ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA MAKALESİ

Evaluation of Lipid Emulsion-Mediated Sequestration and Redistribution of the Highly Lipophilic Carbamazepine in the Plasma of Rats

Yüksek Lipofilik Karbamazepinin Sıçan Plazmasında Lipid Emülsiyonu Aracılı Sekestrasyonu ve Yeniden Dağılımının Değerlendirilmesi

Merve Ekşioğlu[1], Deniz Algedik Gürsoy[2], Engin Sümer[3], Fadime Canpolat[4], Sezgin Sarıkaya[5]

ABSTRACT

Aim: The idea that intravenous lipid emulsion (ILE) may serve as a "reservoir" for lipophilic drugs has emerged in research as an intravascular "lipid sink" effect. Carbamazepine (CBZ) is a widely used anticonvulsant. This compound has a neutral and highly lipophilic structure and can easily cross body membranes. In this study, our hypothesis focused on the potential efficacy of ILE in modulating blood carbamazepine concentrations.

Material and Methods: 22 adult Sprague-Dawley rats were divided into four groups. All groups received CBZ at a dose of 20 mg/kg orogastrically. The first group was the control group. In the second group (activated charcoal group), activated charcoal (AC) was administered orogastrically at a dose of 1 g/kg five minutes after orogastric administration of carbamazepine. The third group (lipid group) received ILE at a dose of 3 ml/kg/min at the fifth minute. The fourth group was the saline group, in which 16 ml/kg of 0.9% NaCl was infused at the fifth minute. Blood samples of 0.5 ml were collected at 0, 4, 8, and 24 hours. Plasma was separated by centrifugation (4000 rpm, 10 minutes) and stored at -80oC for determination of CBZ concentrations. An Agilent 6410B HP-1200 LC series (USA) liquid chromatography system was used for analysis. Quantitative analysis was performed in the multiple reaction mode with electrospray positive ionization (ES+).

Results: At the 8th hour of orogastric CBZ administration, CBZ concentration was significantly lower in the activated charcoal group than in the lipid and saline groups (p: 0.021; p: 0.023; p<0.05, respectively). There was no significant difference in CBZ concentrations between the other groups at 8 hours (p>0.05). In the lipid group, the increase in CBZ plasma concentrations was statistically significant at 4 and 8 hours compared to 0 hours (p: 0.005; p: 0.005, respectively).

Conclusion: In the lipid group, plasma CBZ concentrations increased at 4 and 8 hours in plasma samples from which lipids were separated by differential centrifugation. In the lipid group, no effects favoring drug-lipid sequestration on the plasma distribution of CBZ were observed.

Keywords: Intralipid, lipid emulsion, carbamazepine, liquid chromatography, lipid sink

ÖZ

Amaç: İntravenöz lipid emülsiyonunun (ILE) lipofilik ilaçlar için bir "rezervuar" görevi görebileceği fikri, intravasküler "lipid sink" etkisi olarak araştırmalarda ortaya çıkmıştır. Karbamazepin (CBZ) yaygın olarak kullanılan bir antikonvülzandır. Bu bileşik nötral ve oldukça lipofilik bir yapıya sahiptir ve vücut membranlarını kolayca geçebilir. Bu çalışmada hipotezimiz, ILE'nin kan karbamazepin konsantrasyonlarını modüle etmedeki potansiyel etkinliğine odaklanmıştır.

Gereç ve Yöntemler: 22 yetişkin Sprague-Dawley sıçan dört gruba ayrıldı. Tüm gruplara 20 mg/kg dozunda CBZ orogastrik olarak verildi. Birinci grup kontrol grubuydu. İkinci grupta (aktif kömür grubu), karbamazepinin orogastrik uygulamasından beş dakika sonra aktif kömür (AC) 1 g/kg dozunda orogastrik olarak uygulandı. Üçüncü gruba (lipid grubu) beşinci dakikada 3 ml/kg/dk dozunda ILE verildi. Dördüncü grup, beşinci dakikada 16 ml/kg %0,9 NaCl infüze edilen salin grubuydu. 0, 4, 8 ve 24. saatlerde 0,5 ml kan örnekleri toplandı. Plazma santrifüj (4000 rpm, 10 dakika) ile ayrıldı ve CBZ konsantrasyonlarının belirlenmesi için -80oC'de saklandı. Analiz için bir Agilent 6410B HP-1200 LC serisi (ABD) sıvı kromatografi sistemi kullanıldı. Kantitatif analiz elektrosprey pozitif iyonizasyon (ES+) ile çoklu reaksiyon modunda gerçekleştirildi.

