH level	7.40±3.53	7.39±2.58	7.39±3.09	0.726
Initial Ca^{++} level (mg/dl)	8.97±0.35	9.39±0.34	9.44±0.86	0.154
Post exposure pH level	7.37±0.02	7.19±0.139	7.17±0.204	0.006
Post exposure Ca^{++} level (mg/dl)	9.28±0.456	14.08±4.25	17.01±4.26	0.000

NHO_3: Nitric acid; HCl: Hydrochloric acid.

Table 2. Median values of pathologic scores for each group and total

	Control (n=10)	NHO_3 (n=10)	HCl (n=10)	*p*
Esophagus (median)	0	0.5	1	0.01
Stomach	1	2	2	0.013

NHO_3: Nitric acid; HCl: Hydrochloric acid.

between each group was performed using Kruskal-Wallis test instead of Chi-Square test, because 50% of cells in Chi-Square test have an expected count of <5. A two-sided p value <0.05 was considered significant.

RESULTS

Mean values of weight, initial pH and serum Ca levels of each group are shown in Table 1. There was no statistically significant difference concerning the mean values of weight (231±17,88; p=0.814), initial Ca level (7,39±3,02; p=0.154) and initial pH (9,26±0,59; p=0.726) between the groups. There was a statistically significant difference between the initial and post-ingestion Ca and pH levels of NHO_3 (p=0.006 for Ca increase, p=0.001 for pH decrease) and HCl (p=0.007 for Ca increase, p=0.023 for pH decrease) groups. There was also no statistically significant change in the control group in terms of Ca increase (p=0.079) and pH decrease (p=0.249). Furthermore, there was a statistically significant difference between the three groups for post-ingestion calcium levels (9,28±0,456 for control group, 14,08±4,25 for NHO_3 group, 17,01±4,26 for HCl; p=0.000) and pH (7,37±0,02 for control group, 7,19±0,139 for NHO_3 group, 7,17±0,204 for HCl; p=0.006).

After the post-hoc analysis using Mann-Whitney U-test, the difference between the groups was found to be originated from the placebo group, because NHO_3 and HCl groups were similar according to the post-ingestion Ca levels (p=0.083) and pH (p=0.884) and there was a significant difference between HCl and control group for post-ingestion Ca levels (p=0.001) and pH (p=0.025). In conclusion, the pH and the Ca levels changed after administration of the acid solution, but there was no difference between acid groups according to their effects on the pH and the Ca levels.

A blinded pathologist evaluated all tissue samples including distal esophagus (E) and stomach (S). There was a statistically significant difference between the pathological scores of each group for esophagus and stomach after administration of the solutions (p=0.01 (E) and p=0.013 (S), Table 2). After the post-hoc analysis with Mann-Whitney U-test, the difference was found to be originated from the control group. There was no statistically significant difference between the NHO_3 and HCl groups (p=0.543 (E) and p=0.244 (S)), but the difference between HCl and control groups were statistically significant (p=0.009 (E) and p=0.016 (S)). Tissue samples are shown in Fig. 1.

The 30-minute mortality rates were, 0,2 in the NHO_3 group, 0,6 in the HCl group and 0 in the control group. There was statistically a significant difference between three groups (p=0.01). The difference was found to be originated form the control group (p=0.075 for HCl and NHO_3 groups, p=0.004 for HCl and control groups).