Bulgular: Orogastrik CBZ uygulamasının 8. saatinde, CBZ konsantrasyonu aktif kömür grubunda lipid ve salin gruplarına göre anlamlı derecede düşüktü (sırasıyla p: 0.021; p: 0.023; p<0.05). Diğer gruplar arasında 8. saatte CBZ konsantrasyonları açısından anlamlı bir fark bulunmadı (p>0.05). Lipid grubunda, CBZ plazma konsantrasyonlarındaki artış başlangıca kıyasla 4 ve 8. saatlerde istatistiksel olarak anlamlıydı (sırasıyla p: 0.005; p: 0.005).

Sonuç: Lipid grubunda, lipidlerin diferansiyel santrifüjleme ile ayrıldığı plazma örneklerinde plazma CBZ konsantrasyonları 4 ve 8. saatlerde artmıştır. Lipid grubunda, CBZ'nin plazma dağılımı üzerinde ilaç-lipid sekestrasyonunu destekleyen herhangi bir etki gözlenmedi.

Anahtar Kelimeler: İntralipid, lipid emülsiyonu, karbamazepin, sıvı kromatografisi, lipid sink

Received: 04 September 2023 Accepted: 07 February 2024

[1] University of Health Sciences, Fatih Sultan Mehmet Training and Research Hospital, Department of Emergency Medicine, Istanbul, Turkiye.

[2] Cerkezkoy State Hospital, Emergency Service, Tekirdag, Turkiye.

[3] Yeditepe University, Faculty of Medicine, Experimental Research Center, Istanbul, Turkiye.

[4] Canakkale Onsekiz Mart University, Canakkale Vocational School of Health Services, Pharmacy Services, Canakkale, Turkiye.

[5] Yeditepe University, School of Medicine, Department of Emergency Medicine, Istanbul, Turkiye.

Corresponding Author: Merve Eksioglu, MD **Adress:** University of Health Sciences, Fatih Sultan Mehmet Training and Research Hospital Department of Emergency Medicine, Atasehir-Istanbul Turkiye. **Phone:** +905052953687 **e-mail**: mervekoyunoglu@gmail.com

Atıf için/Cited as: Eksioglu M, Algedik Gürsoy D, Sumer E, Canpolat F, Sarıkaya S. Evaluation Of Lipid Emulsion-Mediated Sequestration and Redistribution of The Highly Lipophilic Carbamazepine in The Plasma of Rats. *Anatolian J Emerg Med* 2024;7(1):1-7. https://doi.org/10.54996/anatolianjem.1354717.

Introduction

Carbamazepine (CBZ) is a broad-spectrum anticonvulsant that is widely prescribed for the treatment of epilepsy, neuropathic pain, hyperactivity, and bipolar disorder (1-2). This compound has a neutral and highly lipophilic structure and can easily cross body membranes (3). Absorption of a single oral dose is highly efficient, with a known elimination half-life of approximately 35 hours (4). Following administration of a single oral dose of carbamazepine to volunteers or patients, the apparent volume of distribution has been observed to range from 0.79 to 1.86 liters per body weight (4). CBZ undergoes extensive hepatic metabolism via the cytochrome P450 enzyme system, with its major metabolite, carbamazepine-10,11-epoxide, exhibiting 50% protein binding and equal anticonvulsant and toxic properties (5). There is no specific antidote for CBZ poisoning and the drug is difficult to eliminate (6). Gastrointestinal decontamination (e.g., a single dose of activated charcoal) is indicated in patients who present within 1-2 hours of ingestion and who have no contraindications (7).

The idea that an intravenous lipid emulsion (ILE) could serve as a "depot" for lipophilic drugs has emerged in research as an intravascular "lipid sink" effect (8,9). Intralipid is an aqueous emulsion of neutral triglycerides derived from soybean oil and made isotonic with glycerol (10). The emulsion, which is composed of particles approximately 0.5 microns in diameter, contains egg yolk phospholipid at a concentration of approximately 1% as its emulsifying component (9). These fat droplets form a lipid compartment in the blood, separate from the aqueous plasma phase, in which lipophilic substances can be solubilized and drawn into the "lipid pool" (10). Among the mechanisms proposed for ILE action, the "lipid sink" coupled with the "lipid shuttle" has been one of the earliest and most enduring explanations (11).

Goals of this investigation

In this study, our hypothesis centered on the potential efficacy of ILE in modulating blood carbamazepine concentrations. The primary objective of our investigation was to evaluate the effect of ILE on blood CBZ concentrations. The second objective is to evaluate the effect of activated charcoal on the absorption of CBZ.

Material and Methods

Ethical Considerations

The study (decision number: 634, approval date: 22.12.2017) was initiated following the approval of the Ethics Committee of the Local Ethics Committee for Animal Experiments at Yeditepe University. The research was conducted at the Laboratory for Experimental Animal Research at the Faculty of Medicine, Yeditepe University, Istanbul, Turkiye. The study followed all relevant international, national, and institutional guidelines for the appropriate care and use of animals in research.

Design and Subject Selection

A total of 22 adult Sprague-Dawley rats weighing between 200 and 300 grams were included in the study. All experimental animals were maintained on a 12-hour day/night cycle at a temperature of 24 ± 4 °C for 7 days prior to the start of the experiment. Rats were given standard diet and water.

Experimental Protocol and Groups

Rats were randomly divided into four equal groups as follows:

- Group 1 (n = 4) was the control group in which CBZ (Sigma-Aldrich Co., St. Louis, MO) was administered orally at 20 mg/kg.
- Group 2 (n = 6) was the activated charcoal (AC) group, in which activated charcoal at a dose of 1 gr/kg was administered orogastrically in the fifth minute after orogastric administration of CBZ at a dose of 20 mg/kg.
- Group 3 (n = 6) was the ILE group, in which ILE at a dose of 3 ml/kg/day was administered in the fifth minute after orogastric administration of CBZ at a dose of 20 mg/kg.
- Group 4 (n = 6) was the saline group in which 16 ml/kg 0.9% NaCl was infused in the fifth minute after orogastric administration of CBZ at a dose of 20 mg/kg.

CBZ and AC were administered via orogastric tube. The tail vein cannulation was performed with a 26-G cannula (i.v. NEO ALPHA, La-med Healthcare, Hayrana, India). ILE (ClinOleic 20% lipid 500 mL, ECZACIBASI Baxter/Belgium) and 0.9% NaCl were administered with an infusion pump (Swiss Made, Arcomed AG Volumed VP 7000, Kloten, Switzerland). Drug administration methods were determined, and the experimental protocol was designed based on study reports in the literature and previously explained references (12,13). CBZ was dissolved in saline for the oral pharmacokinetic (PK) study. Isoflurane inhalation anesthesia (Isoflurane, Isofludem 100 mL) was used in all rats with an animal anesthesia machine. Blood samples of 0.5 ml were collected at 0, 4, 8, 24 h. Plasma was separated from blood by centrifugation (4000 rpm, 10 min) and stored at -80oC for determination of CBZ concentrations. All rats were decapitated by guillotine under anesthesia.

Measurement of Carbamazepine

CBZ and desipramine hydrochloride (purity > 99, as internal standard) were purchased from Sigma (Sigma Aldrich, USA). HPLC grade methanol, HPLC grade acetonitrile, formic acid and ammonium formate were purchased from Merck (Merck, USA).

LC-MS/MS Conditions

An Agilent 6410B HP-1200 LC series (USA) liquid chromatography system was used for the analysis. The study of Canbolat F et al. was modified for the quantitative analysis of CBZ (10). ACE-3 C 8 (3 µm, 3.0 mm 150 mm) column was used for analytical separation. The column temperature was 45 °C. Mobile phase A was 0.001 M ammonium formate in water and mobile phase B is 0.001 M ammonium formate in methanol: acetonitrile (50:50 v/v). The gradient system conditions were as follows 50% mobile phase A for 2 min. From 2.10 min to 5 min, 10% mobile phase A. From 5.10 min to 8 min, 50% mobile phase A. Total analysis time was 8 min at a flow rate of 0.5 mL/min. An Agilent 6410B triple quadrupole detector was used.

Anatolian J Emerg Med 2024;7(1):1-7. https://doi.org/10.54996/anatolianjem.1354717

Molecule	Parent ion (m/z)	Daughter ion(m/z)	Fragmanter voltage (Volt)	Collision Energy (eV)
Carbamazepine	237.0	194.0	120	15
Desipramine (as IS)	267.0	72.0	120	15

Table 1. MRM condition for Carbamazepine and desipramine (as IS) by LC-MS/MS

Quantitative analysis was performed in multiple reaction mode with electrospray positive ionization (ES+). Table 1 shows the parent ion, daughter ion, fragmentor voltage, collision energy for each molecule (analyte).

Preparation of Standard and Quality Control (QC) Samples

Stock standard solutions were prepared by dissolving 50 mg of CBZ in methanol (c: 5.00 mg/mL). A diluted stock standard solution was then prepared by taking the stock standard solution and diluting it with methanol in a 50 ml volumetric flask (c: 25 µg/mL). The prepared solution was labeled and stored at -20°C.

To prepare eight calibration standards and three quality control samples (QCs) for CBZ, diluted stock standard solutions were spiked into plasma at different volumes. The calibration range for CBZ was 5.0 to 12590.0 ng/mL.