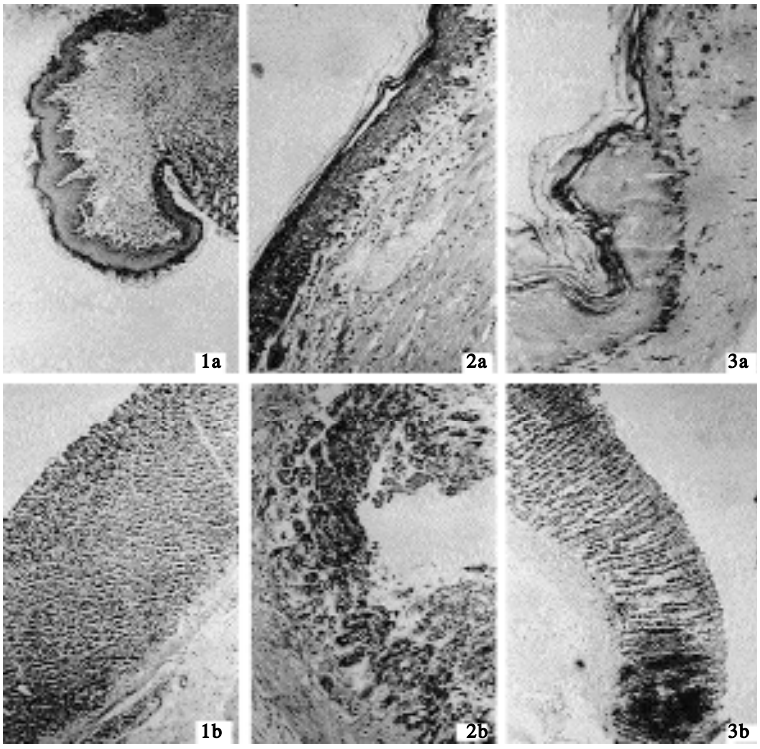

Fig. 1. Tissue samples of each group. (1a: Control esophagus; 1b: Control stomach; 2a: Nitric acid (NO_3) esophagus, 2b: NO_3 stomach; 3a: HCl acid esophagus; 3b: HCl acid stomach; (Hematoxylin - eozine, 10 mm x 15 mm).

DISCUSSION

Metabolic acidosis and local pathological effects are well-known pathological processes as part of acid ingestion. The metabolic and regional pathological effects of several acids were determined in the medical literature;[11-13] however, there is no data about the effects of NHO_3 used as a household cleaner.

When the strong NHO_3 and HCl solutions were administered orally in this rat model, severe metabolic and mild local pathologic effects were noted. This study showed that ingestion of NHO_3 can also cause serious metabolic alterations and local damage in rats like HCl in spite of poor clinical evidence in our medical practice.

Acute and chronic pathological changes in gastrointestinal tract after acid ingestion were reported in the medical literature. In this study, we evaluated the acute effects of acid solution on distal esophagus and stomach as in the previous reports.[11,14] In a prospective evaluation of 41 patients by Zargar et al.,[11] the burns after ingesting acids were classified as follows: grade 0 in 2 patients, grade 1 in 3 patients, grade 2 in 16 patients and grade 3 in 20 patients. Esophageal injury was seen in 87,8% and gastric injury in 85,4% of patients.[11] In the same way like Zargar's findings, both acid groups had significant pathological findings in our study when compared to the control group. But there was no difference between the acid groups according to the pathologic scoring of tissue damage. Only the acute complications of acid ingestion were evaluated in our study, so we must be aware of the delayed complications of the acid ingestion.[15-17] Citci et al.[17] reported the incidence of gastric outlet obstruction 3,8% among 52 patients ingested acid.

In our study, mortality rate of rats in HCl group is higher than NHO_3 group, while there is no difference between acid solutions for their metabolic effects. It is not easy to explain the reason of high mortality rate in HCl group. Tracheal aspiration due to esophageal tube insertion could be a reason of unexpected deaths. This might be a limitation of our study.

Deaths due to the serious hypocalcaemia after hydrofluoric solution ingestion were recorded in the medical literature.[4,7,8] We found a significant increase in Ca level after acid administration in both acid groups but not in control group. Hypercalcaemia due to acid ingestion in this rat model could be explained by bone buffering system against metabolic acidosis. The evidence that bone participates in H^+ buffering *in vivo*, derives principally from the loss of bone sodium and the depletion of bone carbonate after an acute acid load.[18] On the other hand, Ca increase is not found in patients who ingest hydrofluoric acid because of the low solubility of calcium fluoride.

In conclusion; when strong household NHO_3 and HCl solutions were administrated orally, serious metabolic and mild regional pathological changes can occur. Further studies should be performed to elucidate the causes of death following oral ingestion of these compounds and appropriate public health warnings should be taken.

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