Sample Preparation

100 µL internal standard desipramine (IS) (c: 550 µg/mL) and 100 µL cold acetonitrile were added to 100 µL plasma sample, vortexed for 30 seconds and centrifuged at 16162 x g for 5 minutes. Five µL was injected into the analytical system.

Statistical Analyses

The NCSS (Number Cruncher Statistical System) 2007 Statistical Software (NCSS LLC, Kaysville, Utah, USA) program was used for statistical analysis in the evaluation of the results obtained in the study. In addition to descriptive statistical methods (mean, standard deviation, median, frequency, and ratio), the Kruskal-Wallis test was used for intergroup comparisons of variables that did not show a normal distribution, and the Dunn test with Bonferroni correction was used to determine the group responsible for the difference. The Friedman repeated measures test and the Wilcoxon signed rank test with Bonferroni correction were used in post hoc evaluations for within-group comparisons of parameters that did not have a normal distribution. Results were evaluated at the 95% confidence interval and significance was evaluated at the p<0.05 level.

Results

The median plasma concentration of CBZ at 8 hours after administration varied among the groups studied. The median concentration was 3659.6 ng/ml (interquartile range (IQR): 2653, 5805 ng/ml) in the activated charcoal (AC) group, 18888 ng/ml (IQR: 7061, 24096 ng/ml) in the control group, 15004. 9 ng/ml (IQR: 11087, 16197 ng/ml) and in the saline group it was 15957 ng/ml (IQR: 7445, 19168 ng/ml). Notably, at the 8th hour after orogastric administration of CBZ, the control group had a significantly higher CBZ concentration compared to the AC group ($p < 0.01$) (see Figure 1). Furthermore, the CBZ concentration at the 8th hour in the AC group was significantly lower than that observed in the lipid and saline groups ($p < 0.05$) (see Table 2). However, there were no statistically significant differences in CBZ concentrations between groups at 0, 4, and 24 hours ($p > 0.05$).

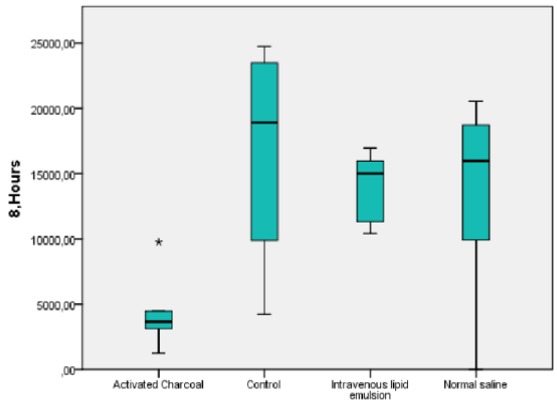

Figure 1. Comparison of CBZ concentration at 8th hour

In the AC group, there was a statistically significant change in CBZ plasma concentrations over time ($p < 0.0001$). Specifically, a significant increase in CBZ concentration was observed at the 4th and 8th hour compared to baseline (hour 0) ($p = 0.001$ and $p = 0.044$, respectively). No significant differences were noted at the 24-hour time point. Similarly, the decrease in CBZ concentration from 4th to 24th hour was statistically significant ($p <0.05$) (see Table 3).

A statistically significant change in CBZ plasma concentrations was also observed in the control group ($p <0.01$). In particular, there were significant increases in CBZ concentrations at the 4th and 8th hour compared to baseline ($p = 0.001$ and $p = 0.028$, respectively), with no significant change at the 24th hour.

A significant change in CBZ plasma concentrations was observed in the lipid group ($p <0.01$). Similar to the AC and control groups, there were significant increases in CBZ concentrations at the 4th and 8th hour compared to baseline ($p = 0.005$ for both). In addition, significant decreases were observed between the time points of the 4th and 8th hour and the time point of the 24th hour ($p <0.05$). No significant differences were observed at other time points ($p> 0.05$).

The saline group also showed a statistically significant change in CBZ plasma concentrations ($p <0.01$). Significant increases in CBZ concentrations were observed at 4 and 8 hours compared to baseline ($p = 0.001$ and $p = 0.014$,

respectively), while no significant change was observed at the 24-hour time point. In addition, a significant decrease was observed between the 4th and 24th hour time points (p <0.05), with no significant differences at other time points (p> 0.05) (Figure 2)

CBZ Concentration	Activated Charcoal-Control	Activated Charcoal- ILE	Activated Charcoal-Saline	Control -ILE	Control-Saline	ILE- Saline
8. hours	0.009**	0.021*	0.023*	1.000	1.000	0.965

Table 2. Post hoc evaluation of plasma CBZ concentrations by groups

*Dunn test with the Bonferroni Correction; *p<0,05 **p<0,01*

CBZ Concentration		Activated Charcoal	Control	ILE	Saline
0.Hours	*Q1-Q3 (median)*	0.73-1.07 (0.80)	0.76-1.23(0.91)	0.57-1.00 (0.69)	0.68-210.4 (1.11)
	Mean ± SD	0.9±0.23	0.97±0.25	0.82±0.40	140.15±340.54
4.Hours	*Q1-Q3 (median)*	10309-15054 (11910)	13920-28303(21901)	11535-17947 (12348)	13068-21736 (18835)
	Mean ± SD	12392.3±3653.1	21341.7±7408.0	13880.4±3448.4	16925.0±8837.9
8.Hours	*Q1-Q3 (median)*	2653-5805 (3659)	7061-24096 (18888)	11087-16197 (15004)	7445-19168 (15958)
	Mean ± SD	4323.7±2874.5	16681.8±9158.6	14105.6±2692.4	13516.0±7590.9
24.Hours	*Q1-Q3 (median)*	10.7-180 (84)	150-9514 (3672)	179-1509 (868)	49-11927 (528)
	Mean ± SD	91.6±77.6	4445.6±5095.7	856.1±660.4	5108.9±8302.2
[a]p		***0.0001****	***0.007****	***0.001****	***0.002****
[b]0 – 4. hours		***0.001****	***0.001****	***0.005****	***0.001****
[b]0 – 8. hours		***0.044****	***0.028****	***0.005****	***0.014****
[b]0 – 24. hours		*0.180*	*0.273*	*0.180*	*0.442*
[b]4 – 8. hours		*0.180*	*0.273*	*1.000*	*0.180*
[b]4 – 24. hours		***0.044****	***0.028****	***0.044****	***0.044****
[b]8 – 24. hours		*0.180*	*0.273*	***0.044****	*0.502*

Table 3. Evaluation of carbamazepine measurements in groups according to follow-up

*[a]Related Samples Friedman's Two-Way Analysis of Variance,[b] Bonferroni corrected Wilcoxon signed Rank test. Q1: First quarter. Q3: Third quarter. *p<0.05 **p<0.01*

Anatolian J Emerg Med 2024;7(1):1-7. https://doi.org/10.54996/anatolianjem.1354717

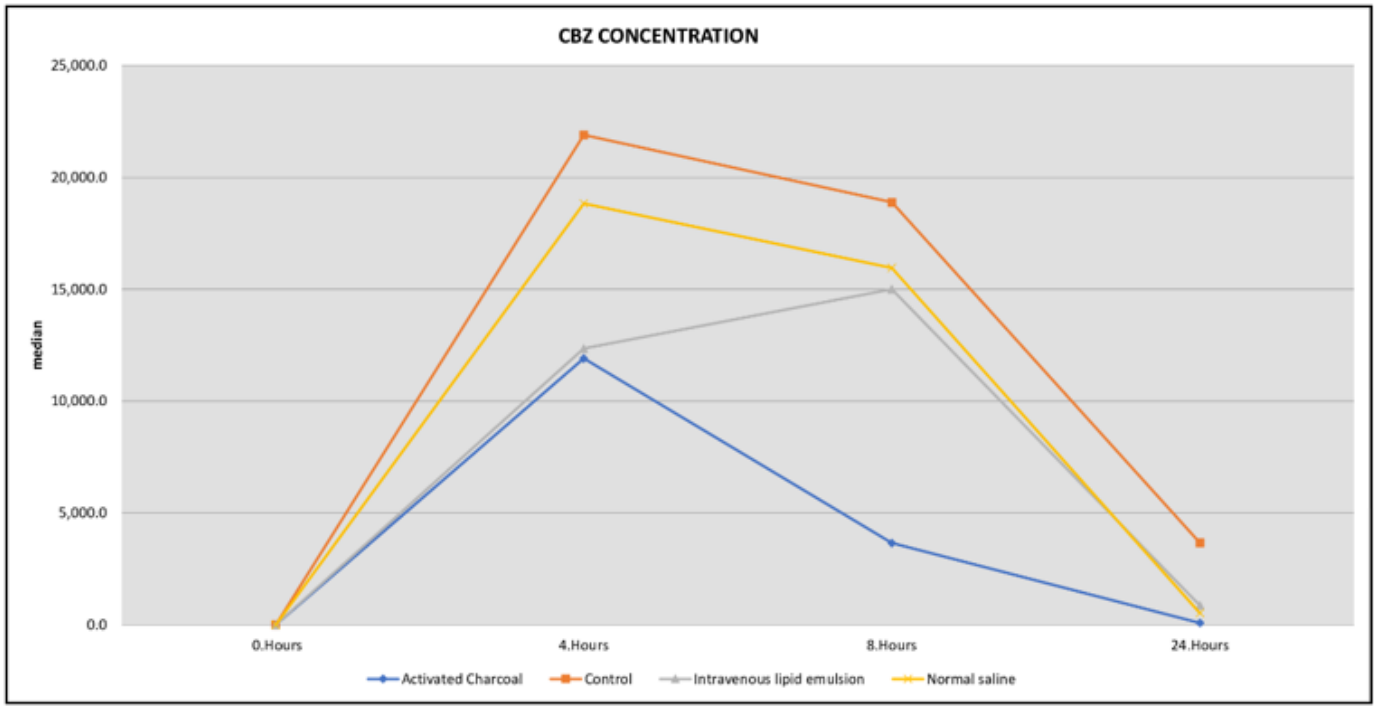

Figure 2. Distribution of carbamezapine concentration according to follow-up

Discussion

The primary objective of this study was to evaluate the effect of ILE on plasma CBZ concentrations after orogastric administration. It is important to emphasize that the model used in our study was not designed as a toxicity model. The dose of carbamazepine administered was carefully chosen to avoid inducing acute toxicity. The decision to use a subtoxic dose of CBZ was intentional, with the goal of elucidating the potential effects of ILE on CBZ pharmacokinetics without the confounding effects associated with acute toxicity. Although the charcoal-treated group showed a significant decrease in CBZ concentrations, this result must be interpreted with caution in the absence of overt toxicity. While there is currently no established antidote to reverse CBZ toxicity, anecdotal case reports have indicated successful treatment of CBZ-induced cardiovascular toxicity with lipid resuscitation therapy (14). However, the existing literature lacks a comprehensive investigation of the effects of intravenous lipid emulsion on the pharmacokinetics and plasma concentrations of CBZ. In our study, a notable observation was a significant reduction in plasma CBZ concentrations at the 8-hour mark in the activated charcoal-treated group compared with the lipid-treated group. This finding suggests that ILE alone may not be sufficient to substantially retain CBZ in the circulation.

Several pharmacokinetic (PK) and pharmacodynamic (PD) mechanisms have been postulated to explain the potential antidotal effects of ILE. Among these mechanisms, the "lipid sink" or "PK sequestration" theory has received the most attention (10). This theory posits that the triglyceride lipids present in ILE have a strong affinity for lipophilic drugs, creating a lipid-rich PK compartment within the circulation. This compartment effectively sequesters lipophilic drugs from their intended targets and free blood components. Equilibration between drug molecules and this lipid-rich phase results in reduced tissue concentrations, allowing for restoration of organ function. In vivo studies have supported the lipid sink effect in successful lipid resuscitation. For example, Niiya et al. demonstrated that pretreatment with lipids protected pigs against amiodarone-induced hypotension, with ILE-treated pigs having lower amiodarone concentrations in the lipid-free aqueous phase compared to saline-treated controls (15). Similarly, Litonius et al. observed an increase in total amitriptyline concentrations and a decrease in the free amitriptyline fraction after ILE administration in amitriptyline-poisoned pigs (16). Another study in a rabbit model of intravenous clomipramine toxicity reported increased total blood clomipramine concentrations along with improved blood pressure after ILE infusion, consistent with sequestration of the toxicant in the intravascular lipid phase (17).

However, our study yielded intriguing results. Contrary to findings from other in vivo studies of lipophilic drugs, we observed that intravenous lipid emulsion did not appear to sequester CBZ in plasma, as indicated by the lack of a statistically significant difference in carbamazepine concentrations between the lipid-treated group and other groups at 4 and 24 hours. This discrepancy with previous studies may be due to differences in the routes of drug administration. In particular, previous studies predominantly used parenteral drug administration, whereas our study favored orogastric administration. It's also important to note that our study did not evaluate an important metabolite, carbamazepine-10,11-epoxide, which has toxicity similar to CBZ (5). The exclusion of this metabolite underscores the need for comprehensive studies to capture its effects. In addition, it's important to recognize that the response to intravenous lipid emulsion may be different for different drugs, influenced by their different chemical properties and pharmacokinetic profiles. Therefore, it is important to emphasize that no single

dominant mechanism can explain the different results observed with different drugs.

To add to the discussion, activated charcoal was used in our study because of its proven ability to cover the surface of the digestive tract and prevent the absorption of certain drugs (18). AC therapy is still the most commonly used method of GI decontamination for acute drug overdose in developed countries. With a highly developed internal pore structure, the enormous surface area of AC allows adsorption of drugs and toxins in the GI tract within minutes of contact, reducing their systemic absorption and subsequent toxicity, and enhancing their elimination (19). Administered immediately after CBZ ingestion, activated charcoal served as a prophylactic measure in our study. In summary, the primary objectives of our article were to investigate the influence of early lipid administration on carbamazepine blood levels and the influence of activated charcoal on CBZ absorption. This multifaceted approach contributes to a more comprehensive understanding of the complex interplay between intravenous lipid emulsion, activated charcoal, and carbamazepine pharmacokinetics and enriches the discourse on clinical applications in toxicologic contexts.

In conclusion, despite the well-established "lipid sink" theory associated with ILE and its demonstrated efficacy with certain lipophilic drugs, our findings suggest that CBZ may not conform to this paradigm. Intravenous lipid emulsion did not appear to significantly sequester CBZ in plasma, as evidenced by the lack of a statistically significant difference in CBZ concentrations between the lipid-treated group and other groups at 4 and 24 hours. Notably, AC administration showed a significant decrease in CBZ concentrations at the 8-hour mark compared to the lipid-treated group, highlighting the efficacy of AC in reducing systemic absorption of CBZ. However, caution must be exercised in interpreting these results because the study intentionally used a subtoxic dose of CBZ and the observed decrease in concentrations occurred in the absence of overt toxicity.

Limitations

Our study has notable limitations that should be considered. First, we did not evaluate the therapeutic efficacy of CBZ sequestration by ILE. The focus remained primarily on elucidating the effects of the interventions without extending the investigation to broader clinical implications and therapeutic outcomes associated with CBZ use. Future research should include endpoints that allow assessment of the therapeutic benefit of ILE in mitigating CBZ-induced effects. In addition, the study focused exclusively on plasma concentrations of CBZ and did not include an assessment of tissue concentrations. This limitation highlights the need for future research specifically designed to investigate the influence of lipid emulsion on target tissue concentrations of CBZ, thus providing a more comprehensive understanding of drug distribution dynamics.

In addition, the lack of acute toxicity assessment and monitoring of vital signs in the experimental animals limits the ability to discuss the broader clinical implications of the study. Acute toxicity assessment is critical to understanding the therapeutic effects of ILE in scenarios where toxic manifestations are present. Future studies incorporating acute toxicity models and monitoring of vital parameters are essential for a more holistic view of ILE efficacy. In addition, the lack of evaluation of carbamazepine-10,11-epoxide, an important metabolite with comparable toxicity to CBZ, is a notable limitation. Future research efforts should include the evaluation of this metabolite to contribute to a more comprehensive understanding of the subject matter, considering its potential impact on toxicity and therapeutic response. In conclusion, addressing these limitations will enhance the overall understanding of the clinical applications of ILE and its interactions with CBZ in toxicological contexts.

Conclusion

In conclusion, our study observed no significant sequestration of CBZ in plasma by ILE, challenging the conventional "lipid sink" hypothesis. The limitations, including the absence of acute toxicity models and vital parameter monitoring, underscore the need for further research to comprehensively evaluate the therapeutic efficacy and broader clinical implications of lipid resuscitation therapy in CBZ toxicity scenarios. These findings contribute valuable insights, guiding future investigations and enhancing our understanding of ILE dynamics in the context of lipophilic drug exposure.

Conflict of Interest: This research has not received any specific grant from any public, commercial, or nonprofit funding agency. Each author certifies that he or she has no commercial relationships (e.g., consulting, stock ownership, equity interest, patent/licensing arrangements, etc.) that could pose a conflict of interest related to this article. The authors certify that they have fully disclosed all relevant information about funding and potential conflicts of interest and that there are no financial or commercial relationships that could be perceived as influencing the work reported in this manuscript.

Financial Support: This research was conducted without specific financial support from any public, commercial, or nonprofit funding agency. The authors acknowledge that the study was self-funded, and no external grants or funding sources were utilized in the design, execution, analysis, or reporting of the research presented in this manuscript.

Authors' Contribution: Each author contributed significantly to the research process and preparation of the manuscript. All authors reviewed and approved the final version of the manuscript for submission.

Ethical Approval: The study (decision number: 634, approval date: 22.12.2017) was initiated following the approval of the Ethics Committee of the Local Ethics Committee for Animal Experiments at Yeditepe University.

References

1. Breton H, Cociglio M, Bressolle F, Peyriere H, Blayac JP, Hillaire-Buys D. Liquid chromatography-electrospray mass spectrometry determination of carbamazepine, oxcarbazepine and eight of their metabolites in

human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;828(1-2):80-90. doi:10.1016/j.jchromb.2005.09.019
2. Zita L, Kurhan S, Krunt O, Chmelíková E, Kraus A, Čítek J, Klouček P, Stupka R. The Effect of Carbamazepine on Performance, Carcass Value, Hematological and Biochemical Blood Parameters, and Detection of Carbamazepine and Its Metabolites in Tissues, Internal Organs, and Body Fluids in Growing Rabbits. Animals (Basel). 2023 Jun 20;13(12):2041. doi: 10.3390/ani13122041. PMID: 37370551; PMCID: PMC10295208.
3. Ghannoum M, Yates C, Galvao TF, et al. Extracorporeal treatment for carbamazepine poisoning: systematic review and recommendations from the EXTRIP workgroup. Clin Toxicol (Phila). 2014;52(10):993-1004. doi:10.3109/15563650.2014.973572
4. Bertilsson L. Clinical pharmacokinetics of carbamazepine. Clin Pharmacokinet. 1978;3(2):128-143. doi:10.2165/00003088-197803020-00003
5. Vree TB, Janssen TJ, Hekster YA, et al. Clinical pharmacokinetics of carbamazepine and its epoxy and hydroxy metabolites in humans after an overdose. Ther Drug Monit. 1986;8(3):297-304. doi:10.1097/00007691-198609000-00011
6. Chyka PA, Seger D, Krenzelok EP, Vale JA; American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. Position paper: Single-dose activated charcoal. Clin Toxicol (Phila). 2005;43(2):61-87. doi:10.1081/clt-200051867
7. Krieglstein J, Meffert A, Niemeyer DH. Influence of emulsified fat on chlorpromazine availability in rabbit blood. Experientia. 1974;30(8):924-926. doi:10.1007/BF01938365
8. Weinberg G, Lin B, Zheng S, et al. Partitioning effect in lipid resuscitation: further evidence for the lipid sink. Crit Care Med. 2010;38(11):2268-2269. doi:10.1097/CCM.0b013e3181f17d85
9. Weinberg GL, VadeBoncouer T, Ramaraju GA, et al. Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anesthesiology. 1998;88(4):1071-1075. doi:10.1097/00000542-199804000-00028
10. Jaffal K, Chevillard L, Mégarbane B. Lipid Emulsion to Treat Acute Poisonings: Mechanisms of Action, Indications, and Controversies. Pharmaceutics. 2023 May 3;15(5):1396. doi: 10.3390/pharmaceutics15051396. PMID: 37242638; PMCID: PMC10224337.
11. Kuo I, Akpa BS. Validity of the lipid sink as a mechanism for the reversal of local anesthetic systemic toxicity: a physiologically based pharmacokinetic model study. Anesthesiology. 2013;118(6):1350-1361. doi:10.1097/ALN.0b013e31828ce74d
12. Uzunget SC, Evrin T, Uzunget SB, et al. Evaluation of activated charcoal and lipid emulsion treatment in model of acute rivaroxaban toxicity. Am J Emerg Med. 2018;36(8):1346-1349. doi:10.1016/j.ajem.2017.12.039
13. Turan CA, Ozturk TC, Akoglu EU, et al. The Role of Intralipid Emulsion in the Rat Model of Digoxin Intoxication. Cardiovasc Toxicol. 2018;18(4):329-336. doi:10.1007/s12012-018-9444-4
14. Hurley, W. T., and P. Hanlon. "Lipid emulsion as an antidote at the Washington Poison Center; use in carbamazepine, flecanide, hydroxychloroquine, bupivacaine, and bupropion." Clinical Toxicology. Vol. 47. No. 7. 52 Vanderbilt Ave, New York, NY 10017 USA: Informa Healthcare, 2009.
15. Niiya T, Litonius E, Petäjä L, Neuvonen PJ, Rosenberg PH. Intravenous lipid emulsion sequesters amiodarone in plasma and eliminates its hypotensive action in pigs. Ann Emerg Med. 2010;56(4):402-408.e2. doi:10.1016/j.annemergmed.2010.06.001
16. Litonius E, Niiya T, Neuvonen PJ, Rosenberg PH. No antidotal effect of intravenous lipid emulsion in experimental amitriptyline intoxication despite significant entrapment of amitriptyline. Basic Clin Pharmacol Toxicol. 2012;110(4):378-383. doi:10.1111/j.1742-7843.2011.00826.x
17. Harvey M, Cave G, Hoggett K. Correlation of plasma and peritoneal diasylate clomipramine concentration with hemodynamic recovery after intralipid infusion in rabbits. Acad Emerg Med. 2009;16(2):151-156. doi:10.1111/j.1553-2712.2008.00313.x
18. Brahmi N, Kouraichi N, Thabet H, Amamou M. Influence of activated charcoal on the pharmacokinetics and the clinical features of carbamazepine poisoning. Am J Emerg Med. 2006;24(4):440-443. doi:10.1016/j.ajem.2005.12.025
19. Position statement and practice guidelines on the use of multi-dose activated charcoal in the treatment of acute poisoning. American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. J Toxicol Clin Toxicol. 1999;37(6):731-751. doi:10.1081/clt-100102451